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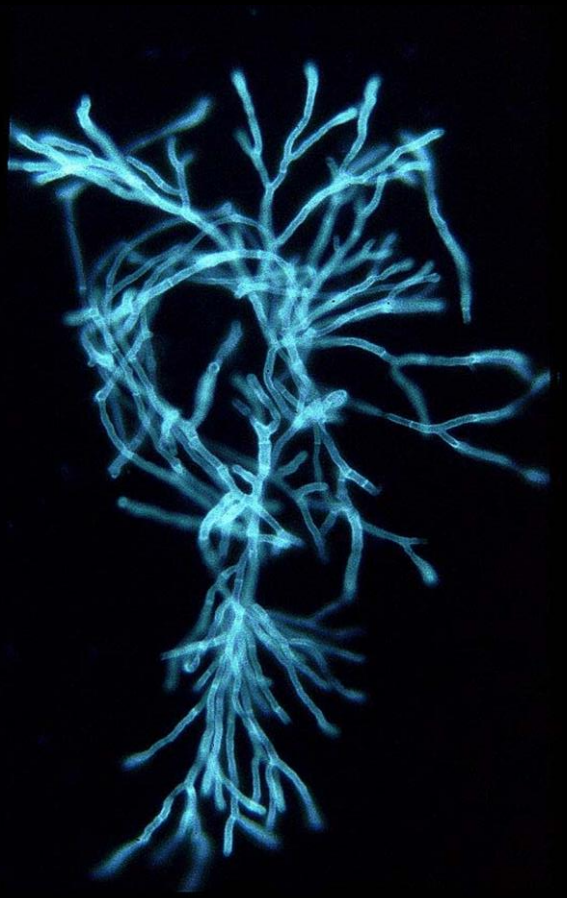
Resistenzentwicklung bei *Candida* und *Aspergillus* - ein klinisches Problem?



PEG-Jahrestagung 2018, Wien

Dr. Oliver Bader

Institut für Medizinische Mikrobiologie, Universitätsmedizin Göttingen

Resistenzentwicklung bei *Candida* und *Aspergillus* - ein klinisches Problem?

Candida auris

Ausbrüche azolresistenter Stämme

Candida glabrata

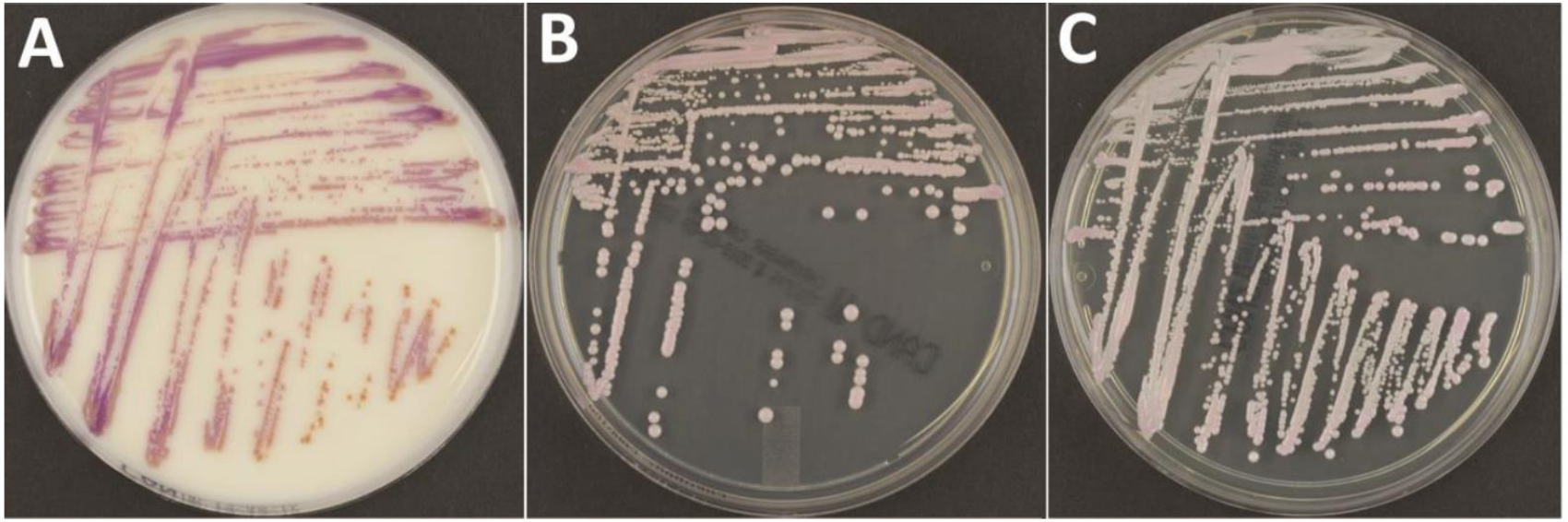
Vermehrtes Auftreten von Isolaten mit erhöhten Echinocandin MHKs

Aspergillus fumgiatus

Pandemisches Auftreten azolresistenter Isolate

Candida auris

- Emerging yeast, first described in Japan 2009
- Nosocomial transmission
- Multidrug resistant
- Rapid dissemination worldwide
- Difficult to identify



Technical Appendix Figure. *Candida auris* colonies from an otherwise healthy patient in Austria after 48 hours at 37°C on various chromogenic media: A) Brilliance Candida Agar; B) CHROMagar Candida; C) Candida ID.

Shiva Pekard-Amenitsch Emerg Infect Dis. 2018 Aug; 24(8): 1596–1597.

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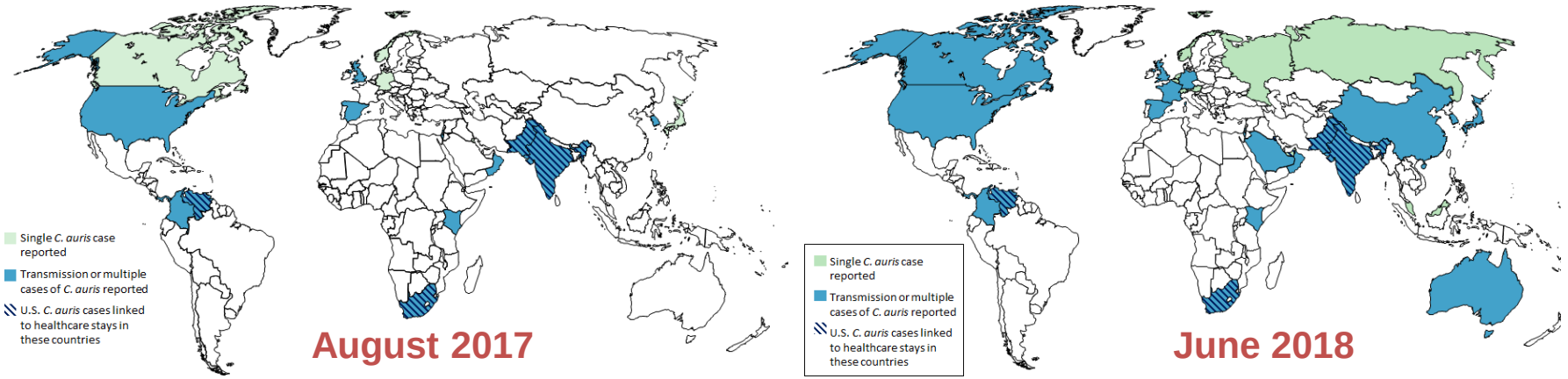
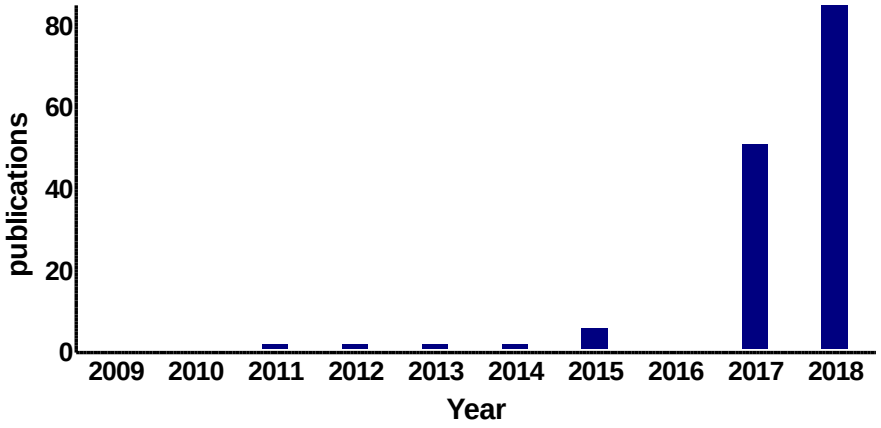
global dissemination



source: US gov't, CDC (https://www.cdc.gov/fungal/diseases/candidiasis/tracking-c-auris.html#world)



Reported cases in DE / AT

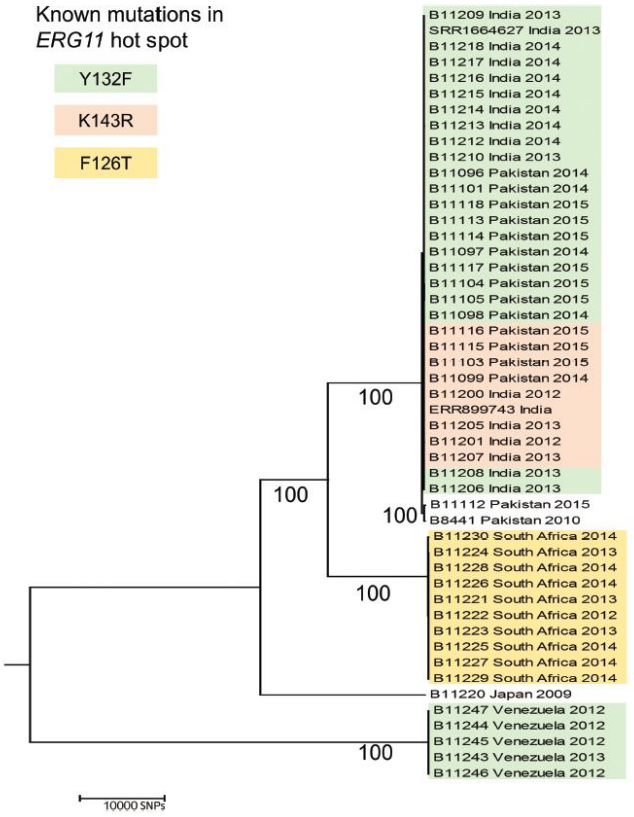
Date	City	clinical background	reference
11/2015	Stuttgart	prosthetic joint infection	Unpublished cases data courtesy of Oliver Kurzai (HKI Jena) and Axel Hamprecht (Uni Cologne)
12/2015	Nuremberg	SIRS	
06/2017	Cologne	intracranial hemorrage	
07/2017	Munich	neurological disorder	
08/2017	Munich	polytrauma	
11/2017	Aachen	gunshot wound	
12/2017	Regensburg	tetraplegia	
01/2018	Vienna	otitis	Pekard-Amenitsch (2018) EID 24(8): 1596–1597

MICs for fluconazole high, but low for amphotericin B
Favourable outcome in all cases
Positive travel history in nearly all cases → imported pathogens

C. auris clades

There are at least 3 different linages of *C. auris*, with geographically biased distribution

→ Epidemiology still unclear !



Lockhart et al., CID 2017:64

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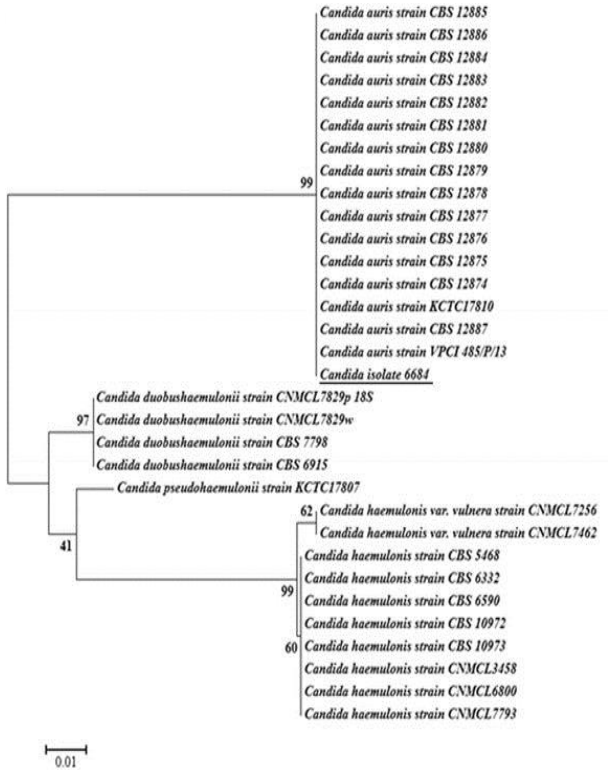
… is now contained in major MALDI-TOF databases



MBT

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts causing bloodstream infections

A. K. Ghosh, S. Paul, P. Sood, S. M. Rudramurthy, A. Rajbanshi, T. J. Jillwin and A. Chakrabarti

Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Ghosh et al, CMI 2015

MBT

Multidrug-Resistant *Candida auris* Misidentified as *Candida haemulonii*: Characterization by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry and DNA Sequencing and Its Antifungal Susceptibility Profile Variability by Vitek 2, CLSI Broth Microdilution, and Etest Method

Shallu Kathuria,[a] Pradeep K. Singh,[a] Cheshta Sharma,[a] Anupam Prakash,[a] Aradhana Masih,[a] Anil Kumar,[b] Jacques F. Meis,[c,d] Anuradha Chowdhary[a]

Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India[a]; Department of Microbiology, Amrita Institute of Medical Sciences & Research Centre, Kochi, Kerala, India[b]; Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands[c]; Department of Medical Microbiology, Radboudumc, Nijmegen, The Netherlands[d]

Kathuria et al, JCM 2015

VITEK-MS

Identification and typing of the emerging pathogen *Candida auris* by matrix-assisted laser desorption ionisation time of flight mass spectrometry

Victoria Girard,[1] Sandrine Mailler,[1] Marion Chetry,[1] Céline Vidal,[2] Géraldine Durand,[1] Alex van Belkum,[1] Arnaldo L. Colombo,[3] Ferry Hagen,[4] Jacques F. Meis[4,5] and Anuradha Chowdhary[6]

[1]*R&D Microbiology, bioMérieux, La Balme les Grottes, France,* [2]*R&D Biomathematics, bioMérieux, Grenoble, France,* [3]*Laboratório Especial de Micologia, Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil,* [4]*Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands,* [5]*Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands and* [6]*Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India*

Girard et al, Mycoses 2016

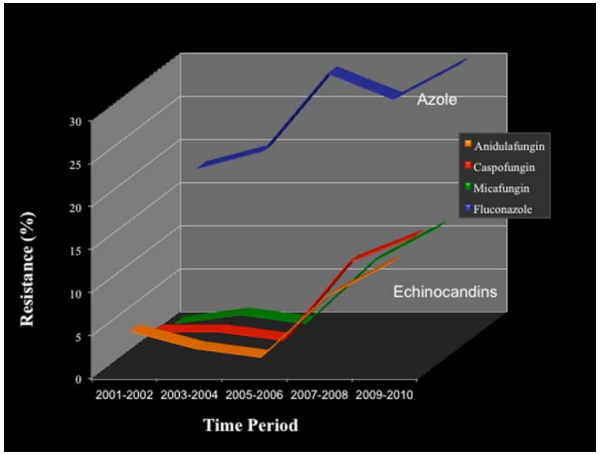
C. auris contained in DB: MBT✓; VITEK-MS RUO ✓; VITEK-IVD: end 2017

Candida glabrata

Rising incidence in echinocandin resistance?

Echinocandin resistance rates

US: (Perlin, Ann N Y Acad Sci 2015)
Echinocandin resistance of 8.0–9.3% was reported in a recent SENTRY program among 1669 bloodstream isolates (BSI) of *C. glabrata*. Similarly, over a 10-year period, echinocandin resistance in *C. glabrata* rose from 2–3% to > 13%.



Italy: (Mencarini, Infection 2018)
Between 2005 and 2015, increased use of echinocandins, but only one Candida glabrata isolate was resistant to caspofungin (1.9%) while 30% of C. glabrata were resistant to fluconazole.

China: (Hou, AAC 2018)
Among 158 *Candida glabrata* bloodstream isolates 8.9% were FLZ^R, 1.9% echinocandin-cross^R.

→ Echinocandin resistance rates in *C. glabrata* vary from 2% to 15%, depending on geography and host population.

Echinocandin resistance

Echinocaninds inhibit fungal glucan synthases, encoded by “fks” genes

Where most yeast only have a single gene, *C. glabrata* has two!

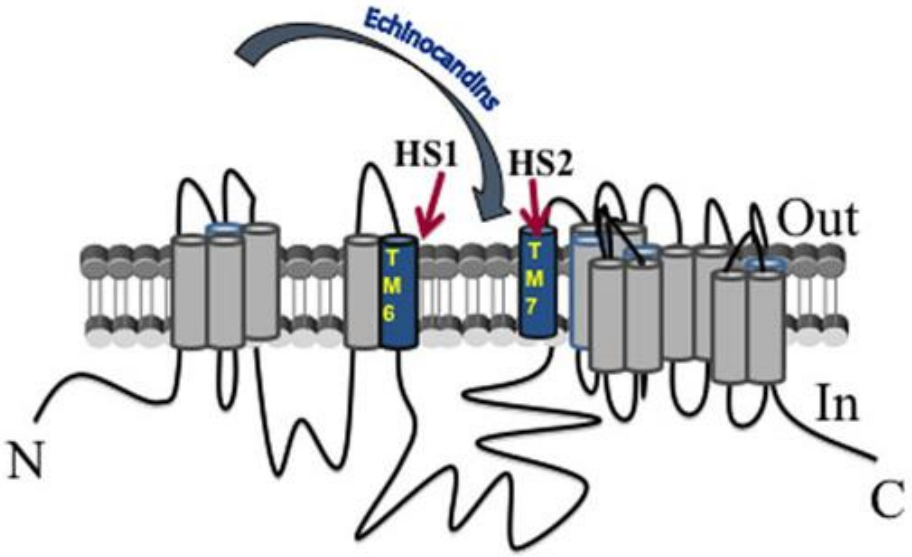


Table 1 Overview of Fks hot spot sequences and amino acid sequence positions resulting in echinocandin resistance

	Fks1p				Fks2p			
Species	Hot spot 1		Hot spot 2		Hot spot 1		Hot spot 2	
C. albicans	641	FLTLSLRDP	1357	DWIRRYTL				
C. dubliniensis	641	FLTLSLRDP	1357	DWIRRYTL				
C. glabrata	625	FLILSLRDP	1340	DWVRRYTL	659	FLILSLRDP	1374	DWIRRYTL

Perlin et al.; Curr Clin Micor Rep (2015) 2:84-95
Perlin; Ann. N.Y. Acad. Sci. 1354 (2015) 1–11

*Cg*FKS mutations seen in Germany

2016/17 @ NRZMyk:

- 41 isolates with ANI^R
- 12 isolates FLU^R, ANI^R with limited remaining treatment options
- 80% with ANI > 0,125 harbour FKS mutations, but only 20% of those with ANI = 0.125

mutation	gene	# isolates
S663P	*FKS2*	4
F659Y	*FKS2*	2
F659del	*FKS2*	2
D666N	*FKS2*	1
D666Y	*FKS2*	1
L630Q	*FKS1*	1
none	-	9

Wagener et al, Der Mikrobiologe 2018
Walther and Kurzai, poster@DMYKG2018 (Innsbruck)

Fitness cost?

- close association exists between drug exposure and the emergence of resistance.
- *FKS*-mediated resistance is directly linked to prior, prolonged, and/or repeated drug exposure.
- chromosomal instability is rapidly observed following exposure to azoles or echinocandins
- cellular stress increases genetic diversity by altering genome integrity

Evolution of MICs and genetic alteration of six related sequentially isolated *Candida glabrata* responsible for invasive infection in a solid organ transplant recipient receiving multiple antifungal therapy

Characteristic		Isolate no.					
		1	2	3	4	5	6
Postoperative day		8	25	97	104	108	119
Source of sample		Blood culture	Liver abscess	Blood culture	Blood culture	Blood culture	Blood culture
Antifungal drug administered at time of sampling (number of days from start of treatment)		Fluconazole (8)	Voriconazole (15) and caspofungin (4)	Micafungin[a] (7)	Liposomal amphotericin B (7)	Liposomal amphotericin B (11)	Liposomal amphotericin B (22)
Determination of MICs (mg/L) by EUCAST method	Amphotericin B	1	1	1	1	1	1
	Fluconazole	8	>64	>64	>64	>64	>64
	Itraconazole	1	>16	>16	>16	>16	>16
	Voriconazole	0.125	4	4	4	4	8
	Posaconazole	0.5	>8	>8	>8	>8	>8
	Caspofungin	0.25	0.5	>16	>16	>16	0.5
	Micafungin	<0.03	<0.03	2	2	1	<0.03
Molecular analysis of genes related to antifungal resistance	*FKS1* (HS1, HS2, HS3)	WT	WT	WT	WT	WT	WT
	FKS2 HS1	WT	WT	S663P	S663P	S663P	WT
	HS2	WT	WT	WT	WT	WT	WT
	FKS3	WT	WT	WT	WT	WT	WT

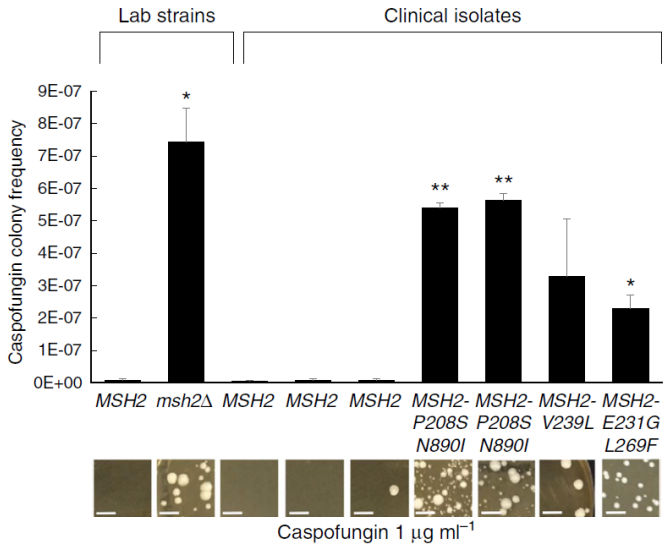
EUCAST, European Committee on Antimicrobial Susceptibility Testing; HS, hot spot; MIC, minimum inhibitory concentration; WT, wild type.

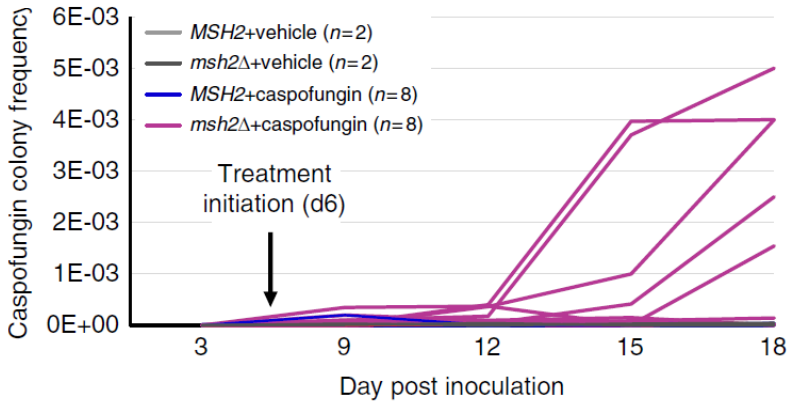
[a] Last dose of micafungin was administrated the day before blood was sampled for culture.

S. Imbert, CMI 2016;22:891.e5e891.e8

DNA mismatch repair

55% of all *C. glabrata* isolates (susceptible and resistant) recovered from patients contain mutations within the MMR gene *MSH2*. Strains with specific *msh2* mutations exhibit a higher frequency of emergence of resistance *in vitro*, and deletion of *MSH2* causes elevated propensity to breakthrough antifungal treatment in a mouse model of gastrointestinal (GI) colonization.





Mouse	Day	*fks* mutation (nucleotide change)
Δ-1	9	Fks1-625delF (1875_1877delCTT)
Δ-1	12	Fks1-S629P (t1885c)
Δ-1	18	Fks1-S629P (t1885c)
Δ-1	21	Fks1-S629P (t1885c)
Δ-2	18	Fks1-S629P (t1885c)
Δ-3	18	Fks1-S629P (t1885c)
Δ-6	15	Fks1-S629P (t1885c)
Δ-6	18	Fks1-S629P (t1885c)
Δ-6	21	Fks1-S629P (t1885c)

Haeley et al, 2016, Nat Comm.

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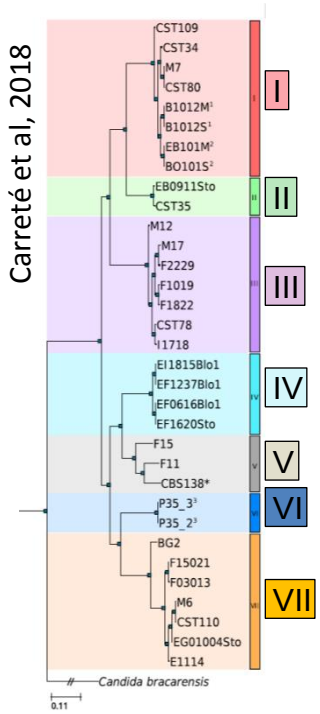
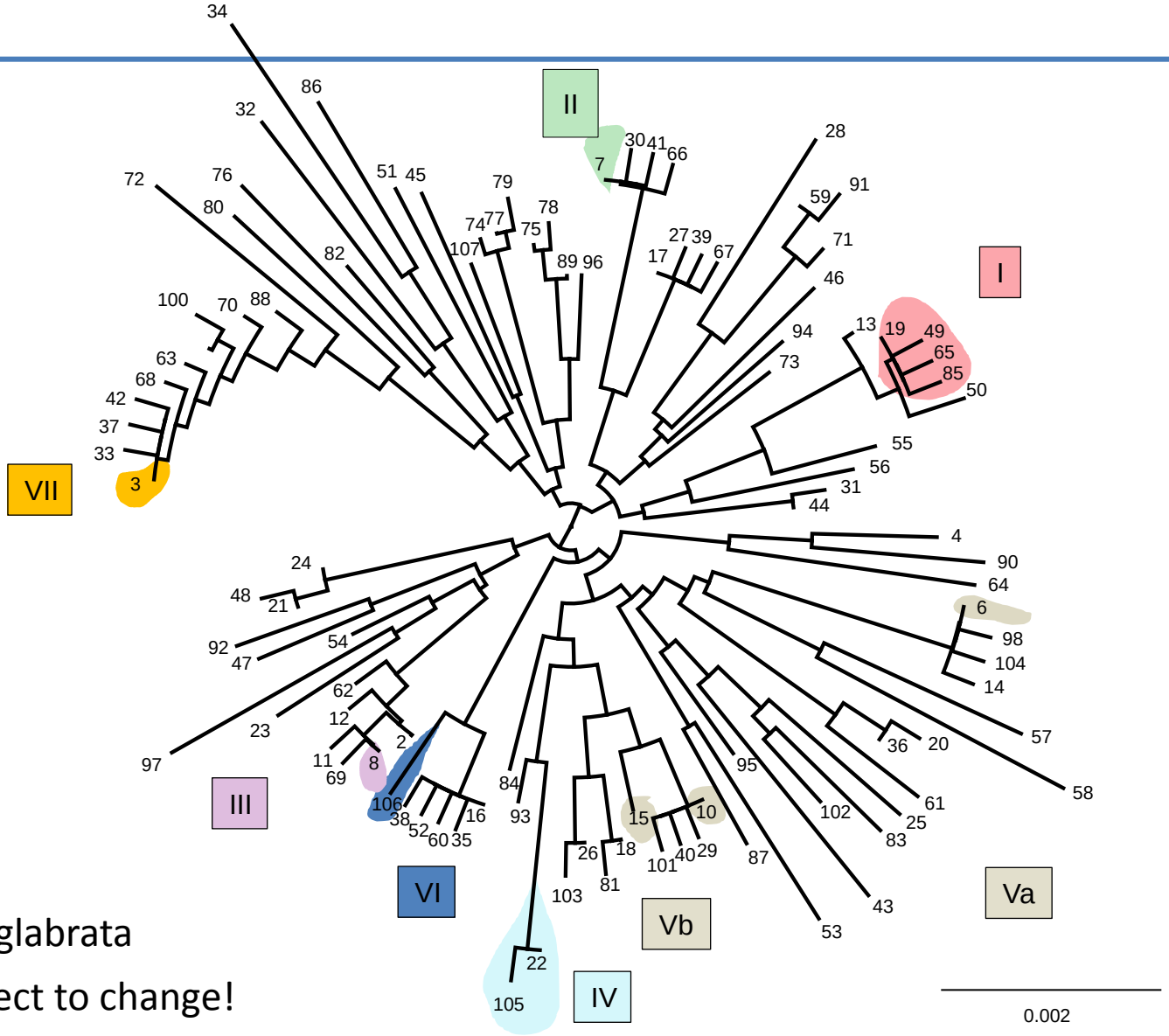
C. glabrata population structure





Database: pubmlst.org/cglabrata

MLST tree structure subject to change!

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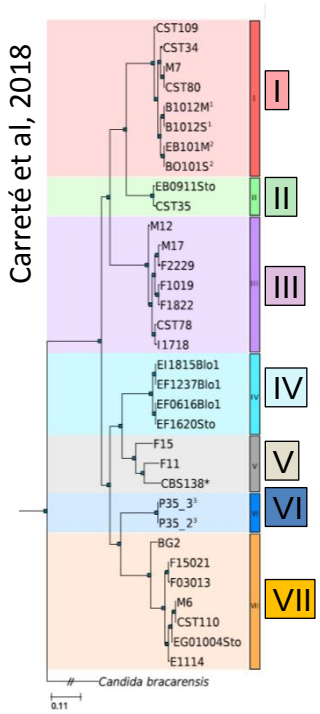
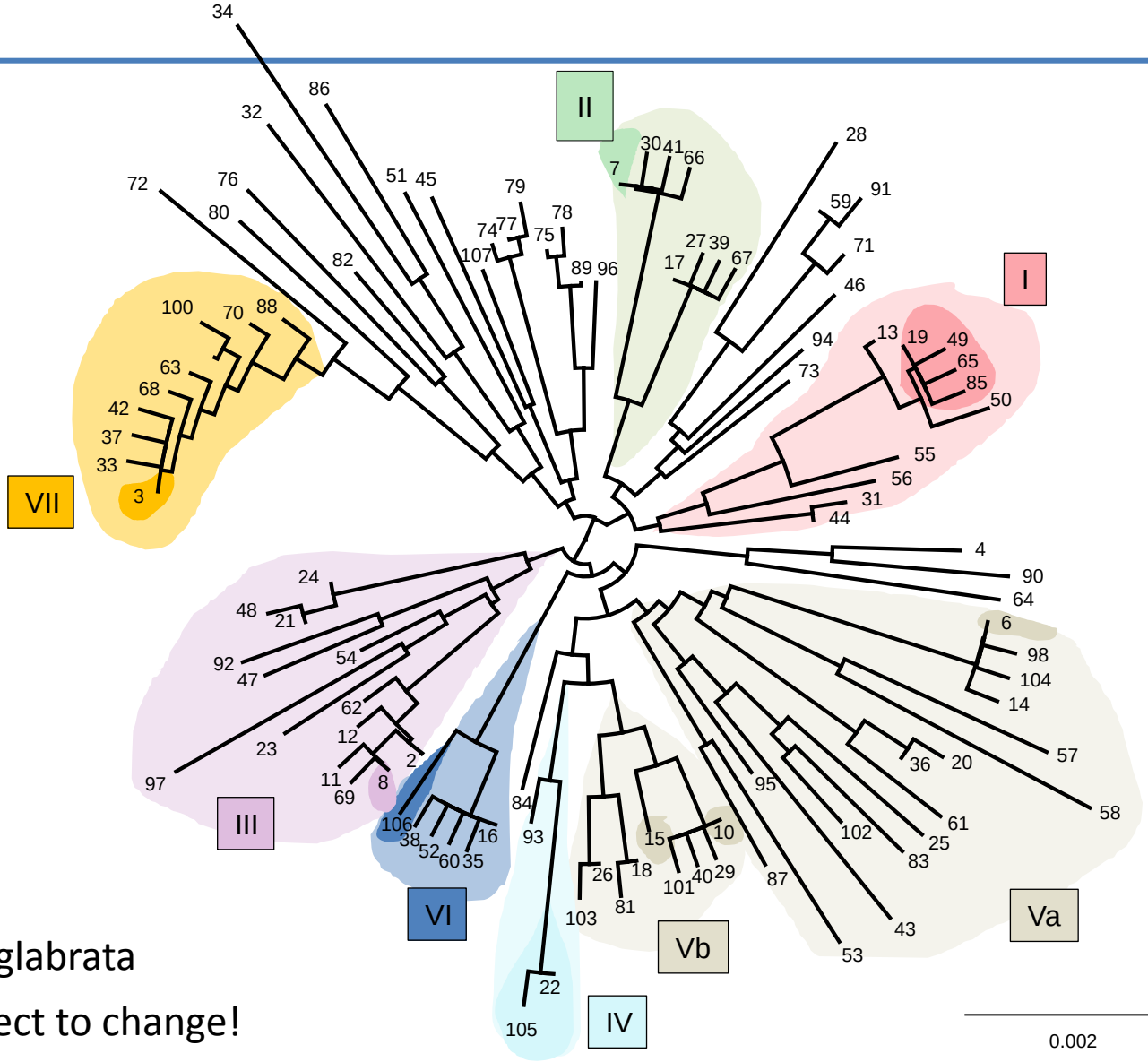
C. glabrata population structure





Database: pubmlst.org/cglabrata

MLST tree structure subject to change!

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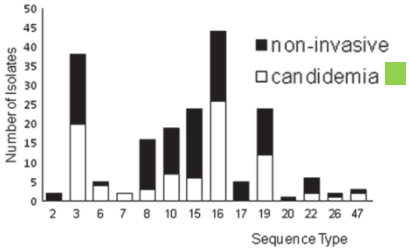
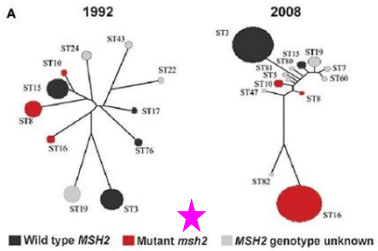
C. glabrata population structure

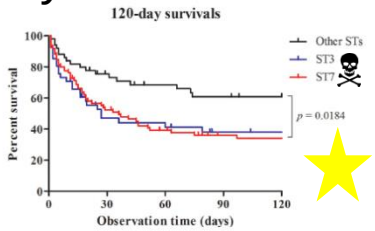
Lott et al 2012



Healey et al 2016



Byun et al 2018



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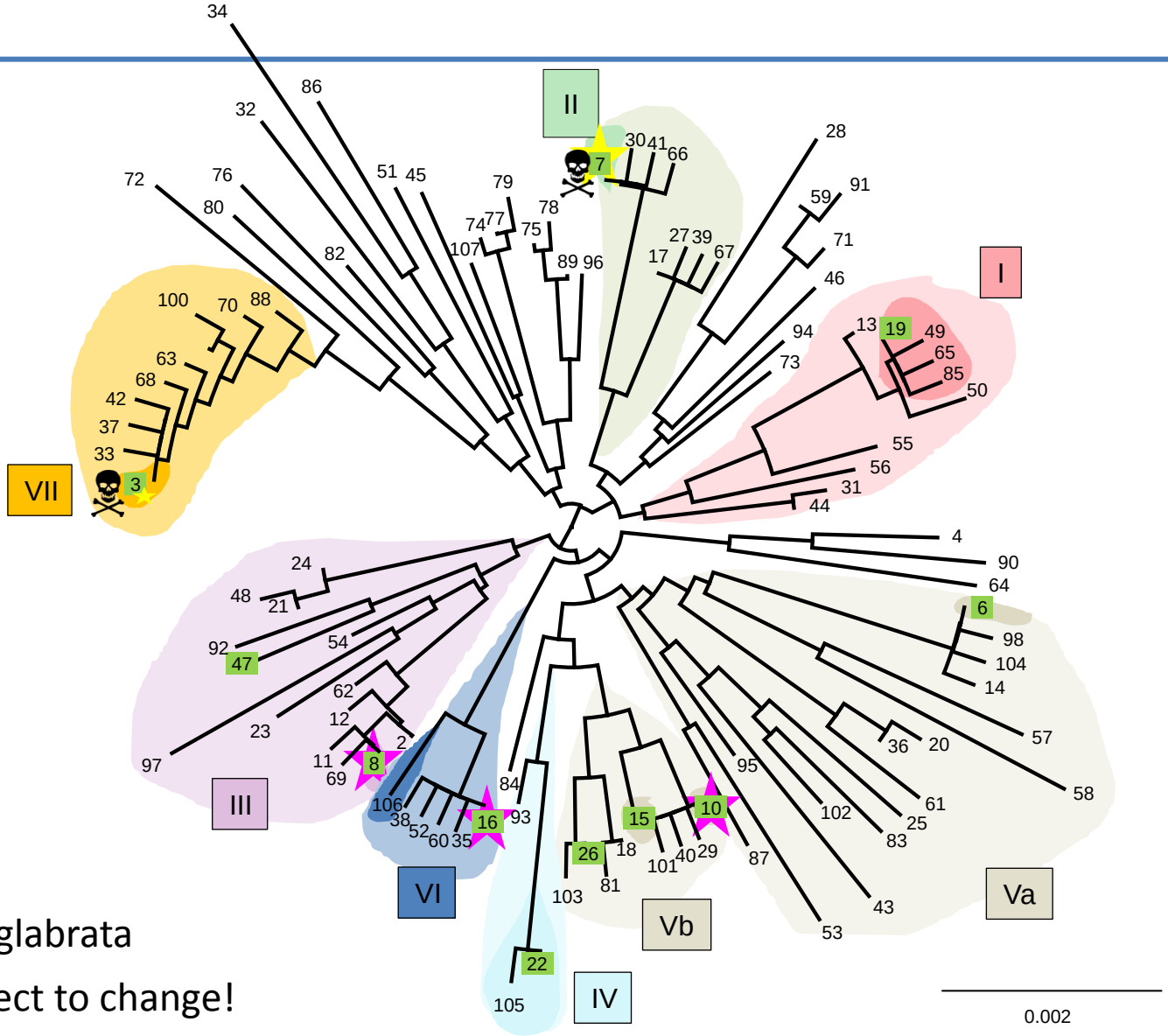
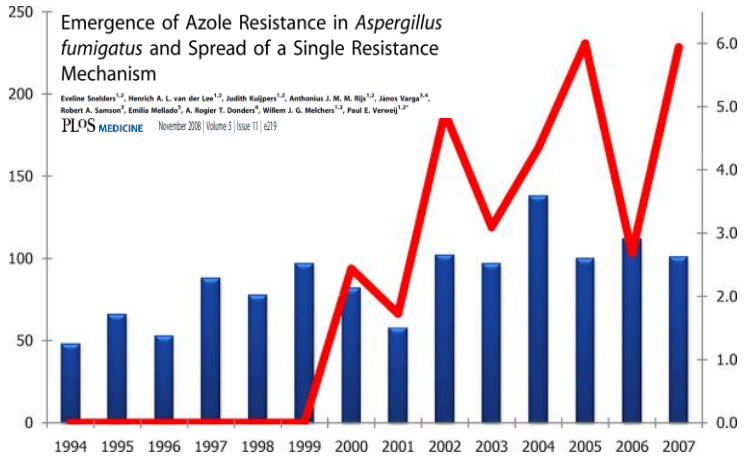


Aspergillus fumgiatus

Global spread of azole resistant linages

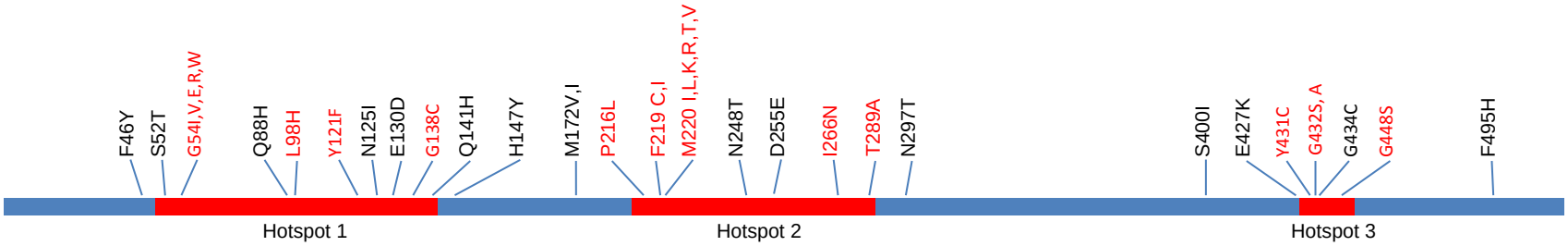
Mechanisms of azole resistance: Cyp51A

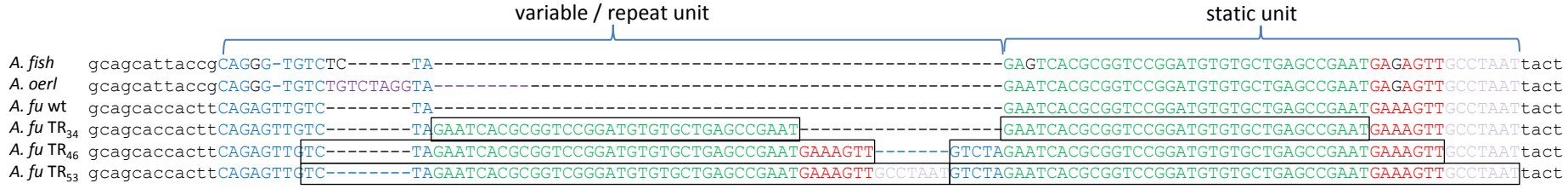


"pandemic" alleles:

TR_{34}/L98H

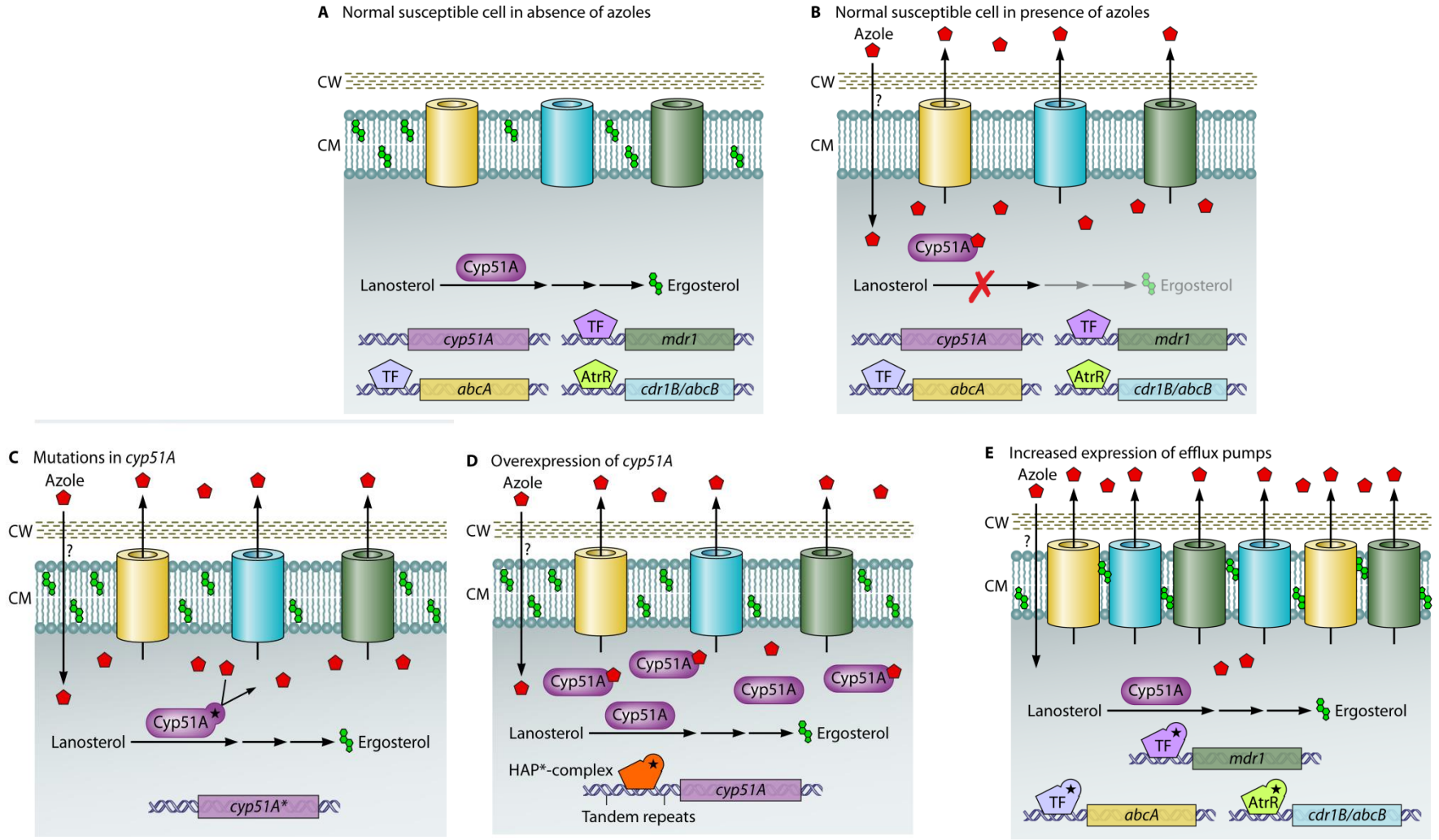
TR_{46}/Y121F/T289A





Dudakova *et al.*, CMR 2017

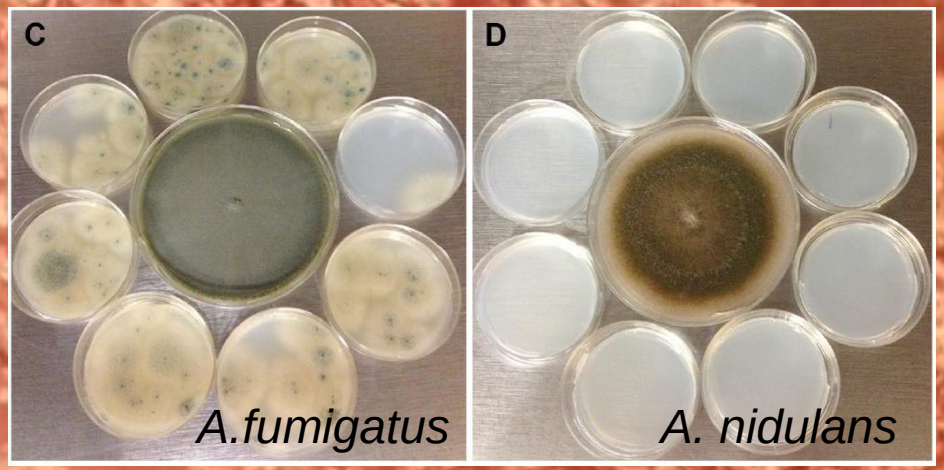
Azole resistance mechanisms in A.f.



Dudakova *et al.*, CMR 2017

Current working hypothesis

A. fumigatus is one of the best-adapted fungi to dispersal, and its condida are found world-wide incl. the upper atmosphere, and both polar regions

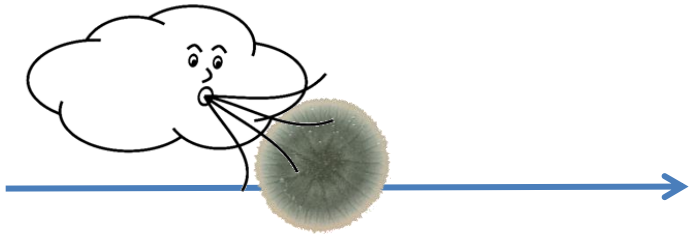


Kwon-Chung and Segui, PPath, 2013

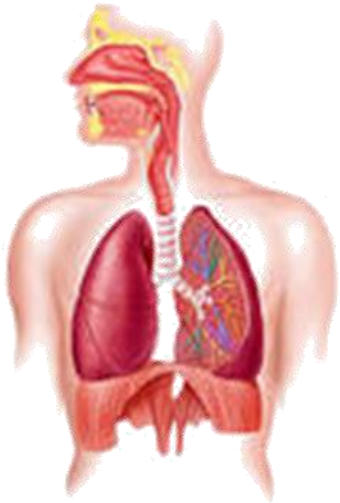
Current working hypothesis

Unintentional induction of resistance in *A. fumigatus* e.g. TR_{34}/L98H or TR_{46}/Y121F/T289A (G54?)

dispersal by wind



selection over susceptible strains through agricultural azoles?



- presence in hosts
- opportunistic infection
- (selection through azole prophylaxis ???)
- not only humans but also wild animals!

conidia also travel the land route…

Intercountry Transfer of Triazole-Resistant *Aspergillus fumigatus* on Plant Bulbs

[1]Department of Clinical Microbiology, Trinity College Dublin, Ireland; [2]Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, and [3]Centre of Expertise in Mycology, Radboud University Medical Center/Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

Katie Dunne,[1] Ferry Hagen,[2,3] Niamh Pomeroy,[1] Jacques F. Meis,[2,3] and Thomas R. Rogers[1]

Sample No.[a]	Date of Sample	Type of Sample	Origin	No. of Triazole-Resistant/Total *A. fumigatus* Colonies/Plant Bulb Pack	MIC, mg/L[b] Itraconazole (ECV = 1) [9]	Voriconazole (ECV = 1) [9]	Posaconazole (ECV = 0.5) [9]	Resistance Mechanism
P1	Jan 2016	Double mixed tulip bulbs (30[c])	Lisse, the Netherlands	1/5	0.5	>8	0.5	TR_{46}/Y121F/T289A
P2, P3	Jan 2016	Bastogne tulip bulbs (6[a])	Lisse, the Netherlands	2/3	1	4	1	TR_{46}/Y121F/T289A
					8	>8	0.5	TR_{34}/L98H
P4	Jan 2016	Triumph tulip bulbs (6[c])	Breezand, the Netherlands	1/4	8	4	0.5	TR_{34}/L98H
P5, P6	Jan 2016	Narcissus bulbs (8[c])	Breezand, the Netherlands	2/5	0.5	>8	1	TR_{46}/Y121F/T289A
					8	4	0.5	TR_{34}/L98H
P7	Jan 2016	Tall Triumph mixed tulip bulbs (10[c])	The Netherlands (region not specified)	1/2	1	>8	1	TR_{46}/Y121F/T289A
D6	Feb 2016	Soil (2 g)	Hospital campus, Dublin, Ireland	…	8	4	0.5	TR_{34}/L98H

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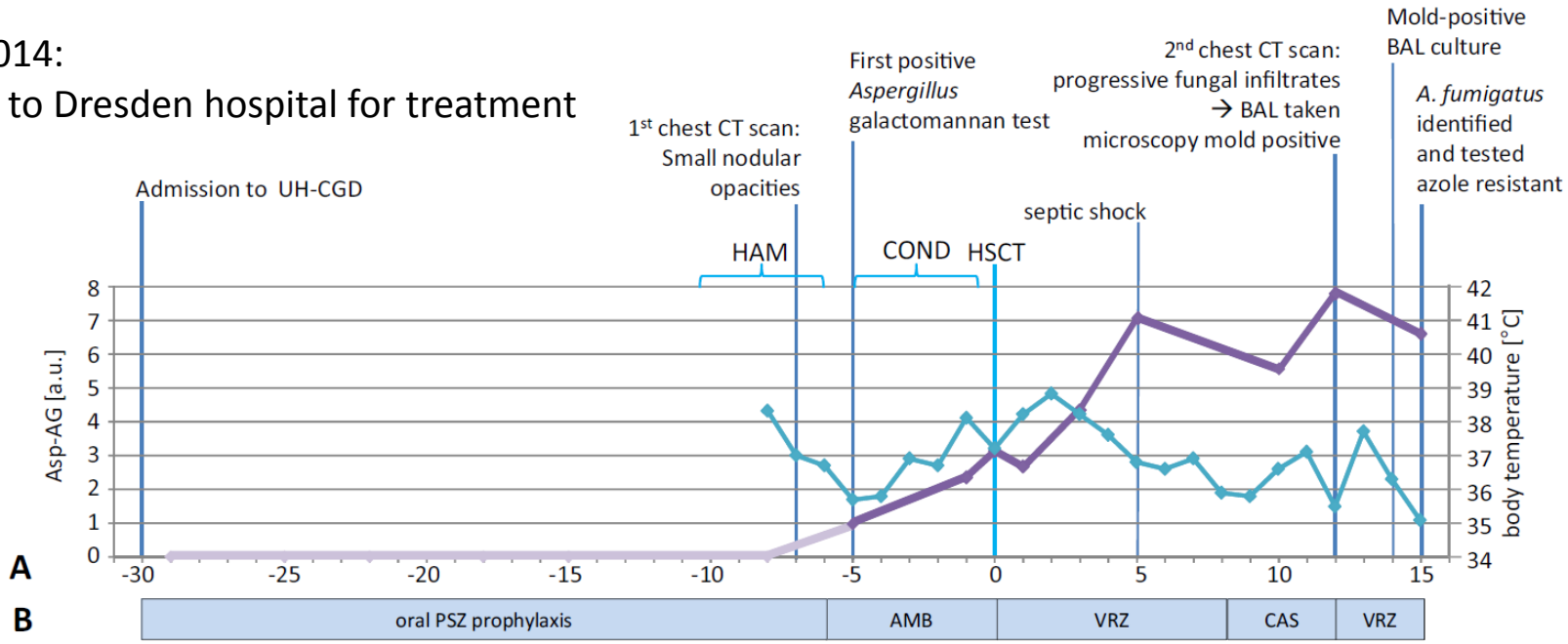
AML Case

Up to June 2014:

- 71-year old male patient
- treated in a general hospital for AML with adverse-risk cytogenetic features.
- received induction chemotherapy and oral posaconazole for antifungal prophylaxis
- Eventually, a new bone marrow aspirate revealed residual AML
- because of progressive AML, decitabine therapy was initiated

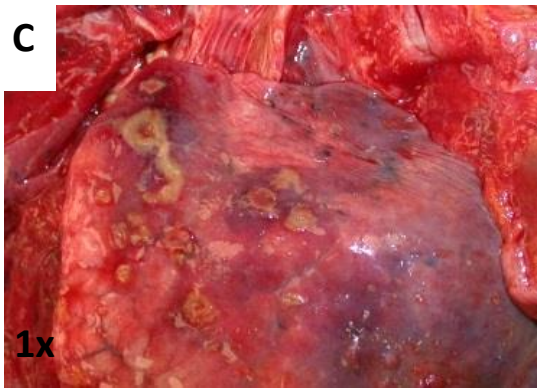
In August 2014:

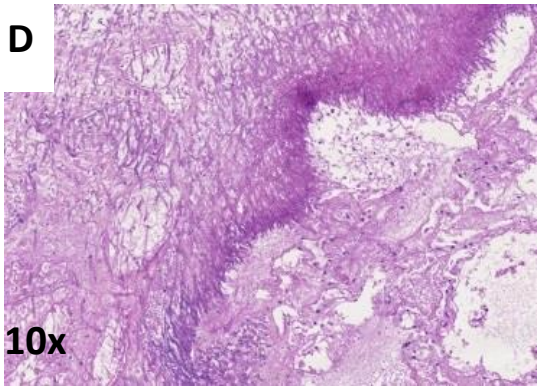
- referred to Dresden hospital for treatment

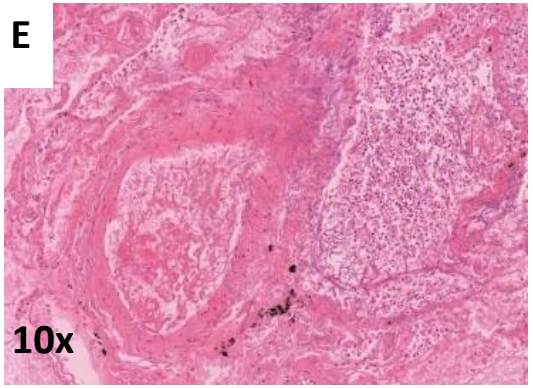


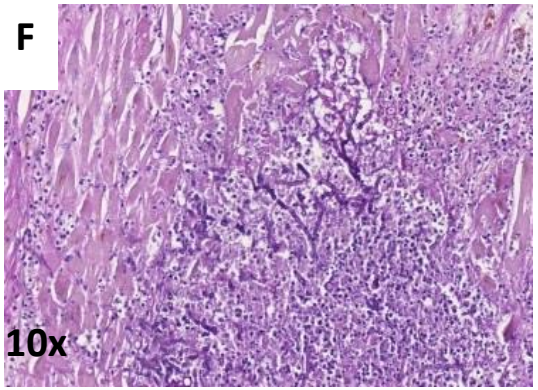
Rößler S, Bader O, *et al.*, AAC 2017

AML Case









(C) multiple fungal lesions on the surface of the lung,
(D) fungal abscess in lung parenchyma (PAS reaction),
(E) vascular invasive growth and detection of dichotomously branched and septated fungi in the lumen of a lung vessel
(F) fungal septipyemic focus in the anterior wall of the heart with surrounding granulocytic reaction (PAS reaction)

Rößler S, Bader O, *et al.*, AAC 2017

AML Case

antifungal susceptibility testing of the culture isolate (BAL)

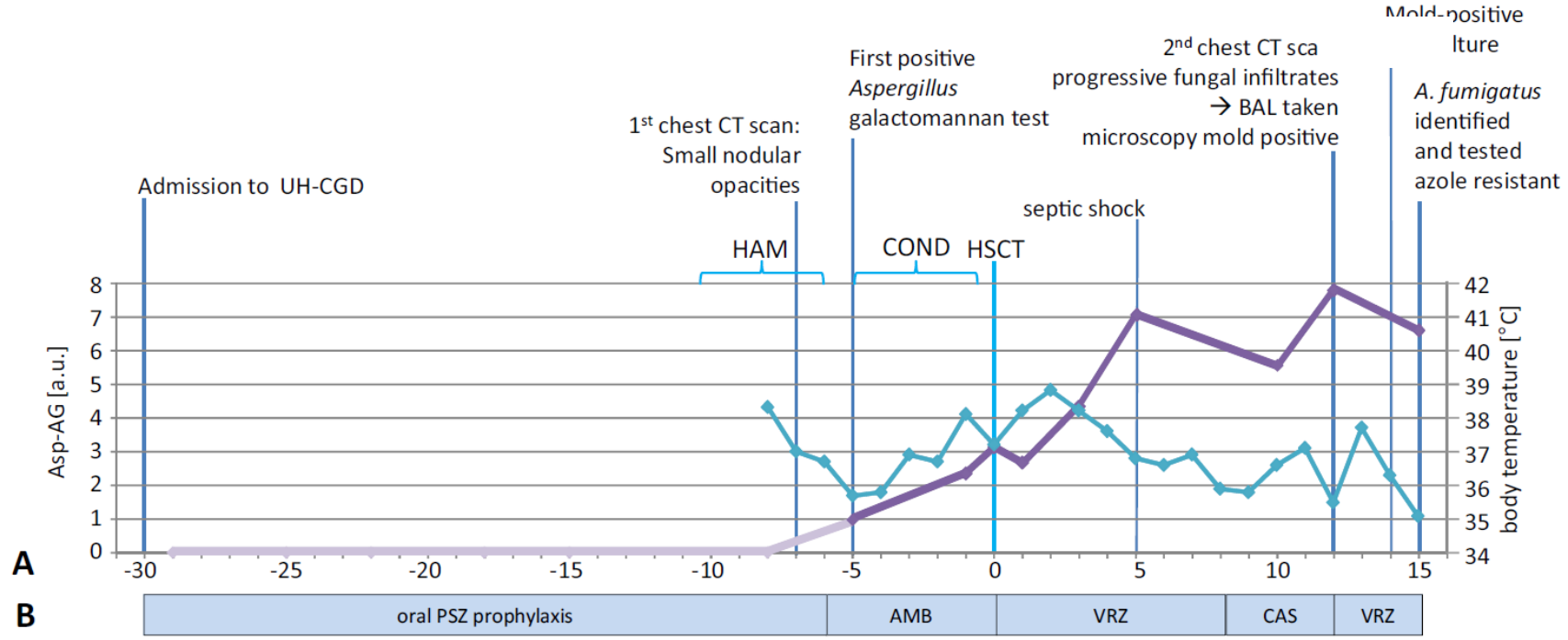
Antifungal agent	E-test		EUCAST borth microdilution		
	MIC	interpretation	MIC	breakpoints	Interpretation
posaconazole	0.5	resistant	0.5	S <= 0.12; R > 0.25	resistant
itraconazole	2	sensitive	1	S <= 1; R > 2	sensitive
voriconazole	>32	resistant	> 32	S <= 1; R > 2	resistant
amphotericin B	0.25	sensitive	< 0.125	S <= 1; R > 2	sensitive

MIC = minimal inhibitory concentration [µg/ml]
EUCAST = European Committee on Antimicrobial Susceptibility Testing

Rößler S, Bader O, *et al.*, AAC 2017

AML Case

PSZ and VRZ ineffective in this patient
Strain with PSZ^{R} and VRZ^{R} strain with TR$_{46}$/Y121F/M172/T289A *cyp51A* mutation

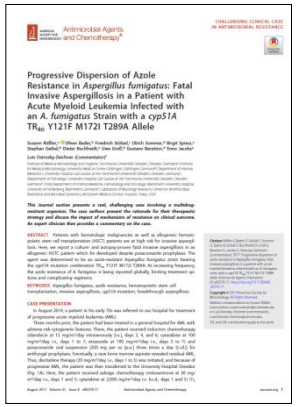


Rößler S, Bader O, *et al.*, AAC 2017

Reported isolates in Germany

clinical

2012 2012 2013 2014 2015



2017

2017

35 reported clinical isolates

environmental

2015

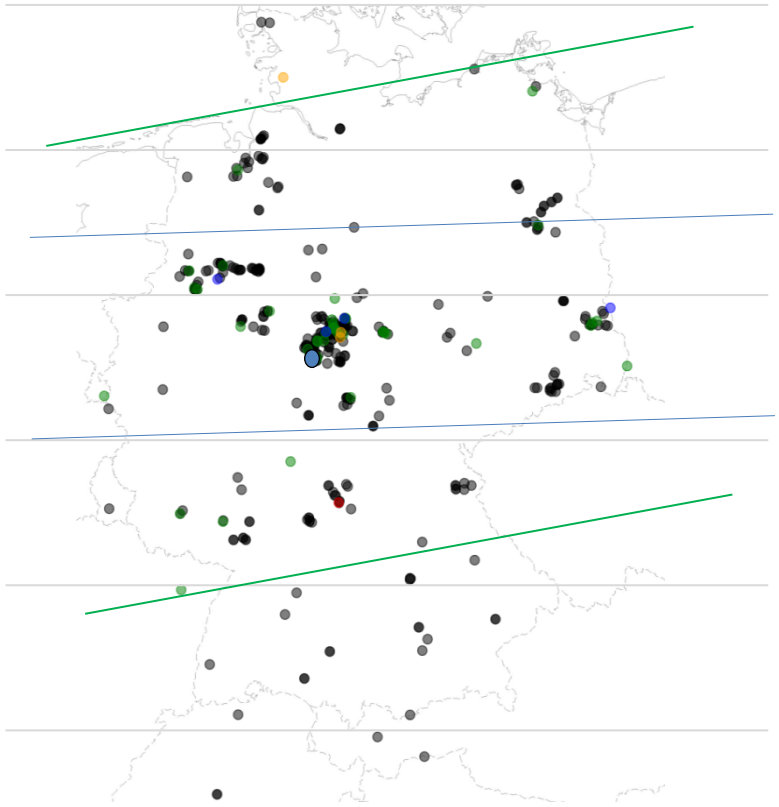
55 reported environ-mental isolates

NRZ data 2017

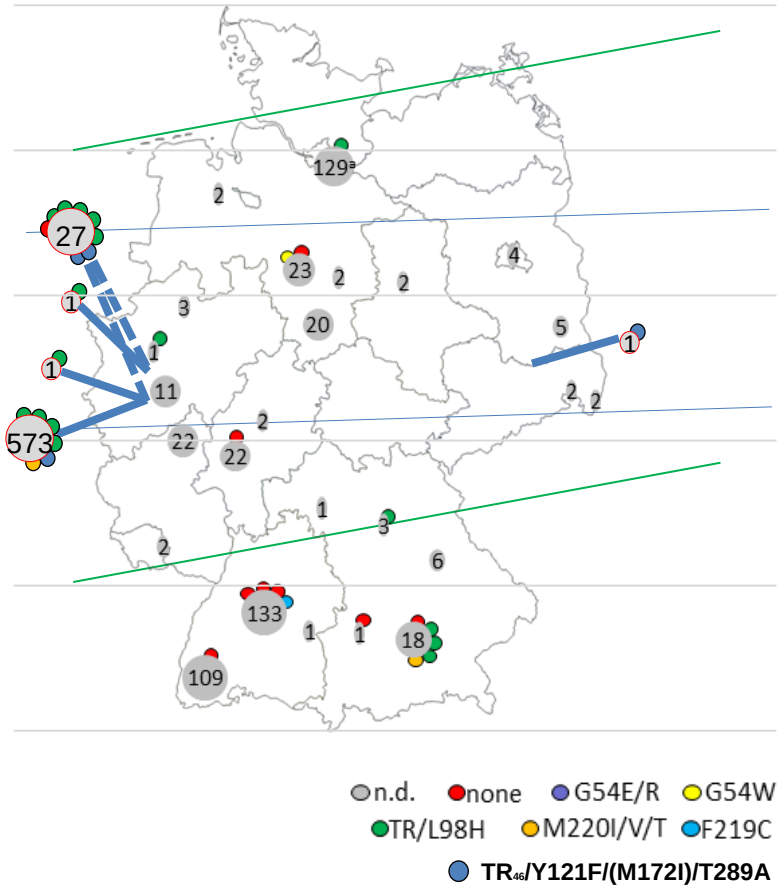
19 isolates, 2/3 with mutations in CYP51A (Walther, Kurzai, Poster@DMYKG2018)

North-South imbalance ?

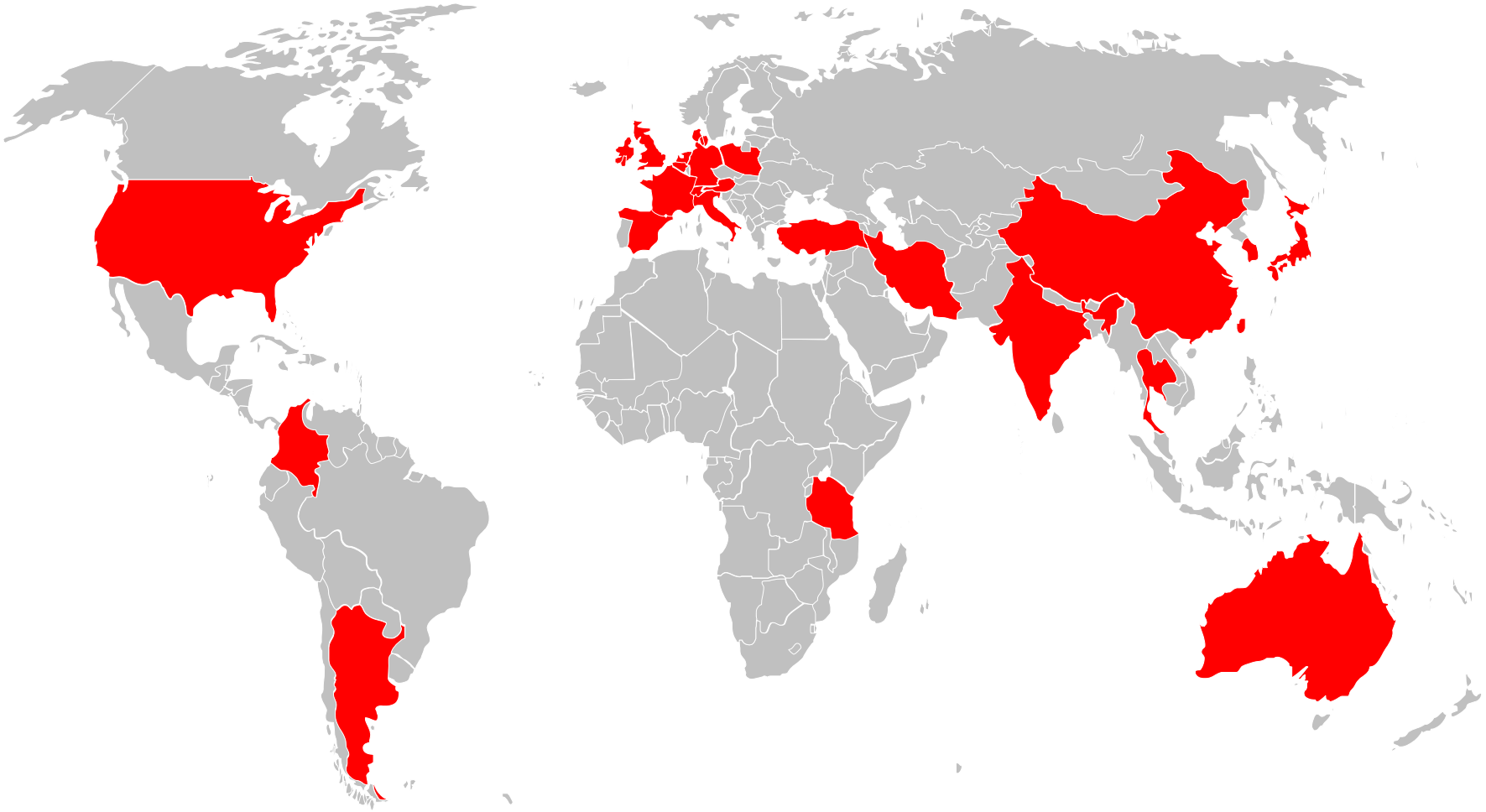
environmental isolates



clinical isolates



ARAf findings by country



→ if people look for ARAf, they will find it !

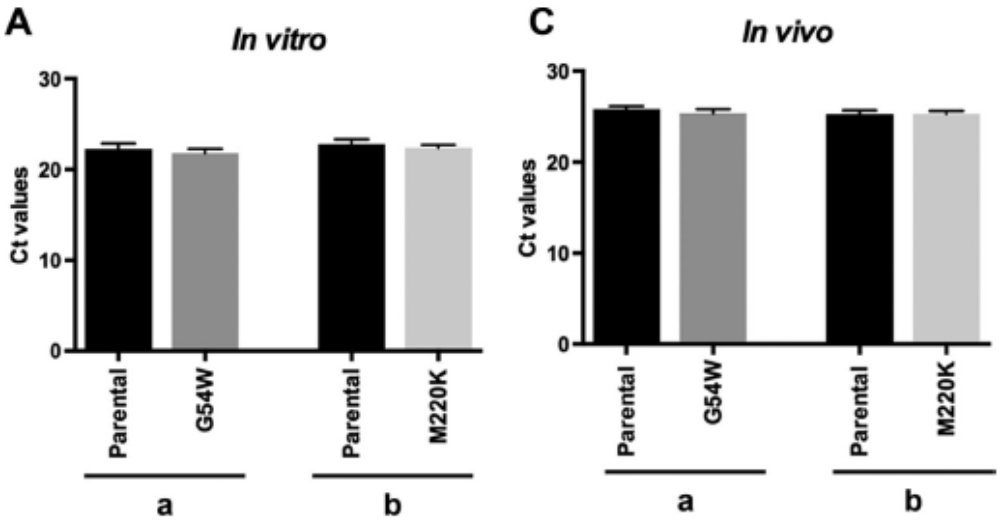
No fitness cost associated with ARAf

Fitness Studies of Azole-Resistant Strains of *Aspergillus fumigatus*

Isabel Valsecchi,[a] Emilia Mellado,[b] Rémi Beau,[a] Shriya Raj,[a] Jean-Paul Latgé[a]

Unité des Aspergillus, Institut Pasteur, Paris, France[a]; Mycology Reference Laboratory, Instituto de Salud Carlos III, Madrid, Spain[b]

Antimicrobial Agents and Chemotherapy December 2015 Volume 59 Number 12



qPCR-based growth estimation after competition

on agar in mouse model

"International expert opinion"

(in the absence of clinical guidelines)

International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*

Paul E. Verweij[a,*], Michelle Ananda-Rajah[b], David Andes[c], Maiken C. Arendrup[d], Roger J. Brüggemann[e], Anuradha Chowdhary[f], Oliver A. Cornely[g], David W. Denning[h], Andreas H. Groll[i], Koichi Izumikawa[j], Bart Jan Kullberg[k], Katrien Lagrou[l], Johan Maertens[m], Jacques F. Meis[a,n], Pippa Newton[h], Iain Page[h], Seyedmojtaba Seyedmousavi[a], Donald C. Sheppard[o], Claudio Viscoli[p], Adilia Warris[q], J. Peter Donnelly[r]

Verweij et al, (2015) Drug Res Updates 21:30-40

- microbiological diagnostics critical to guiding therapy
- if azole resistant isolates are obtained,
  - the underlying mechanism should be identified for epidemiological reasons,
  - but neither therapy initiation/modification nor MIC determination delayed

- 2-10% environmental prevalence →alternative therapies must be considered
- 10 % environmental prevalence → definitely re-evaluate azole therapy

- at least 5 independent colonies should tested for AST

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culture

cave: susceptible / resistant – mixed cultures !!! → screening agar

J Antimicrob Chemother 2015; **70**: 412–415
doi:10.1093/jac/dku410 Advance Access publication 17 October 2014

Journal of Antimicrobial Chemotherapy

Concomitant occurrence of itraconazole-resistant and -susceptible strains of *Aspergillus fumigatus* in routine cultures

Ahmad et al, 2015 JAC 70:412-415

CORRESPONDENCE

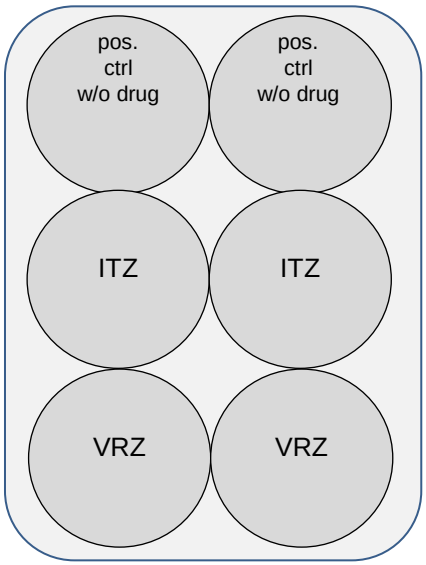
Voriconazole-Susceptible and Voriconazole-Resistant *Aspergillus fumigatus* Coinfection

To the Editor:

Azole resistance is an increasing problem in *Aspergillus fumigatus* infection (1). Two mutations, TR_{34}/L98H and TR_{46}/Y121F/T289A, are frequently recovered from isolates of patients with azole-resistant invasive aspergillosis and are believed to originate from the environment (2, 3). In regions with these environmental mutations, azole-resistant *Aspergillus* diseases may develop in patients not previously treated with azoles, and mortality rates are very high (4–6). We review the clinical course of three patients with proven invasive aspergillosis resulting from voriconazole-susceptible and voriconazole-resistant *A. fumigatus* strains. We hypothesized that in regions with TR_{34}/L98H and TR_{46}/Y121F/T289A environmental mutations, individual pulmonary lesions may arise from *A. fumigatus* strains with different azole resistance profiles.

unit because of pulmonary deterioration requiring mechanical ventilation. The BAL showed heavy growth of *A. fumigatus*. Resistance screening of four colonies indicated voriconazole-susceptible infection, and voriconazole was added to the regimen. Ten days after voriconazole was started, routine follow-up bronchial aspirate yielded a voriconazol-resistant *A. fumigatus* colony (Table 1). Because the patient was improving clinically with adequate voriconazole plasma levels, voriconazole was continued. However, a bronchial aspirate taken on day 13 showed heavy growth of voriconazole-resistant *A. fumigatus*. Despite continued clinical improvement, anidulafungin was added. Almost 2 weeks after intubation, ventilator support could be withdrawn, and immunosuppressive drugs were reintroduced. A follow-up computed tomography scan of the thorax showed cavity formation in the right upper lobe lesion, and multiple nodular lesions. The patient suddenly died after having received voriconazole and anidulafungin combination therapy for 34 days. At autopsy, multiple pulmonary fungal lesions were found, as well as one fungal lesion in the kidney transplant. *A. fumigatus* colonies cultured from the cavitating

Kolwijck et al, 2016
Am J Respir Crit Care Med 193



large-scale screening

AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews®

Molecular Tools for the Detection and Deduction of Azole Antifungal Drug Resistance Phenotypes in *Aspergillus* Species

Anna Dudakova,[a] Birgit Spiess,[b] Marut Tangwattanachuleeporn,[a,c] Christoph Sasse,[d] Dieter Buchheidt,[b] Michael Weig,[a] Uwe Groß,[a] Oliver Bader[a]

Institute for Medical Microbiology, University Medical Center Göttingen, Göttingen, Germany[a]; 3rd Department of Internal Medicine, Hematology and Oncology, Mannheim University Hospital, University of Heidelberg, Mannheim, Germany[b]; Unit of Medical Technology, Faculty of Allied Health Sciences, Burapha University, Chon Buri, Thailand[c]; Institute of Microbiology and Genetics, Department of Molecular Microbiology & Genetics, Georg August Universität Göttingen, Göttingen, Germany[d]

- molecular mechanisms
- a scheme to screen large scale collections
- tools to locally analyse sequencing data

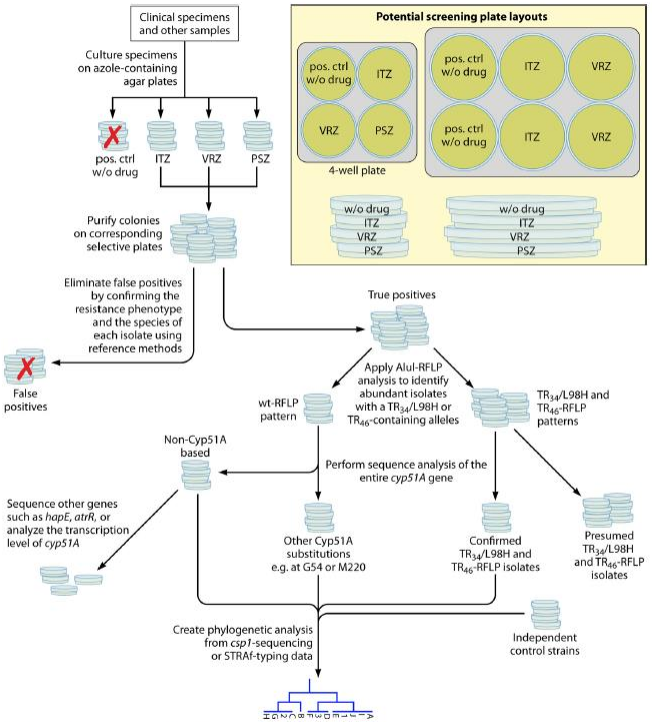


FIG 3 Potential sampling workflow for ARAf screening studies. No standardized scheme for conducting screening studies is established yet, but combining several approaches proposed in the literature gives rise to an efficient workflow that eliminates false-positive results and yields robust numbers on the prevalence and phylogenetic cohesion of resistant isolates.

Take-home messages

- *Candida auris* is a globally emerging, fluconazole resistant species associated to (nosocomial?) outbreaks

- Echinocandin resistance is emerging in *Candida glabrata*
- Potentially tied to hypermutability phenotype (*MSH2*), already present in parts of the *C. glabrata* population

- Spread of azole resistance in *A. fumigatus* is not an academic, but a clinical problem.
- It is not restricted to The Netherlands, but of global concern
- Environmental screening makes sense to estimate the prevalence in local clinics / wards

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***Candida auris* and *Candida glabrata* epidemiologic data:**
O Kurzai (Würzburg/Jena), A. Hamprecht (Köln)

MykoLabNet-D-Partners: (in alphabetical order)
F. Albert, W. Bartelt, A. Beider, O. Benedek, B. Beyreiß, D. Buchheidt, M. Christner, L. Draht, C. Friedrichs, M. Fritzenwanker, N. Guenther, A. Haas, J. Held, M. Klotz, E. Kurig, , S. Peter, R. Pfüller, U Schumacher, L. Sedlacek, M. Seibold, M. Simon, K. Tintelnot A. F. Wendel, M.-G. Weyer, O. Zimmermann

Collectors of environmental samples: (in order of samples contributed)
N. Hoberg, S. Geibel, E. Vogel, J. Büntzel, J. Springer, L. Lehning, Chr. Schädel „Strecker&Weinert“, L. Metzger, A. Zautner, D. Buchheidt, S. Wiegmann, S. Klingebiel, A.-Chr. Loock, J. Hegewald, M. Hassenpflug, A. Aurin, J. Szymczak, „alphaomega Labor“, N. Diffloth, M. Kuhns

Contributions of strains:
J. Steinmann (Nürnberg), A. Hamprecht (Köln), S. Rößler (Dresden)

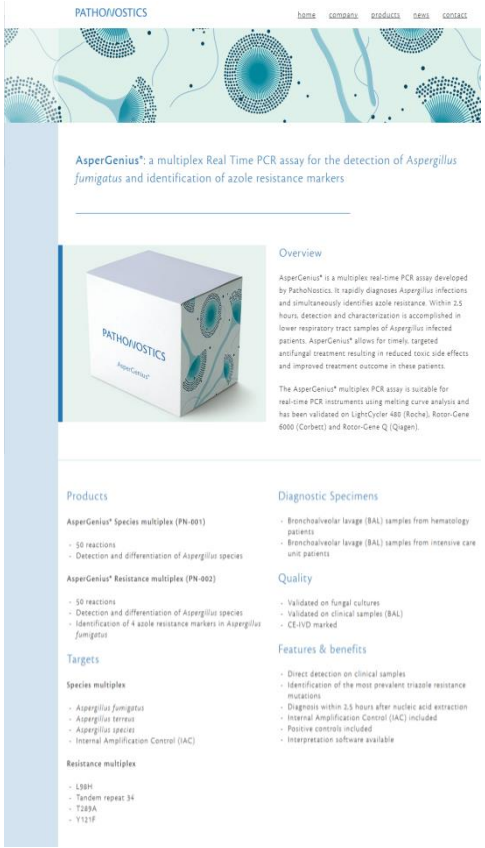
Bundesministerium für Gesundheit

Pfizer

MSD
(Posaconazol)

Multiplex -PCR approach

(AsperGenious Assay, PathoNostics, NL)



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AsperGenius®: a multiplex Real Time PCR assay for the detection of *Aspergillus fumigatus* and identification of azole resistance markers

Overview

AsperGenius® is a multiplex real-time PCR assay developed by PathoNostics. It rapidly diagnoses *Aspergillus* infections and simultaneously identifies azole resistance. Within 2.5 hours, detection and characterization is accomplished in lower respiratory tract samples of *Aspergillus* infected patients. AsperGenius® allows for timely, targeted antifungal treatment resulting in reduced toxic side effects and improved treatment outcome in these patients.

The AsperGenius® multiplex PCR assay is suitable for real-time PCR instruments using melting curve analysis and has been validated on LightCycler 480 (Roche), Rotor-Gene 6000 (Corbett) and Rotor-Gene Q (Qiagen).

Products

AsperGenius® Species multiplex (PN-001)

- 50 reactions
- Detection and differentiation of *Aspergillus* species

AsperGenius® Resistance multiplex (PN-002)

- 50 reactions
- Detection and differentiation of *Aspergillus* species
- Identification of 4 azole resistance markers in *Aspergillus fumigatus*

Targets

Species multiplex

- *Aspergillus fumigatus*
- *Aspergillus terreus*
- *Aspergillus species*
- Internal Amplification Control (IAC)

Resistance multiplex

- L98H
- Tandem repeat 34
- T289A
- Y121F

Diagnostic Specimens

- Bronchoalveolar lavage (BAL) samples from hematology patients
- Bronchoalveolar lavage (BAL) samples from intensive care unit patients

Quality

- Validated on fungal cultures
- Validated on clinical samples (BAL)
- CE-IVD marked

Features & benefits

- Direct detection on clinical samples
- Identification of the most prevalent triazole resistance mutations
- Diagnosis within 2.5 hours after nucleic acid extraction
- Internal Amplification Control (IAC) included
- Positive controls included
- Interpretation software available

<u>company statement</u>: multi-platform RT-PCR
Detects *A. fumigatus*, *A. terreus*, *A. spec*. from BAL samples
From *A. fumigatus*: L98H, TR34, T289A, Y121F

Analytical and Clinical Evaluation of the PathoNostics AsperGenius Assay for Detection of Invasive Aspergillosis and Resistance to Azole Antifungal Drugs during Testing of Serum Samples

P. Lewis White,[a] Raquel B. Posso,[b] Rosemary A. Barnes[b]

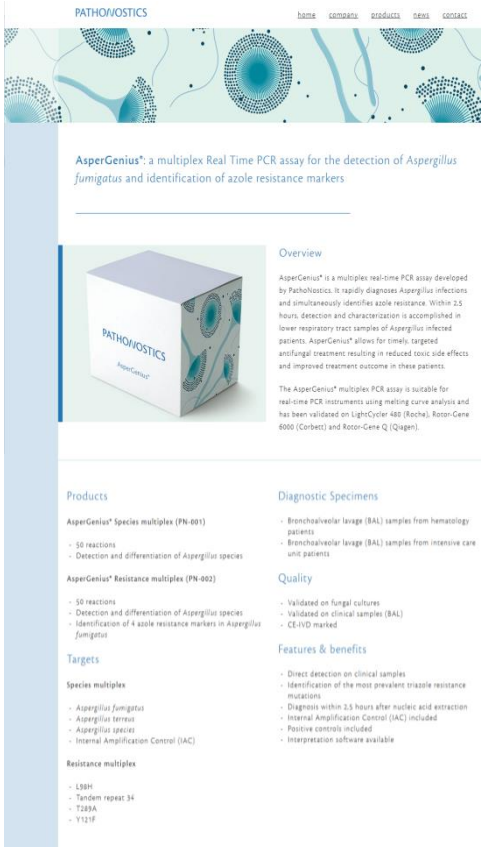
Public Health Wales Microbiology Cardiff, Cardiff, United Kingdom[a]; Infection, Immunity and Biochemistry, School of Medicine, Cardiff University, Cardiff, United Kingdom[b]

The commercially developed PathoNostics AsperGenius species assay is a multiplex real-time PCR capable of detecting aspergillosis and genetic markers associated with azole resistance. The assay is validated for testing bronchoalveolar lavage fluids, replacing the requirement for culture and benefiting patient management. Application of this assay to less invasive, easily obtainable samples (e.g., serum) might be advantageous. The aim of this study was to determine the analytical and clinical performance of the AsperGenius species and resistance assays for testing serum samples. For the analytical evaluations, serum samples were spiked with various concentrations of *Aspergillus* genomic DNA for extraction, following international recommendations. For the clinical study, 124 DNA extracts from 14 proven/probable invasive aspergillosis (IA) cases, 2 possible IA cases, and 33 controls were tested. The resistance assay was performed on *Aspergillus fumigatus* PCR-positive samples when a sufficient fungal burden was evident. The limits of detection of the species and resistance assays for *A. fumigatus* DNA were 10 and ≥75 genomes/sample, respectively. Nonreproducible detection at lower burdens was achievable for all markers. With a positivity threshold of 39 cycles, the sensitivity and specificity of the species assay were 78.6% and 100%, respectively. For 7 IA cases, at least one genetic region potentially associated with azole resistance was successfully amplified, although no resistance markers were detected in this small cohort. The AsperGenius assay provides good clinical performance with the added ability to detect azole resistance directly from noninvasive samples. While the available burden will limit application, it remains a significant advancement in the diagnosis and management of aspergillosis.

White, Posso, Barnes (2015) JCM 53:2155-21

Multiplex -PCR approach

(AsperGenius Assay, PathoNostics, NL)



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Journal of Antimicrobial Chemotherapy Advance Access published August 15, 2016

J Antimicrob Chemother
doi:10.1093/jac/dkw323

Journal of Antimicrobial Chemotherapy

PCR-based detection of *Aspergillus fumigatus Cyp51A* mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay® in 201 patients with haematological disease suspected for invasive aspergillosis

G. M. Chong[1]*, M. T. van der Beek[2], P. A. von dem Borne[3], J. Boelens[4], E. Steel[5], G. A. Kampinga[6], L. F. R. Span[7], K. Lagrou[8], J. A. Maertens[9], G. J. H. Dingemans[10], G. R. Gaajetaan[10], D. W. E. van Tegelen[10], J. J. Cornelissen[11], A. G. Vonk[12] and B. J. A. Rijnders[1]

Results: Two hundred and one patients each contributed one BAL sample, of which 88 were positive controls and 113 were negative controls. The optimal cycle threshold cut-off value for the *Aspergillus* species PCR was <38. With this cut-off, the PCR was positive in 74/88 positive controls. The sensitivity, specificity, positive predictive value and negative predictive value were 84%, 80%, 76% and 87%, respectively. 32/74 BAL samples were culture negative. Azole treatment failure was observed in 6/8 patients with a RAM compared with 12/45 patients without RAMs ($P=0.01$). Six week mortality was 2.7 times higher in patients with RAMs (50.0% versus 18.6%; $P=0.07$).

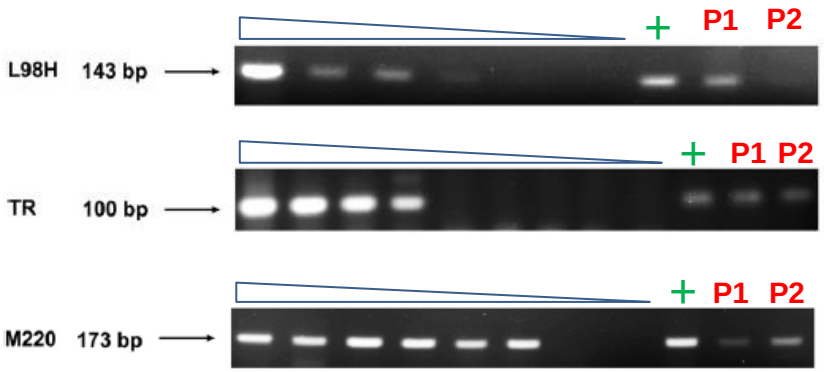
nested-PCR approach / AML case

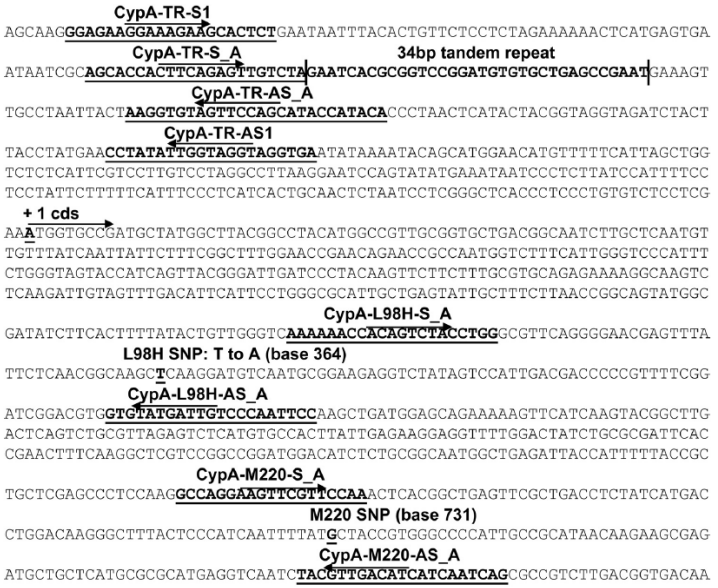
(Spieß & Buchheidt; Mannheim)

Development of Novel PCR Assays To Detect Azole Resistance-Mediating Mutations of the *Aspergillus fumigatus cyp51A* Gene in Primary Clinical Samples from Neutropenic Patients

Birgit Spiess,[a] Wolfgang Seifarth,[a] Natalia Merker,[a] Susan J. Howard,[b] Mark Reinwald,[a] Anne Dietz,[c] Wolf-Karsten Hofmann,[a] and Dieter Buchheidt[a]

Spiess et al. (2012) AAC 56:3309-10





sequencing!

Amplification of specific *cyp51*A fragments from autopsy material (lung, heart) of the patient shown at this talk's beginning

→ confirms TR_{46}, Y121F, and T289A (region with M172 not covered)

Rößler S, Bader O, *et al.*, AAC 2017