Global status of azole resistance in Europe and Asia

Prof. Sevtap ARIKAN-AKDAGLI, MD
Hacettepe University Medical School
Ankara Turkey

Agenda

- Background
- *Azole resistance in Aspergillus:* The first report
- Proposed and frequently used ECOFFs (azoles vs. *Aspergillus*)
- National reports
  - The Netherlands
  - UK
  - Japan
  - India
- Global surveillance studies
  - ARTEMIS
  - SCARE
- Azole resistance in strains of *A. fumigatus* isolated from patients with specific underlying disorders
- Is azole resistance increasing in strains of *A. fumigatus*?
- Data available for non-*fumigatus* Aspergilli
- Possible and potential sources for acquisition of azole resistance
- Conclusions

Antifungal drugs against Aspergillus

POLYENES

TRIAZOLES

CANDINS

- Availability of ORAL formulations
- Significant therapeutic options for patients with Chronic Pulmonary Aspergillosis & ABPA who require long-term therapy

Denning & Perlin. Future Microbiol 2011; 6: 1229

Medical Mycology
2008, 1–7, iFirst article

informa
healthcare

Comparative *in vitro* activities of posaconazole, voriconazole, itraconazole, and amphotericin B against *Aspergillus* and *Rhizopus*, and synergy testing for *Rhizopus*

SEVTAP ARIKAN*, BANU SANCAK*, SEHNAZ ALP*, GULSEN HASCELIK* & PAUL MCNICHOLAS†

Table 1 MICs of posaconazole, voriconazole, itraconazole, and amphotericin B against clinical *Aspergillus* and *Rhizopus* isolates.

Test isolates (number tested) Incubation time	Posaconazole CLSI microdilution		Posaconazole Etest		Voriconazole CLSI microdilution		Itraconazole CLSI microdilution		Amphotericin B CLSI microdilution	
	GM	Range	GM	Range	GM	Range	GM	Range	GM	Range
Aspergillus (Total, *n* =82)										
24 h	0.96	0.5–1	0.02	0.002–0.03	0.49	0.125–1	0.93	0.25–2	1.82	0.5–4
48h	1.01	0.5–2	0.02	0.0075–0.125	0.80	0.125–2	1.13	0.25–2	2.51	1–8
A. fumigatus (43)										
24 h	0.94	0.5–1	0.01	0.002–0.03	0.45	0.125–1	1	0.5–2	1.73	1–4
48 h	0.97	0.5–2	0.03	0.0075–0.125	0.72	0.125–2	1.21	0.5–2	2.39	2–8
A. flavus (29)										
24 h	1	1	0.02	0.0075–0.03	0.65	0.25–1	0.83	0.25–1	2.2	1–4
48 h	1.02	1–2	0.06	0.03–0.125	1	0.5–2	0.98	0.25–2	2.93	2–4
A. niger (7)										
24 h	0.91	0.5–1	0.01	0.002–0.03	0.30	0.125–0.5	0.91	0.5–2	1.21	0.5–2
48 h	1.22	1–2	0.07	0.06–0.125	0.67	0.25–1	1.35	0.5–2	1.81	1–2
A. terreus (2)										
24 h	–	1	–	0.0075–0.03	–	0.5	–	1	–	2
48 h	–	1	–	0.015–0.125	–	1	–	1	–	2–4
A. nidulans (1)										
24 h	–	1	–	0.03	–	0.125	–	1	–	2
48 h	–	1	–	0.125	–	0.25	–	1	–	2
R.oryzae (*n* =11)										
24 h	1.13	1–2	ND	ND	15.02	8 →8	2	2	2.57	2–4
48h	1.55	1–2	ND	ND	15.02	8 →8	3.75	2–8	3.53	2–8

GM, Geometric mean; ND, not determined.

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1997, p. 1364–1368 Vol. 41, No. 6
0066-4804/97/$04.00+0

Itraconazole Resistance in *Aspergillus fumigatus*

DAVID W. DENNING,[1,2*] K. VENKATESWARLU,[3] KAREN L. OAKLEY,[2,4] M. J. ANDERSON,[2]
N. J. MANNING,[5] DAVID A. STEVENS,[6] DAVID W. WARNOCK,[7] AND STEVEN L. KELLY[3]

3 clinical isolates with high (>16 µg/ml) Itra MICs

AF72
AF90
AF91

→ Verification of the MICs in a neutropenic murine model

Isolates from California, USA in late 1980s

Journal of Antimicrobial Chemotherapy (1997) **40**, 401–414

JAC

Correlation between in-vitro susceptibility testing to itraconazole and in-vivo outcome of *Aspergillus fumigatus* infection

D. W. Denning[a,b,c*], S. A. Radford[d], K. L. Oakley[b], L. Hall[a], E. M. Johnson[d] and D. W. Warnock[d]

Initial efforts for standardization of test parameters for generation of reproducible MIC data and prediction of clinical outcome

Table IV. Proposed methods for susceptibility testing of *Aspergillus* spp. to itraconazole

	Agar dilution	Microtitre method
Medium	RPMI agar with 0.03% L-glutamine	RPMI-1640 with L-glutamine, $NaHCO_3$, and 2% glucose buffered with MOPS to pH 7.0
Inoculum	Multipoint inoculation from 10^6 to 10^7 conidia/mL	10^5 conidia (100 μL)
Itraconazole dilution series	0.03–64 mg/L	0.06–16 mg/L
Temperature	28 or 35°C	35°C
Duration	48–72 h	48 h
Endpoint determination	Visual, no growth	Visual, no growth

MOPS, 3-(*N*-morpholino) propanesulphonic acid.

MICs:

S strains: 0.12-1 μg/ml

R strains: $\geq$ 16

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Azole-resistance in *Aspergillus*: Proposed nomenclature and breakpoints

Paul E. Verweij[a], Susan J. Howard[b], Willem J.G. Melchers[a], David W. Denning[b,*]

Drug Resistance Updates 12 (2009) 141–147

EUCAST

CLSI

Table 3
Proposed interpretative breakpoints (MIC, mg/L) for *A. fumigatus* and clinically licensed, active azoles.

Drug	Susceptible	Intermediate	Resistant
Itraconazole	<2	2	>2
Voriconazole	<2	2	>2
Posaconazole	<0.5	0.5	>0.5

Table 4
Proposed nomenclature of resistance in *Aspergilli* to clinically licensed azoles.

	Name	Description
Phenotype	Pan-azole resistant	The MICs of the isolate are in the resistant range for all available active azoles.
	Multi-azole resistant	The MICs are in the resistant range for more than one, but not all, azoles.
	Itraconazole, voriconazole or posaconazole resistant	The MICs are in the resistant range for a single azole.
Genotype	ITZgR, VCZgI, POSgR (G54W)	Isolate has a G54W substitution with a corresponding phenotype of resistance to itraconazole and posaconazole and intermediate susceptibility to voriconazole.

JOURNAL OF CLINICAL MICROBIOLOG
0095-1137/10/$12.00 doi:10.1128/JC

Vol. 48, No. 9

Wild-Type MIC ... off Values for the Triazol... I Broth Micr...)

A. Espinel-Ir... Pelaez,[5]
M. A...

TABLE 3. MIC distribution and ECVs for three triazoles and six *Aspergillus* spp. from five laboratories as determined by the CLSI M38-A2 microdilution method at 48 h

Species and antifungal agent (no. of isolates)	MIC (μg/ml)		ECV (μg/ml)	ECV (%)[b]
	Range	Mode[a]		
Aspergillus fumigatus				
Itraconazole (2,554)	≤0.03–2	0.5	1	98.8
Posaconazole (1,647)	≤0.015–4	0.06	0.5(0.25)[c]	99.2 (NA)[c]
Voriconazole (2,778)	0.03–≥4	0.25	1	97.7
A. flavus				
Itraconazole (536)	0.03–2	0.5	1	99.6
Posaconazole (321)	≤0.03–2	0.06	0.25	94.7
Voriconazole (590)	0.06–4	0.5	1	98.1
A. terreus				
Itraconazole (369)	0.03–1	0.25	1	100
Posaconazole (330)	≤0.03–2	0.25	0.5	99.7
Voriconazole (462)	0.03–>4	0.5	1	99.1
A. niger				
Itraconazole (427)	0.03–2	1	2	100
Posaconazole (325)	≤0.03–2	0.5	0.5	96.9
Voriconazole (479)	≤0.03–4	0.5	2	99.4
A. nidulans				
Itraconazole (141)	0.03–2	0.5	1	95
Posaconazole (129)	0.03–8	0.25	1	97.7
Voriconazole (139)	0.03–≥4	0.125	2	99.3
A. versicolor				
Itraconazole (68)	0.03–2	1	2	100
Posaconazole (41)	0.03–≥4	0.5	1[d]	NA
Voriconazole (80)	0.03–>4	0.25	2	97.5

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The Netherlands

JOURNAL OF CLINICAL MICROBIOLOGY, July 2002, p. 2648–2650 Vol. 40, No. 7
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.7.2648–2650.2002

Nationwide Survey of In Vitro Activities of Itraconazole and Voriconazole against Clinical *Aspergillus fumigatus* Isolates Cultured between 1945 and 1998

Paul E. Verweij,[1*] Debbie T. A. Te Dorsthorst,[1,2] Anthonius J. M. M. Rijs,[1]
Hilly G. De Vries-Hospers,[3] Jacques F. G. M. Meis,[2] and the Dutch Interuniversity Working Party for Invasive Mycoses

The isolates in a collection of 170 *Aspergillus fumigatus* isolates recovered from 114 patients and 21 different medical centers in The Netherlands over a period of 53 years were tested for the presence of resistance to itraconazole and voriconazole according to the guidelines of NCCLS document M38-P and by the E-test. Three isolates were highly resistant to itraconazole, and voriconazole MICs were low for all isolates.

A. fumigatus (n=170)

Multicenter, **the Netherlands**

53 years, 1945-1998

MIC-1

- **Itra MIC of 64 µg/ml in 3 isolates**
- **No isolates with high vori MICs**

TABLE 1. Comparison of itraconazole and voriconazole MICs for 170 *A. fumigatus* isolates

Antifungal agent	Test method[a]	MIC (µg/ml)			
		Geometric mean	Range	50%	90%
Itraconazole	Broth micro (24 h)	1	0.5–64	1	1
	Broth micro (48 h)	1	0.5–64	1	1
	Etest (24 h)	0.6	0.03–64	0.5	0.75
	Etest (48 h)	0.7	0.13–64	0.5	0.75
Voriconazole	Broth micro (24 h)	0.17	0.06–0.5	0.13	0.25
	Broth micro (48 h)	0.25	0.06–1	0.25	0.5

The Netherlands

PLoS Medicine November 2008 | Volume 5 | Issue 11 | e219

Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism

-I

Eveline Snelders[1,2], Henrich A. L. van der Lee[1,2], Judith Kuijpers[1,2], Anthonius J. M. M. Rijs[1,2], János Varga[3,4], Robert A. Samson[3], Emilia Mellado[5], A. Rogier T. Donders[6], Willem J. G. Melchers[1,2], Paul E. Verweij[1,2*]

ITRA RESISTANCE

Isolates of years 1994-2007

n=1912

The Netherlands, single center

Screening test: Growth in presence of 8 μg/ml itra

MIC: ≥ 4 μg/ml

Itra R: %1.7-6

Figure 1. Epidemiology of ITZ Resistanc[...] in the *A. fumigatus* Isolates

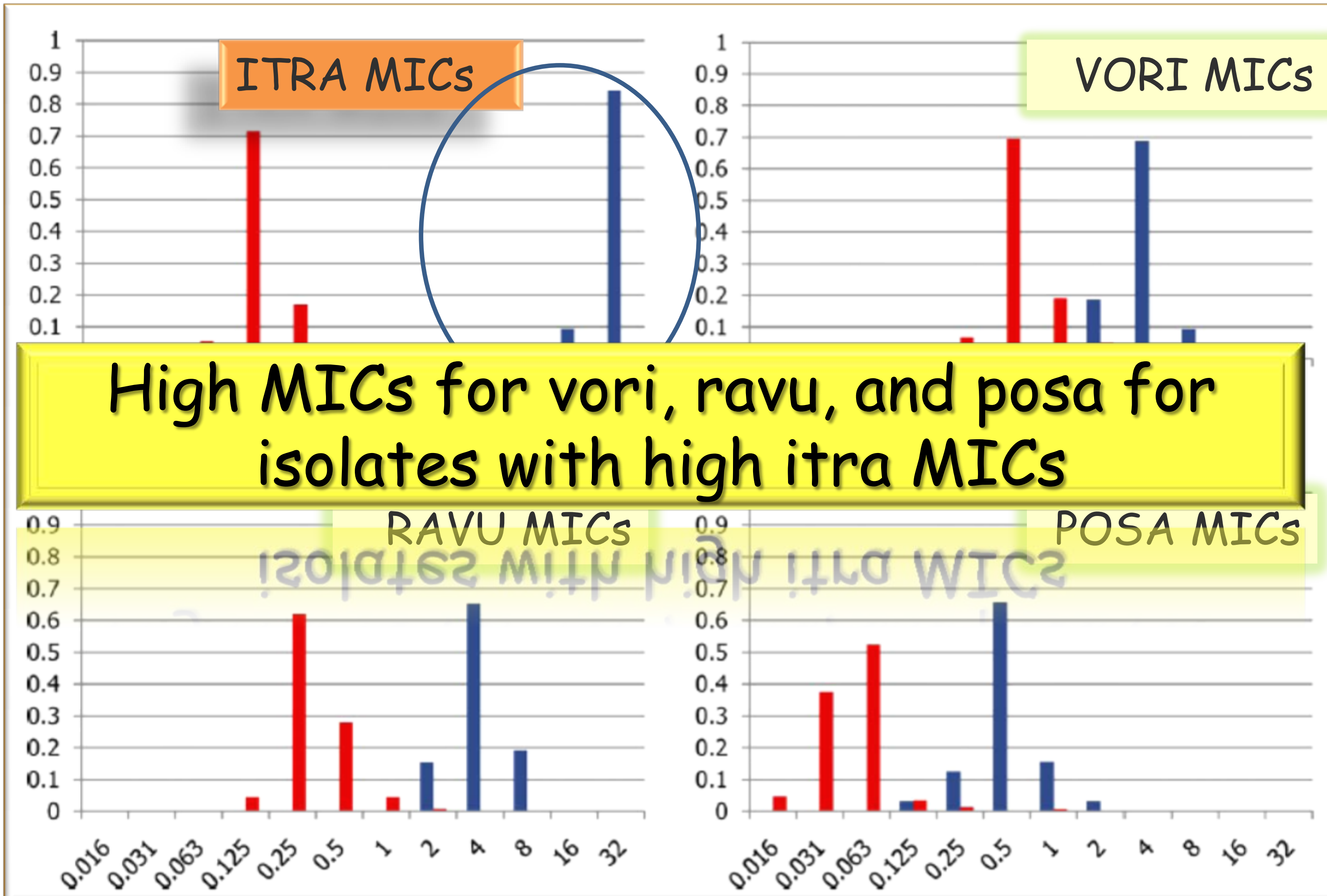
Blue bars represent the number of patie[...]s with a positive *A. fumigatus* culture (left *y*-axis) and the red line represents the percentage of those patients with an ITZ+ isolate (right *y*-axis). The *x*-[...]xis is the year.

doi:10.1371/[...]

Emergence and increase in itra resistance after 1999

PLoS Medicine November 2008 | Volume 5 | Issue 11 | e219

Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism -II



PLoS Medicine
November 2008 | Volume 5 | Issue 11 | e219

Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism -III

Eveline Snelders[1,2], Henrich A. L. van der Lee[1,2], Judith Kuijpers[1,2], Anthonius J. M. M. Rijs[1,2], János Varga[3,4], Robert A. Samson[3], Emilia Mellado[5], A. Rogier T. Donders[6], Willem J. G. Melchers[1,2], Paul E. Verweij[1,2*]

BLACK: Itra-S strains (Control group)

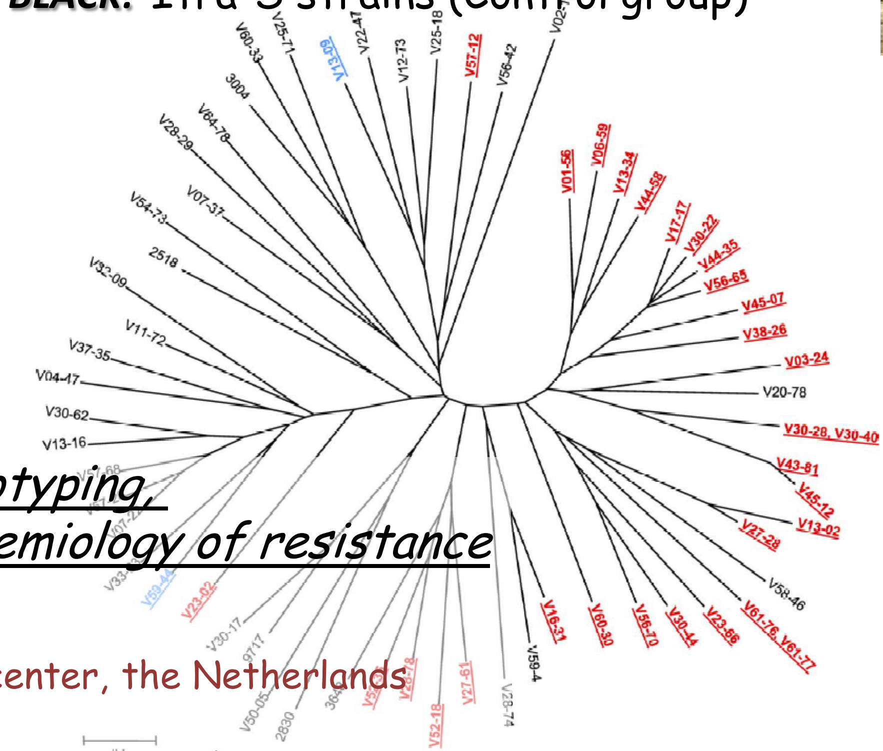


Figure 3. Genotypic Relatedness of 32 ITZ+ *A. fumigatus* Isolates and 32 ITZ− Controls

Genotyping, epidemiology of resistance

n=464
Multicenter, the Netherlands and
6 European countries

Belgium, France, Greece, Norway, Sweden, and UK

GENOTYPING:

RED: L98H modification in *Cyp51A* gene together with 34 bp tandem repeat in the gene promoter (TR/L98H)

Most of the isolates. Genetically distinct but clustered. Some from azole-naive patients.

SOURCE ? ENVIRONMENTAL ?

Agricultural use of azoles as fungicides ?

The Netherlands, multicenter

Clinical implications of azole resistance in Aspergillus fumigatus, The Netherlands, 2007-2009

Va n der Linden et al.
Emerg Infect Dis 2011 Oct;17:1846

✓June 2007 through January 2009

✓ All clinical *Aspergillus* spp. isolates were screened for itraconazole resistance (2,062 isolates from 1,385 patients)

✓The prevalence of itraconazole resistance in *A. fumigatus* : **5.3%** (range 0.8%-9.5%)

✓ 64.0% of patients from whom a resistant isolate was identified were azole-naive

ARTEMIS, Global antifungal surveillance program (isolates of 2000-2006)

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2008, p. 2568–2572 Vol. 46, No. 8
0095-1137/08/$08.00+0 doi:10.1128/JCM.00535-08

In Vitro Survey of Triazole Cross-Resistance among More than 700 Clinical Isolates of *Aspergillus* Species

-I

M. A. Pfaller,[1,2]* S. A. Messer,[1] L. Boyken,[1] C. Rice,[1] S. Tendolkar,[1] R. J. Hollis,[1] and D. J. Diekema[1,3]

771 *Aspergillus* strains

Most (88% of them for itra, 98% of them for vori & posa) have MICs of $\leq$ 1 μg/ml

ARTEMIS, Global antifungal surveillance program (isolates of 2000-2006)

In Vitro Survey of Triazole Cross-Resistance among More than 700 Clinical Isolates of *Aspergillus* Species[∇] -II

M. A. Pfaller,[1,2*] S. A. Messer,[1] L. Boyken,[1] C. Rice,[1] S. Tendolkar,[1] R. J. Hollis,[1] and D. J. Diekema[1,3]

JCM 2008; 46: 2568

		Cumulative % of isolates inhibited at MIC of (μg/ml)			
		0.5	1	2	4
A. fumigatus (n=553)	Itraconazole	51	93	100	
	Voriconazole	98	>99	>99	100
	Posaconazole	97	99	100	
All *Aspergillus* spp. (n=771)	Itraconazole	53	88	98	98
	Voriconazole	90	98	>99	>99
	Posaconazole	95	98	>99	>99

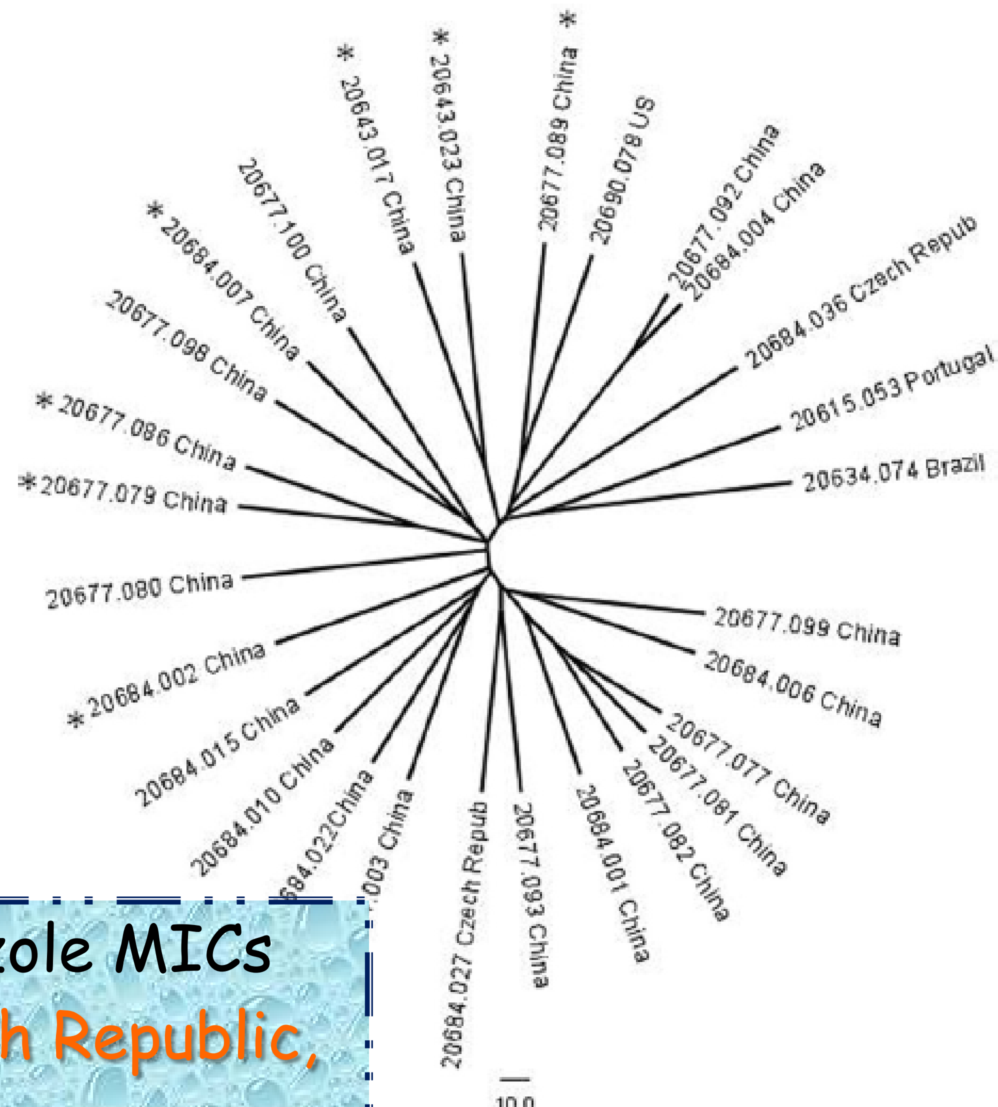
ARTEMIS, Global antifungal surveillance program (isolates of 2008-2009)

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p
0066-4804/11/$12.00 doi:10.1128/AAC.00185-11

Azole Resistance in *As*
ARTEMIS Global
Due to the
the

Shawn R. Lockhart,[1]* João P.
Daniel J. Dieke

- Survey of 497 *A. fumigatus*
- Years 2008-2009
- Part of ARTEMIS global surveillance study



28 isolates with high triazole MICs
From: Brazil, China, Czech Republic, Portugal, USA

of all 28 isolates with elevated triazole MIC values. Isolates with the TR/L98H mutation are marked with an asterisk.

UK

Emerg Infect Dis 2009; 15: 1068

Frequency and Evolution of Azole Resistance in *Aspergillus fumigatus* Associated with Treatment Failure[1]

Susan J. Howard, Dasa Cerar, Michael J. Anderson, Ahmed Albarrag, Matthew C. Fisher, Alessandro C. Pasqualotto, Michel Laverdiere, Maiken C. Arendrup, David S. Perlin, and David W. Denning

-I

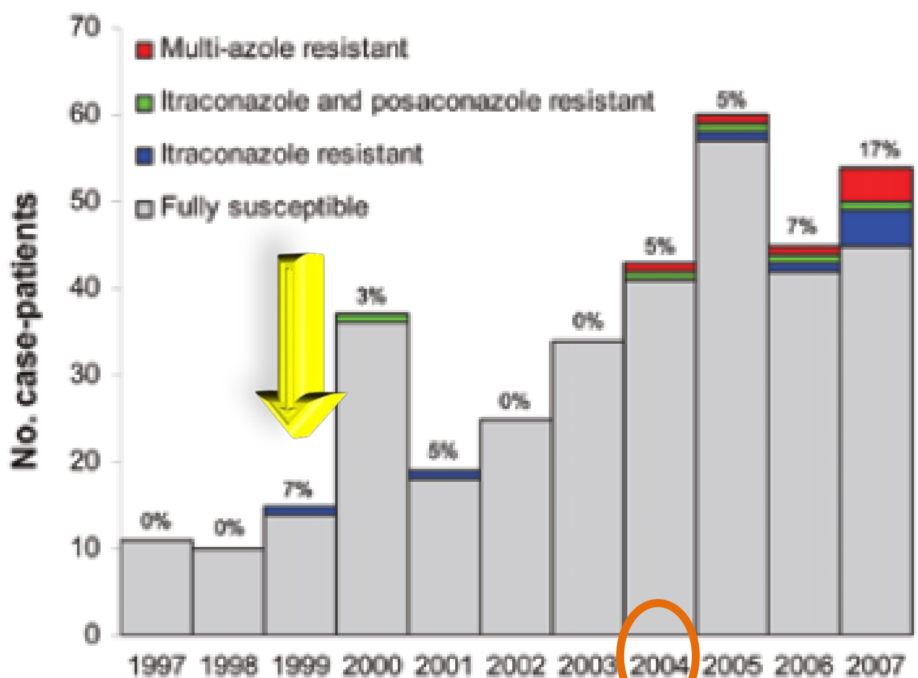


Figure 1. Azole resistance in clinical *Aspergillus fumigatus* isolates received in the Regional Mycology Laboratory Manchester, UK, 1997–2007. Overall azole resistance for each year is shown above each column as a percentage. Data do not include sequential isolates from the same patient.

Overall frequency of itraconazole resistance :
5%
(n=400, excluding duplicate isolates)

1999: First itra-R isolate
Significant increase in the frequency of resistance since 2004 (8%) as compared to the R rate prior to 2004

UK

Emerg Infect Dis 2009; 15: 1068

Frequency and Evolution of Azole Resistance in *Aspergillus fumigatus* Associated with Treatment Failure[1] -II

Susan J. Howard, Dasa Cerar, Michael J. Anderson, Ahmed Albarrag, Matthew C. Fisher, Alessandro C. Pasqualotto, Michel Laverdiere, Maiken C. Arendrup, David S. Perlin, and David W. Denning

Itra-R isolates

→ 65% VORI-R

→ 74% POSA-R

ECOFF used:

Itra: > 2 μg/ml
Vori: > 2
Posa: > 0.5

SCARE, Prospective international surveillance of azole resistance (Isolates of May 2009 - June 2010)

Prospective international surveillance of azole resistance in *Aspergillus fumigatus*. SCARE-Network

JWM van der Linden, MC Arendrup, PE Verweij, SCARE Network

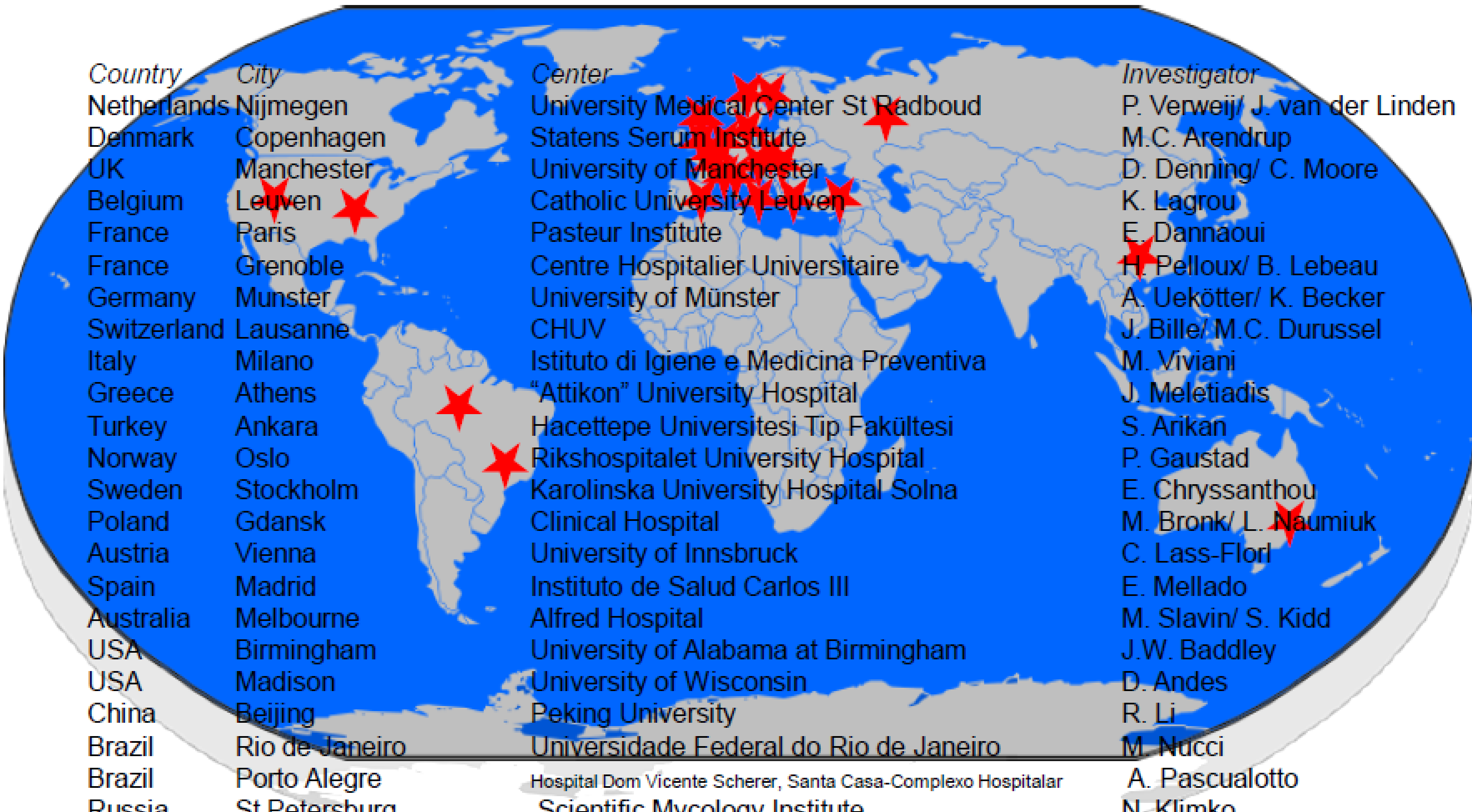
ICAAC 2011, Chicago

M-490

UMC St Radboud

Courtesy of Paul Verweij & Jan van der Linden

23 participating centers from 20 countries

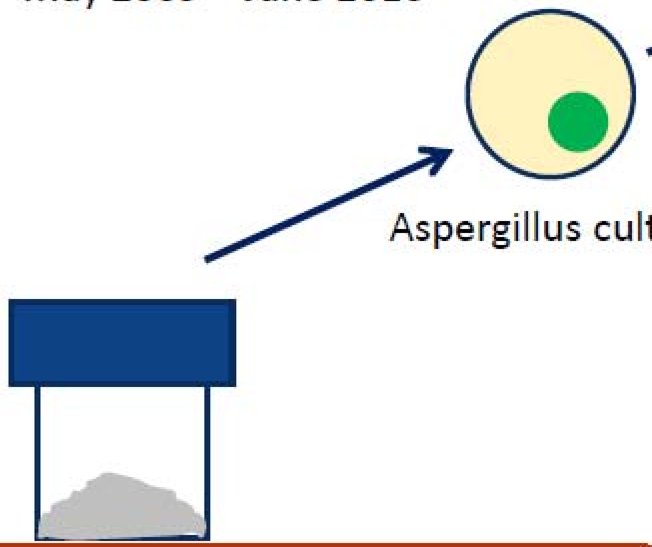


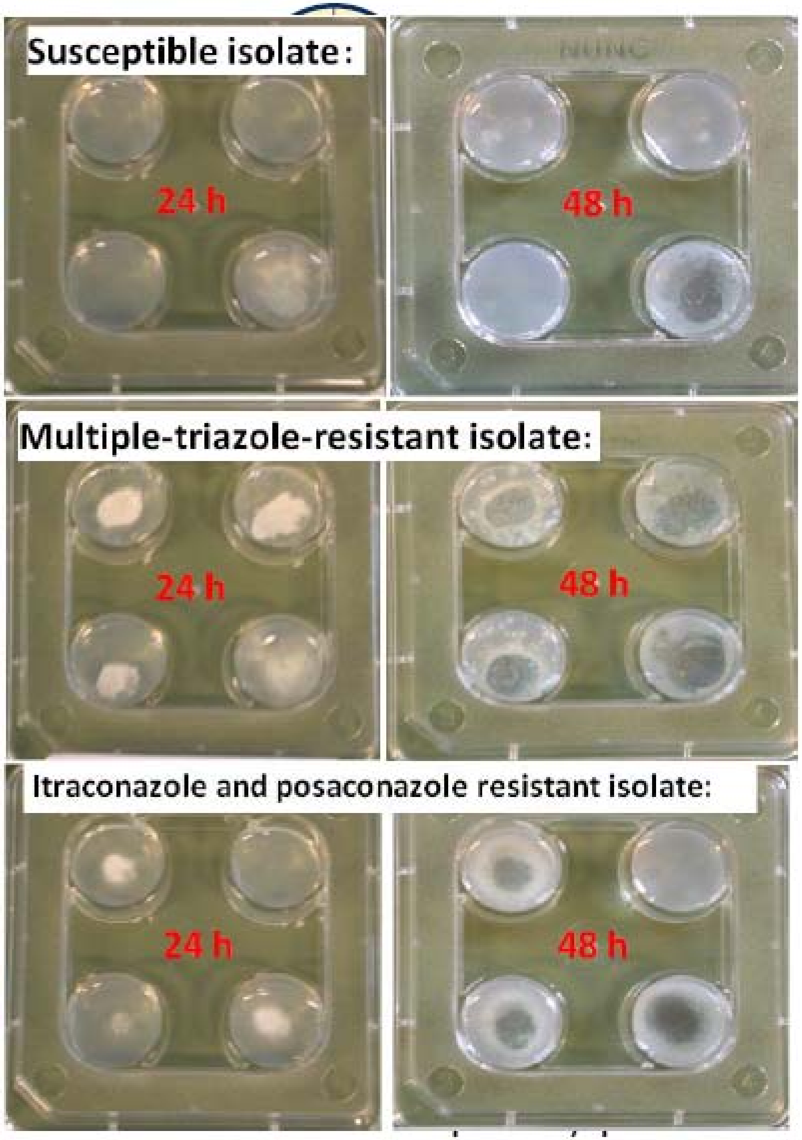
Country	*City*	*Center*	*Investigator*
Netherlands	Nijmegen	University Medical Center St Radboud	P. Verweij/ J. van der Linden
Denmark	Copenhagen	Statens Serum Institute	M.C. Arendrup
UK	Manchester	University of Manchester	D. Denning/ C. Moore
Belgium	Leuven	Catholic University Leuven	K. Lagrou
France	Paris	Pasteur Institute	E. Dannaoui
France	Grenoble	Centre Hospitalier Universitaire	H. Pelloux/ B. Lebeau
Germany	Munster	University of Münster	A. Uekötter/ K. Becker
Switzerland	Lausanne	CHUV	J. Bille/ M.C. Durussel
Italy	Milano	Istituto di Igiene e Medicina Preventiva	M. Viviani
Greece	Athens	"Attikon" University Hospital	J. Meletiadis
Turkey	Ankara	Hacettepe Universitesi Tip Fakültesi	S. Arikan
Norway	Oslo	Rikshospitalet University Hospital	P. Gaustad
Sweden	Stockholm	Karolinska University Hospital Solna	E. Chryssanthou
Poland	Gdansk	Clinical Hospital	M. Bronk/ L. Naumiuk
Austria	Vienna	University of Innsbruck	C. Lass-Florl
Spain	Madrid	Instituto de Salud Carlos III	E. Mellado
Australia	Melbourne	Alfred Hospital	M. Slavin/ S. Kidd
USA	Birmingham	University of Alabama at Birmingham	J.W. Baddley
USA	Madison	University of Wisconsin	D. Andes
China	Beijing	Peking University	R. Li
Brazil	Rio de Janeiro	Universidade Federal do Rio de Janeiro	M. Nucci
Brazil	Porto Alegre	Hospital Dom Vicente Scherer, Santa Casa-Complexo Hospitalar	A. Pascualotto
Russia	St Petersburg	Scientific Mycology Institute	N. Klimko

Courtesy of Paul Verweij & Jan van der Linden

Surveillance-method

Prospective, multicenter surveillance
May 2009 – June 2010





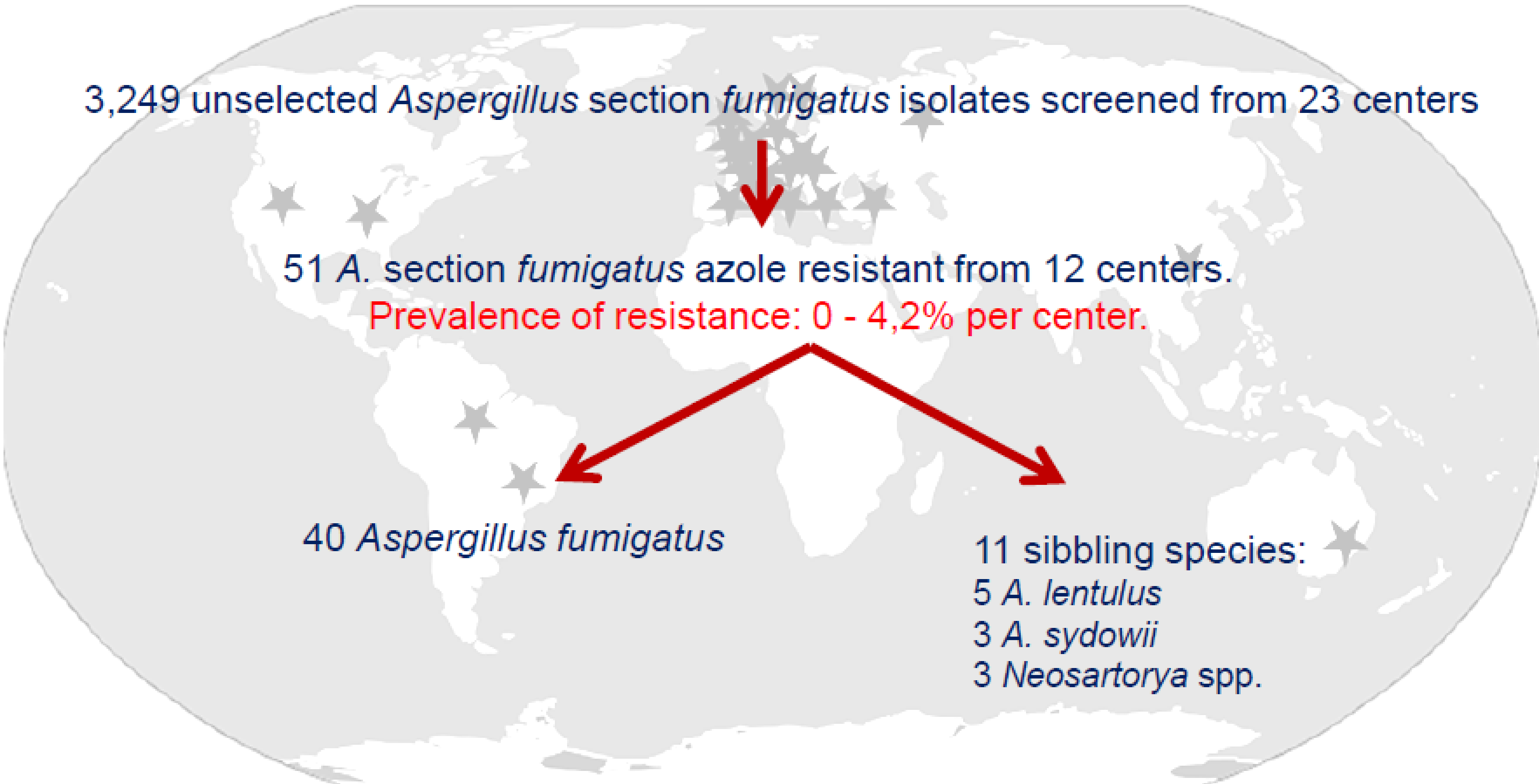
- Complet
questionn

- Cyp-sequencing

www.umcn.nl

Courtesy of Paul Verweij & Jan van der Linden

Results: isolates



Courtesy of Paul Verweij & Jan van der Linden

Results: phenotypic analysis

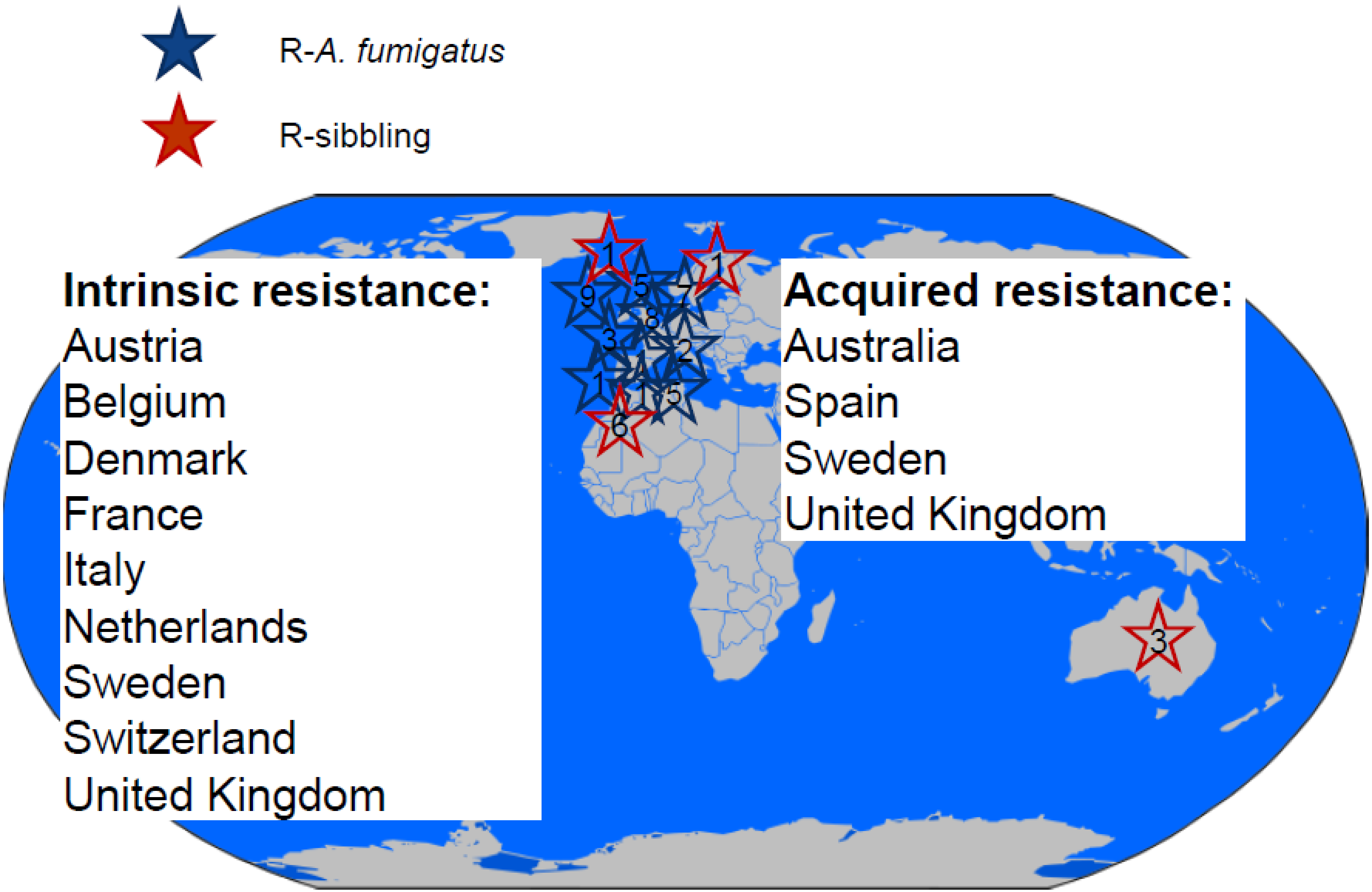
A. fumigatus:

ITZ-resistant (>2 mg/L)	100%
VCZ-resistant (>2 mg/L)	60%
PCZ-resistant (>0.5 mg/L)	58%

Sibbling species:

ITZ-resistant (>2 mg/L)	100%
VCZ-resistant (>2 mg/L)	82%
PCZ-resistant (>0.5 mg/L)	18%

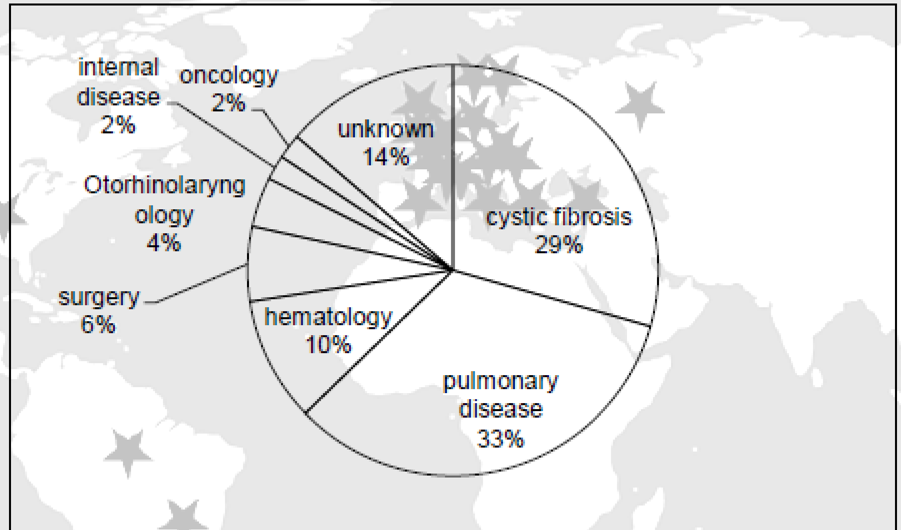
Courtesy of Paul Verweij & Jan van der Linden



Courtesy of Paul Verweij & Jan van der Linden

Clinical data: resistant isolates

Underlying diseases:



***Aspergillus* diseases:**

Invasive aspergillosis in 6 patients:
3 proven
1 probable
2 possible

Non-invasive aspergillosis in 6 patients:
2 ABPA
2 aspergilloma
2 otomycosis

82% azole naïve within 3 months prior to culture

Courtesy of Paul Verweij & Jan van der Linden

Japan

Antifungal Susceptibilities of *Aspergillus fumigatus* Clinical Isolates Obtained in Nagasaki, Japan

AAC Jan 2012; 56: 584

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- Nagasaki University Hospital, Nagasaki, Japan
- 196 clinical *A. fumigatus* isolates

G54 mutation in *cyp51A in 64 and 100 % of non-wild type isolates for itra and posa, respectively*

TRIAZOLE	ECOFF (μg/ml) used	% non-wild type
Itraconazole	1	7.1
Voriconazole	1	4.1
Posaconazole	0.5	2.6

India

Journal of Antimicrobial Chemotherapy Advance Access published October 25, 2011

2012 Febr; 67: 362

J Antimicrob Chemother
doi:10.1093/jac/dkr443

Journal of Antimicrobial Chemotherapy

Isolation of multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR/L98H mutations in the *cyp51A* gene in India

Anuradha Chowdhary[1*], Shallu Kathuria[1], Harbans S. Randhawa[1], Shailendra N. Gaur[2], Corné H. Klaassen[3] and Jacques F. Meis[3,4]

103 isolates
Years: 2005-2010

2 isolates
(1.9%)
with high triazole MICs
Itra MIC: >16
Vori MIC: 2
Posa MIC: 2
Isavu MIC: 8
Isolates are from patients with chronic respiratory disease who are **azole-naive**

Agenda

- Background
- Azole resistance in *Aspergillus:* The first report
- Proposed and frequently used ECOFFs (azoles vs. *Aspergillus*)
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  - The Netherlands
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  - ARTEMIS
  - SCARE
- Azole resistance in strains of *A. fumigatus* isolated from patients with specific underlying disorders
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- Conclusions

France, heamotological malignancies

J Antimicrob Chemother 2011; **66**: 371–374

Low prevalence of resistance to azoles in *Aspergillus fumigatus* in a French cohort of patients treated for haematological malignancies

Alexandre Alanio[1], Emilie Sitterlé[1], Martine Liance[1,2], Cécile Farrugia[1,2], Françoise Foulet[1], Françoise Botterel[1,2], Yosr Hicheri[3], Catherine Cordonnier[2,3], Jean-Marc Costa[1,4] and Stéphane Bretagne[1,2,5*]

Prospective
4 years
89 consecutive pat.s

118 strains
Etest
Breakpoints: > 2 (Itra), > 2 (Vori), > 0.25 (Posa)

Itra-R: 0.85%
(ONE ISOLATE)
Itra MIC: 16 μg/ml
Vori MIC: 0.38
Posa: 0.25

Azole-R Aspergillus & CF - France

Title: High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole.

Antimicrob Agents Chemother 2012; 56: 869

Pierre-Régis Burgel[1,2], Marie-Thérèse Baixench[1,3], Michaël Amsellem[1,3], Etienne Audureau[1,4], Jeanne Chapron[1,2], Reem Kanaan[1,2], Isabelle Honoré[1,2], Jean Dupouy-Camet[1,3], Daniel Dusser[1,2], Corné H. Klaassen[5], Jacques F Meis[5,6], Dominique Hubert[1,2], André Paugam[1,3]

Cochin Univ. Hospital -France

ITRA MICs of ≥ 2 µg/ml

→ in isolates of **4.6%** of CF patients

! Azole resistant *A. fumigatus* strains were detected in 20% of subjects who have received itraconazole within the previous 3 years.

Azole-R Aspergillus & CF - Denmark

Journal of Clinical Microbiology, June 2011, p. 2243–2251 Vol. 49, No. 6
0095-1137/11/$12.00 doi:10.1128/JCM.00213-11

Aspergillus Species and Other Molds in Respiratory Samples from Patients with Cystic Fibrosis: a Laboratory-Based Study with Focus on *Aspergillus fumigatus* Azole Resistance

Klaus Leth Mortensen,[1]* Rasmus Hare Jensen,[1] Helle Krogh Johansen,[2] Marianne Skov,[3] Tacjana Pressler,[3] Susan Julie Howard,[4] Howard Leatherbarrow,[4] Emilia Mellado,[5] and Maiken Cavling Arendrup[1]

(6 of 133 patients had azole-non-S or R *A. fumigatus*)

4.5 %

All 6 patients were **previously exposed to azoles** (46 to 278 wk.s prior to detection of resistant isolate) - - longer than that for pat.s with no mould or with azole-S *Aspergillus* isolates

	S	I	R
Itraconazole	< 1	2	> 4
Voriconazole	< 1	2	> 4
Posaconazole	< 0.5	0.5	> 1

PCR (+), culture (-) Aspergillus… (very low organism burden)

High-frequency triazole resistance found in nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease

Denning et al. CID 2011 May; 52: 1123

Further amplification of the CYP51A gene in a subset of PCR-positive, culture negative samples for detection of key single-nucleotide polymorphisms (SNPs) associated with triazole resistance

↓

In **culture-negative, PCR-positive samples**, triazole-resistance mutations (L98H/TR and M220) were detected within the drug target CYP51A in **55 %** of samples.

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The Netherlands

PLoS Medicine November 2008 | Volume 5 | Issue 11 | e219

Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism

Eveline Snelders[1,2], Henrich A. L. van der Lee[1,2], Judith Kuijpers[1,2], Anthonius J. M. M. Rijs[1,2], János Varga[3,4], Robert A. Samson[3], Emilia Mellado[5], A. Rogier T. Donders[6], Willem J. G. Melchers[1,2], Paul E. Verweij[1,2*]

-I

ITRA RESISTANCE

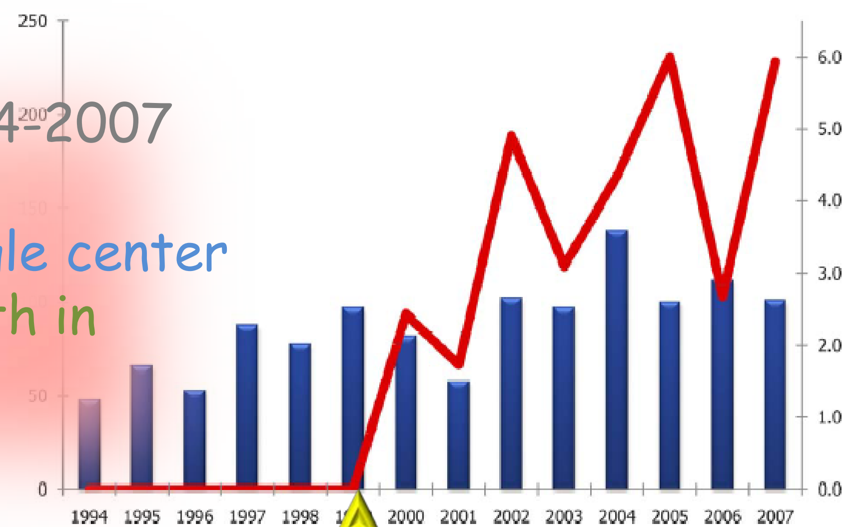
Isolates of years 1994-2007

n=1912

The Netherlands, single center

Screening test: Growth in presence of 8 µg/ml itra

MIC: > 4 µg/ml



Itra R: %1.7-6

Figure 1. Epidemiology of ITZ Resistance in the *A. fumigatus* Isolates

Blue bars represent the number of patients with a positive *A. fumigatus* culture (left *y*-axis) and the red line represents the percentage of those patients with an ITZ+ isolate (right *y*-axis). The *x*-axis is the year.

doi:10.1371/...

Emergence and increase in itra resistance after 1999

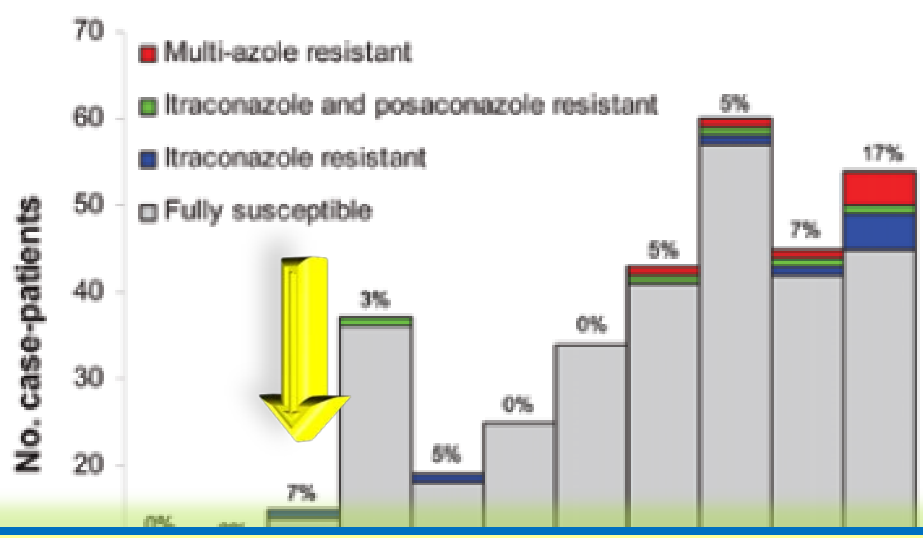
UK

Emerg Infect Dis 2009; 15: 1068

Frequency and Evolution of Azole Resistance in *Aspergillus fumigatus* Associated with Treatment Failure[1]

-I

Susan J. Howard, Dasa Cerar, Michael J. Anderson, Ahmed Albarrag, Matthew C. Fisher, Alessandro C. Pasqualotto, Michel Laverdiere, Maiken C. Arendrup, David S. Perlin, and David W. Denning



Overall frequency of itraconazole resistance : **5%** (n=400, excluding duplicate isolates)

Increase in resistance since 2004 (8%) as compared to the R rate prior to 2004

Figure 1. Azole resistance in clinical *Aspergillus fumigatus* isolates received in the Regional Mycology Laboratory Manchester, UK, 1997–2007. Overall azole resistance for each year is shown above each column as a percentage. Data do not include sequential isolates from the same patient.

UK

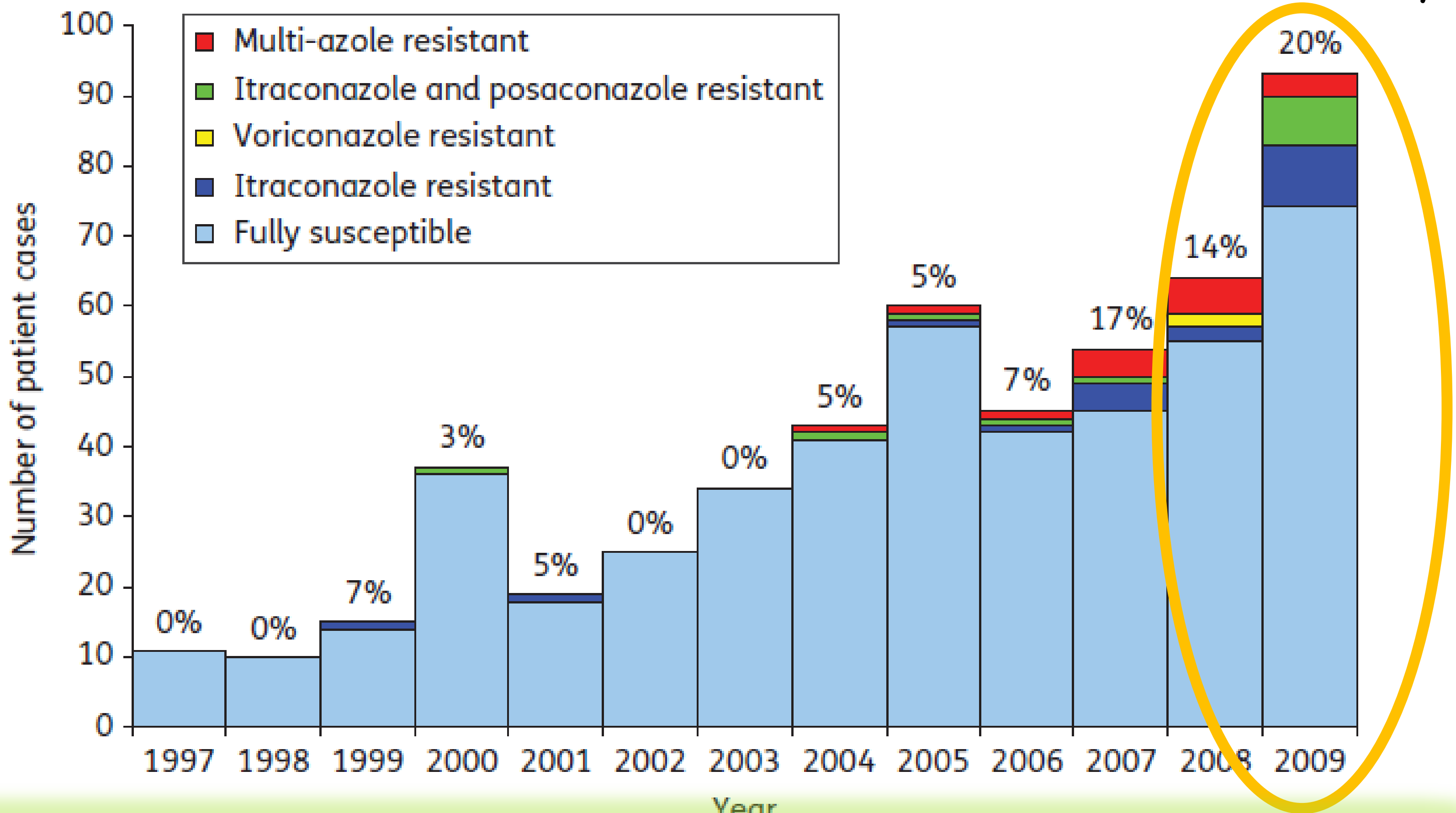
J Antimicrob Chemother 2010; **65**: 2116–2118

Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009

Ahmed Bueid[1], Susan J. Howard[1,2], Caroline B. Moore[1,2], Malcolm D. Richardson[1,2], Elizabeth Harrison[1], Paul Bowyer[1] and David W. Denning[1,2*]

Azole R freq. by patient

%: Overall azole R for that year



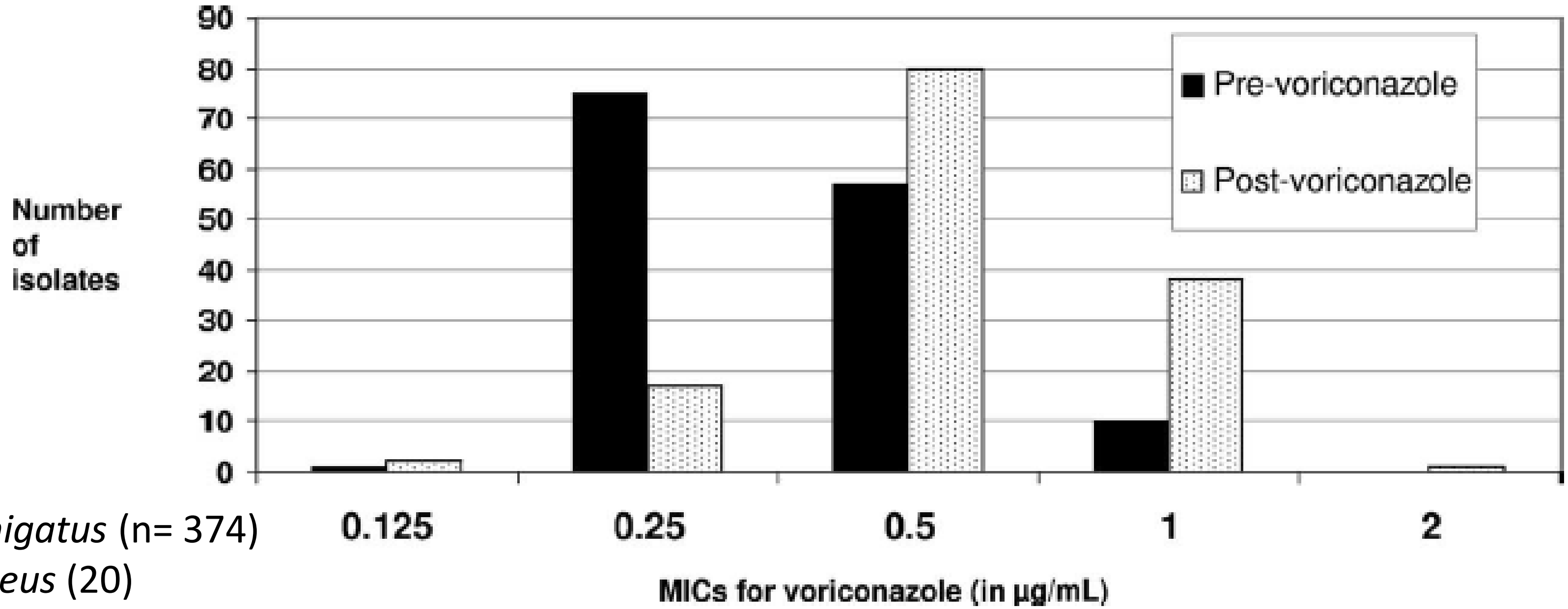
Azole resistance has continued to increase after 2007

Spain

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2008, p. 3444–3446 Vol. 52, No. 9

Clinical Isolates of *Aspergillus* Species Remain Fully Susceptible to Voriconazole in the Post-Voriconazole Era

Jesús Guinea,[1,2]* Sandra Recio,[1] Teresa Peláez,[1,2] Marta Torres-Narbona,[1] and Emilio Bouza[1,2]



A. fumigatus (n= 374)
A. terreus (20)
A. niger (3)
A. flavus (3)

No increase in voriconazole MICs in post- vs. pre-voriconazole era

Part of ARTEMIS (isolates of 2001-2009)

Vol. 49, No. 2

0095-1137/11/$12.00 doi:10.1128/JCM.02136-10

Use of Epidemiological Cutoff Values To Examine 9-Year Trends in Susceptibility of *Aspergillus* Species to the Triazoles

M. Pfaller,* L. Boyken, R. Hollis, J. Kroeger, S. Messer, S. Tendolkar, and D. Diekema

University of Iowa, Iowa City, Iowa

TABLE 3. Trends in susceptibility of *A. fumigatus* and *A. flavus* respiratory tract isolates to itraconazole, posaconazole, and voriconazole as determined by CLSI BMD methods

Antifungal agent	Species (ECV [μg/ml])	Years	No. tested	MIC (μg/ml) Range	MIC (μg/ml) Mode	% of MICs >ECV
Itraconazole	*A. fumigatus* (1)	2001–2003	173	0.12–2	0.5	4.0
	A. fumigatus (1)	2004–2006	441	0.03–>8	0.25	0.7
	A. fumigatus (0.5)	2004–2006	532	0.007–1	0.03	1.1
	A. fumigatus (0.5)	2007–2009	607	0.015–2	0.03	4.9
	A. flavus (0.5)	2001–2003	32	0.12–2	0.25	9.4
	A. flavus (0.5)	2004–2006	78	0.015–>8	0.06	1.3
	A. flavus (0.5)	2007–2009	125	0.03–1	0.06	6.4
Voriconazole	*A. fumigatus* (1)	2001–2003	173	0.06–1	0.25	0.0
	A. fumigatus (1)	2004–2006	532	0.12–4	0.25	1.6
	A. fumigatus (1)	2007–2009	607	0.12–4	0.5	1.6
	A. flavus (1)	2001–2003	32	0.12–1	0.5	0.0
	A. flavus (1)	2004–2006	78	0.25–>8	0.5	2.6
	A. flavus (1)	2007–2009	125	0.25–2	1	1.6

No consistent trend towards decreased susceptibility for any triazole and *A. fumigatus*

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2011, p. 4802–4809 Vol. 55, No. 10

Cryptic Species and Azole Resistance in the *Aspergillus niger* Complex[∇†]

Susan J. Howard,[1,2]* Elizabeth Harrison,[1] Paul Bowyer,[1] Janos Varga,[3‡] and David W. Denning[1,2]

n=50 (45: clinical)

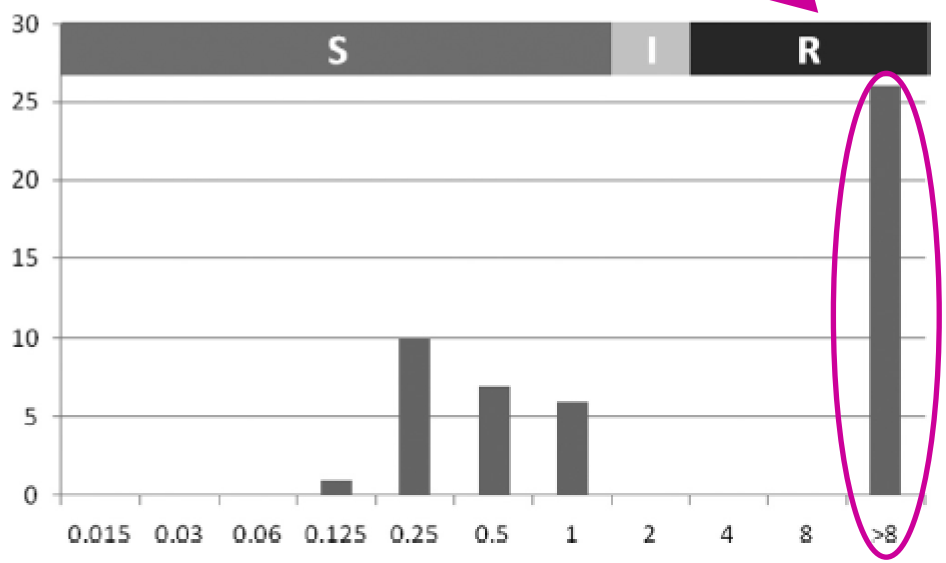

FIG. 1. Itraconazole MICs of *Aspergillus* section *Nigri* from the Mycology Reference Centre Manchester culture collection and proposed ECVs (S, susceptible; I, intermediate; R, resistant). The MIC is shown on the *x* axis, and the number of isolates is shown on the *y* axis.

5 clades

Clade	Itra R %
A. awamori	36
A. tubingensis	90
A. niger	33
A. acidus	100
Unknown	67

Overall, 52% of the isolates are itra-R

Itra-R: Common

Azole cross-R: unusual

cyp51A mutations may not be playing an important role

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Possible acquisition of azole-resistant *Aspergillus* strains

Selection in the individual patient during azole therapy
- - Selection of resistance *in vivo*

RISK FACTORS : Chronic *Aspergillus* inf.s, aspergilloma (high fungal burden), azole exposure

De novo acquisition of resistant strains from the environment

SOURCE: Agricultural use of azole compounds

Howard & Arendrup. Med Mycol 2011;49 (Suppl. 1): S90; Bowyer et al. Curr Infect Dis Rep. 2011;13: 485

Rapid Induction of Multiple Resistance Mechanisms in *Aspergillus fumigatus* during Azole Therapy: a Case Study and Review of the Literature

Antimicrob. Agents Chemother. **2012, 56(1):10**

Simone M. T. Camps,[a,b] Jan W. M. van der Linden,[a,b] Yi Li,[a,b] Ed J. Kuijper,[c] Jaap T. van Dissel,[d] Paul E. Verweij,[a,b] and Willem J. G. Melchers[a,b]

Review of the literature showed that in patients who develop azole resistance during therapy, multiple resistance mechanisms commonly emerge. Furthermore, the median time between the last cultured wild-type isolate and the first azole-resistant isolate was 4 months (range, 3 weeks to 23 months), indicating a rapid induction of resistance.

Development of Azole Resistance in *Aspergillus fumigatus* during Azole Therapy Associated with Change in Virulence

PLoS ONE April 2010 | Volume 5 | Issue 4 | e10080

Maiken Cavling Arendrup[1]*, Eleftheria Mavridou[2], Klaus Leth Mortensen[1], Eveline Snelders[2], Niels Frimodt-Møller[3], Humara Khan[4], Willem J. G. Melchers[2], Paul E. Verweij[4]

In Vitro Acquisition of Secondary Azole Resistance in *Aspergillus fumigatus* Isolates after Prolonged Exposure to Itraconazole: Presence of Heteroresistant Populations

Antimicrob. Agents Chemother. 2012, 56(1):174. DOI: 10.1128/AAC.00301-11.
Published Ahead of Print 17 October 2011.

Pilar Escribano,[a,b,c] Sandra Recio,[a,b] Teresa Peláez,[a,b,c,d] Milagros González-Rivera,[b,e] Emilio Bouza,[a,b,c,d] and Jesús Guinea[a,b,c,d]

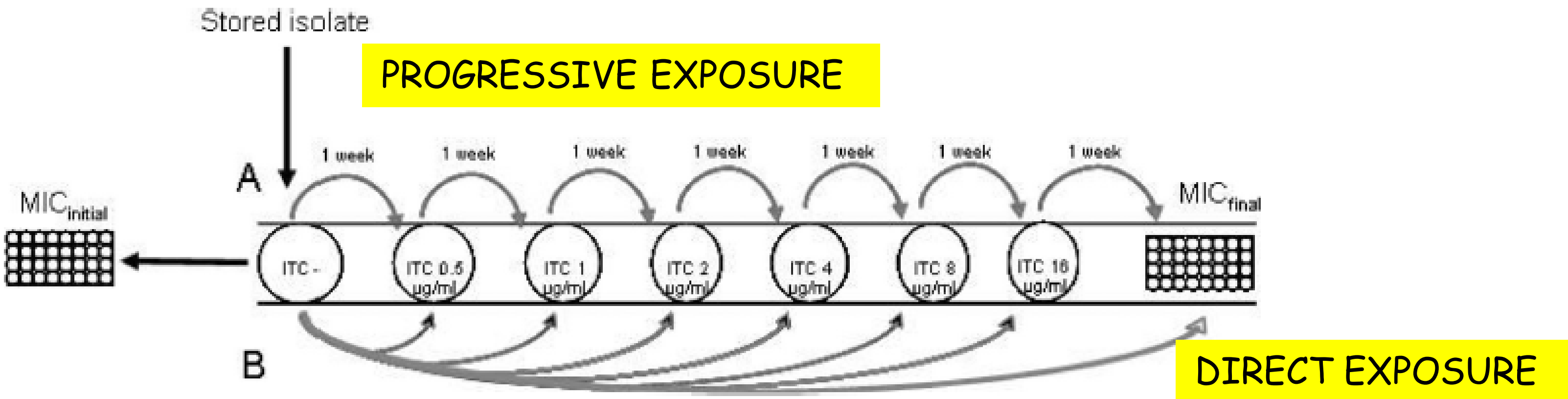

FIG 1 Representation of progressive exposure (experiment A) or direct exposure (experiment B) to itraconazole (ITC) to induce secondary azole resistance in 20 *Aspergillus fumigatus* isolates. In both experiments, 20 µl of the adjusted conidium suspension of 3×10^7 CFU/ml was used. All plates were incubated at 35°C for 1 week.

Itra, Vori, and Posa MICs are higher after progressive exposure as compared to direct exposure

Using concentrated (2×10^9 cfu/ml) conidium suspension and an itraconazole concentration of 4 µg/ml, selection of heteroresistant population in vitro (with G54 mutation) is possible

Azole fungicide use in the agricultural world and emergence of resistance

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2001, p. 2987–2990 Vol. 45, No. 11
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.11.2987–2990.2001

Critical Annotations to the Use of Azole Antifungals for Plant Protection

HERBERT HOF*

Institute for Medical Microbiology and Hygiene, University of Heidelberg, Mannheim, Germany

… In the final risk assessment for the use of azoles, not only the toxicological aspects but also the possibility of induction and/or selection of resistant human pathogenic fungi should be taken into account. …….

Applied and Environmental Microbiology, June 2009, p. 4053–4057 Vol. 75, No. 12

The Netherlands

Possible Environmental Origin of Resistance of *Aspergillus fumigatus* to Medical Triazoles

Eveline Snelders,[1,2] Robert A. G. Huis in 't Veld,[1,2] Anthonius J. M. M. Rijs,[1,2] Gert H. J. Kema,[3] Willem J. G. Melchers,[1,2] and Paul E. Verweij[1,2]*

Aim : To investigate whether azole resistance emerges through exposure to azole in the *environment* rather than *in vivo due to* azole therapy

Itra-R *A. fumigatus* were cultured from:
Indoor hospital env.
Soil obtained from flower beds
Commercial compost
Leaves
Seeds

Cross-resistance was observed to:
Voriconazole
Posaconazole
Azole fungicides (Metcunazole, tebuconazole)

Genetic clustering of resistant environmental & clinical isolates (and they were apart from non-R isolates)

Colonization with azole-R isolates from the environment is possible

Austria, Denmark, Spain

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2010, p. 4545–4549 Vol. 54, No. 11

Environmental Study of Azole-Resistant *Aspergillus fumigatus* and Other Aspergilli in Austria, Denmark, and Spain▽

Klaus Leth Mortensen,[1]* Emilia Mellado,[2] Cornelia Lass-Flörl,[3] Juan Luis Rodriguez-Tudela,[2] Helle Krogh Johansen,[4] and Maiken Cavling Arendrup[1]

SAMPLES FROM: Soil from flowerbeds surrounding the hospitals, soil from Tivoli Gardens (Copenhangen), and compost bags

DENMARK SOIL SAMPLES

A.fumigatus strains (n=4) with high azole MICs (all with TR/L98H)

SPAIN SOIL SAMPLE

A. lentulus strain with high vori MIC

Multi-azole-R *A. fumigatus* in the environment in Denmark

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The isolation rate of *Aspergillus* from clinical samples is low and the true incidence of acquired triazole resistance remains partly unknown. A better understanding of the extent of the resistance is required.

Azole-resistant strains of *A. fumigatus* have so far been reported from European and Asian countries at varying frequencies [high rates in UK (Manchester) and The Netherlands (Nijmegen)].

Available data are mostly of *A. fumigatus* (complex). The situation for non-*fumigatus Aspergilli* is less clear.

Continued surveillance of azole resistance should be maintained in each center to determine the resistance rates and any possible trend of increase in the isolation frequency of resistant clinical strains.

Based on the association with agricultural azole use, environmental sampling remains significant as well.