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LEUVEN**



# **EMERGENCE OF ASPERGILLUS FUMIGATUS RESISTANCE IN BELGIUM**

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## Azole resistance in *Aspergillus*: a growing public health menace



“A ‘perfect storm’ combining extensive antifungal fungal exposure ... along with a highly efficient, evolutionarily perfected dispersal system, has led to our current situation.”

David W Denning<sup>1,2</sup> & David S Perlin<sup>3</sup>

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<sup>3</sup>Public Health Research Institute, New Jersey Medical School-UMDNJ, Newark, NJ, USA

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Future Medicine, 2011, 6, 1229-1232

Future Microbiology  
Editorial

REVIEW

Curr Opin Infect Dis 2013, 26: 493-500



## Azole resistance in *Aspergillus fumigatus*: a growing public health concern

Edith Vermeulen<sup>a</sup>, Katrien Lagrou<sup>a,b</sup>, and Paul E. Verweij<sup>c</sup>

# Eukaryotic Cell

## ***Aspergillus lentulus* sp. nov., a New Sibling Species of *A. fumigatus***


S. Arunmozhi Balajee, Jennifer L. Gribskov, Edward Hanley, David Nickle and Kieren A. Marr  
*Eukaryotic Cell* 2005, 4(3):625. DOI:  
10.1128/EC.4.3.625-632.2005.



## Journal of Clinical Microbiology

## **Mistaken Identity: *Neosartorya pseudofischeri* and Its Anamorph Masquerading as *Aspergillus fumigatus***

S. Arunmozhi Balajee, Jennifer Gribskov, Mary Brandt, James Ito, Annette Fothergill and Kieren A. Marr  
*J. Clin. Microbiol.* 2005, 43(12):5996. DOI:  
10.1128/JCM.43.12.5996-5999.2005.

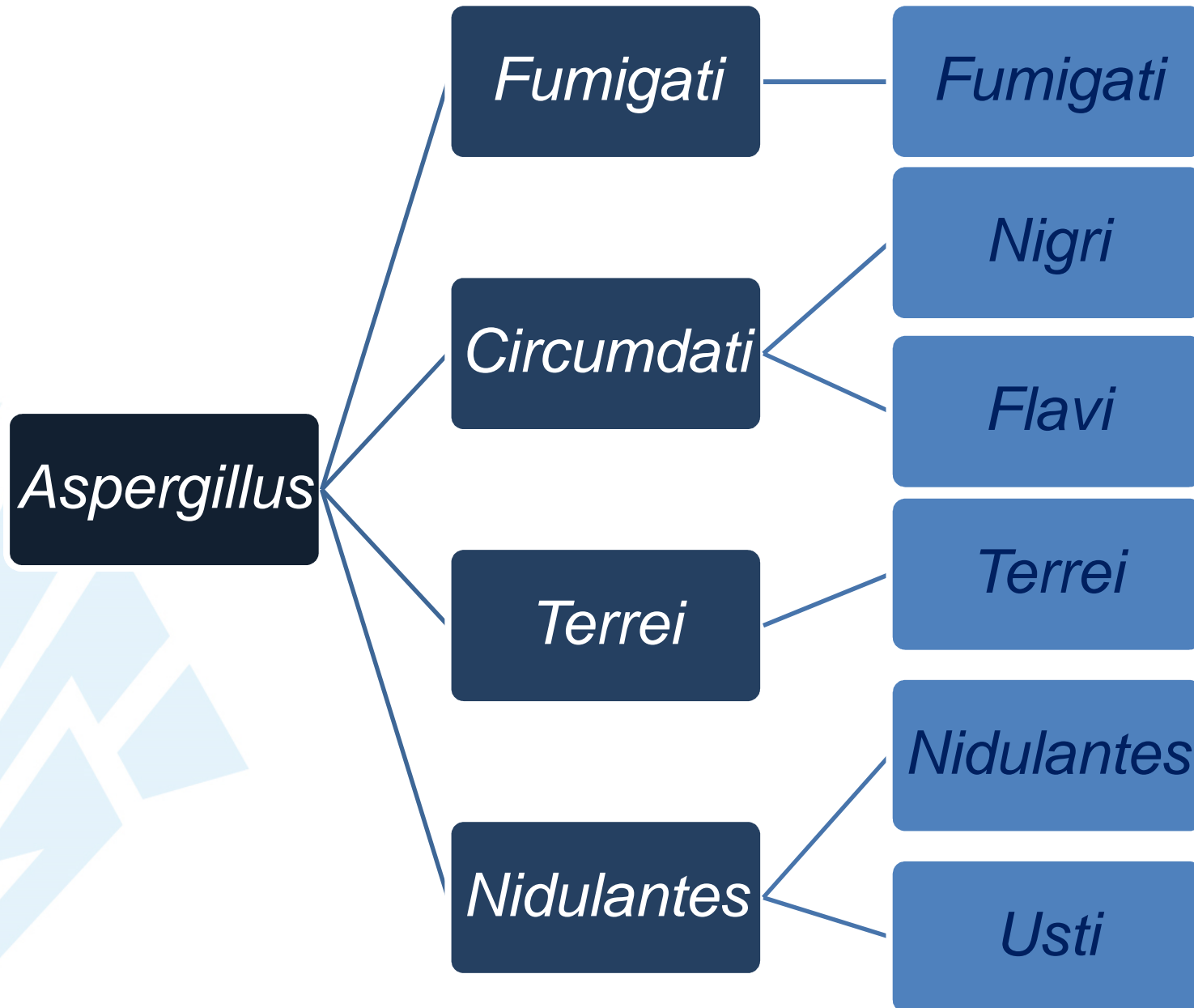




GENUS

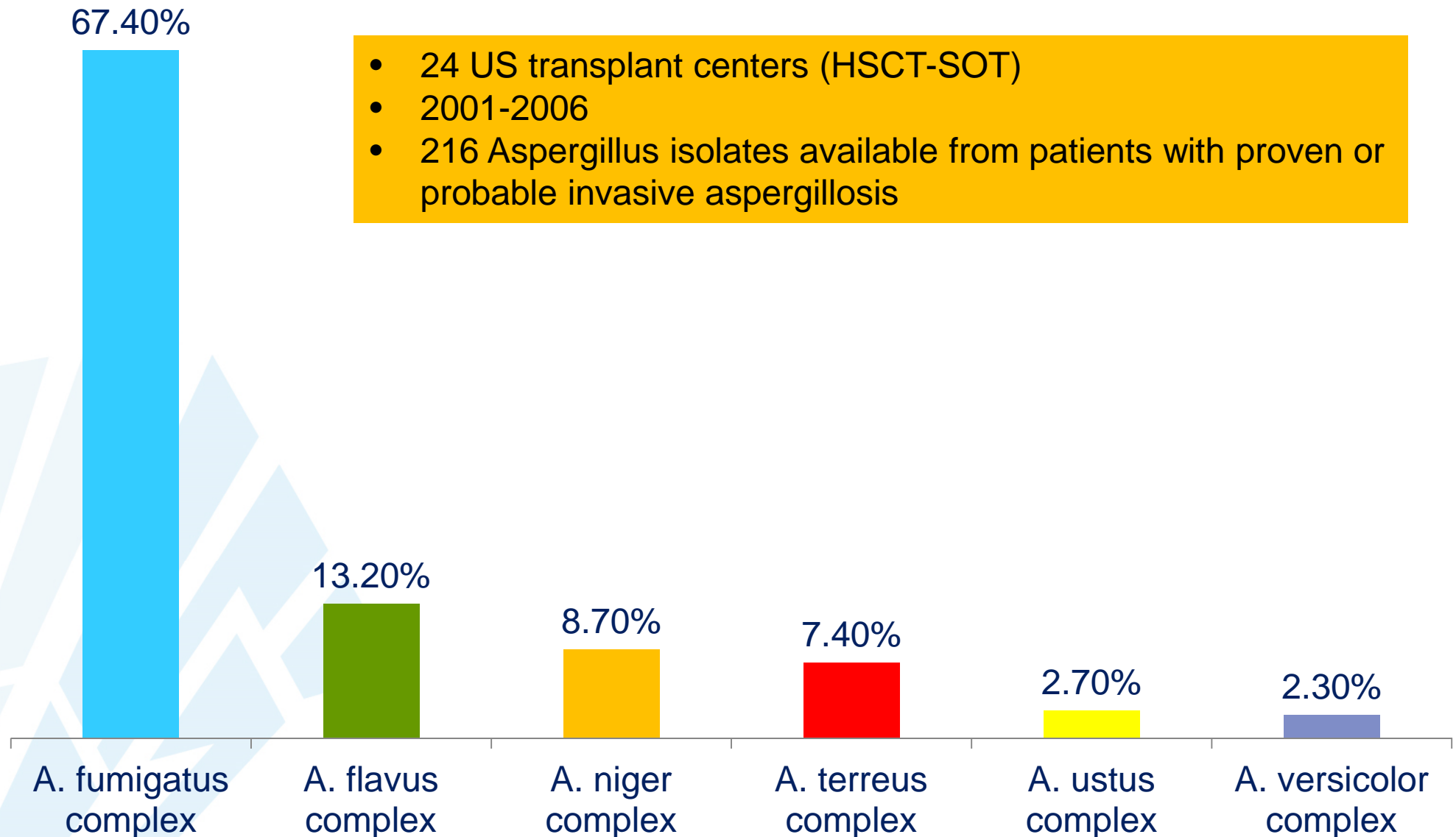
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SECTION



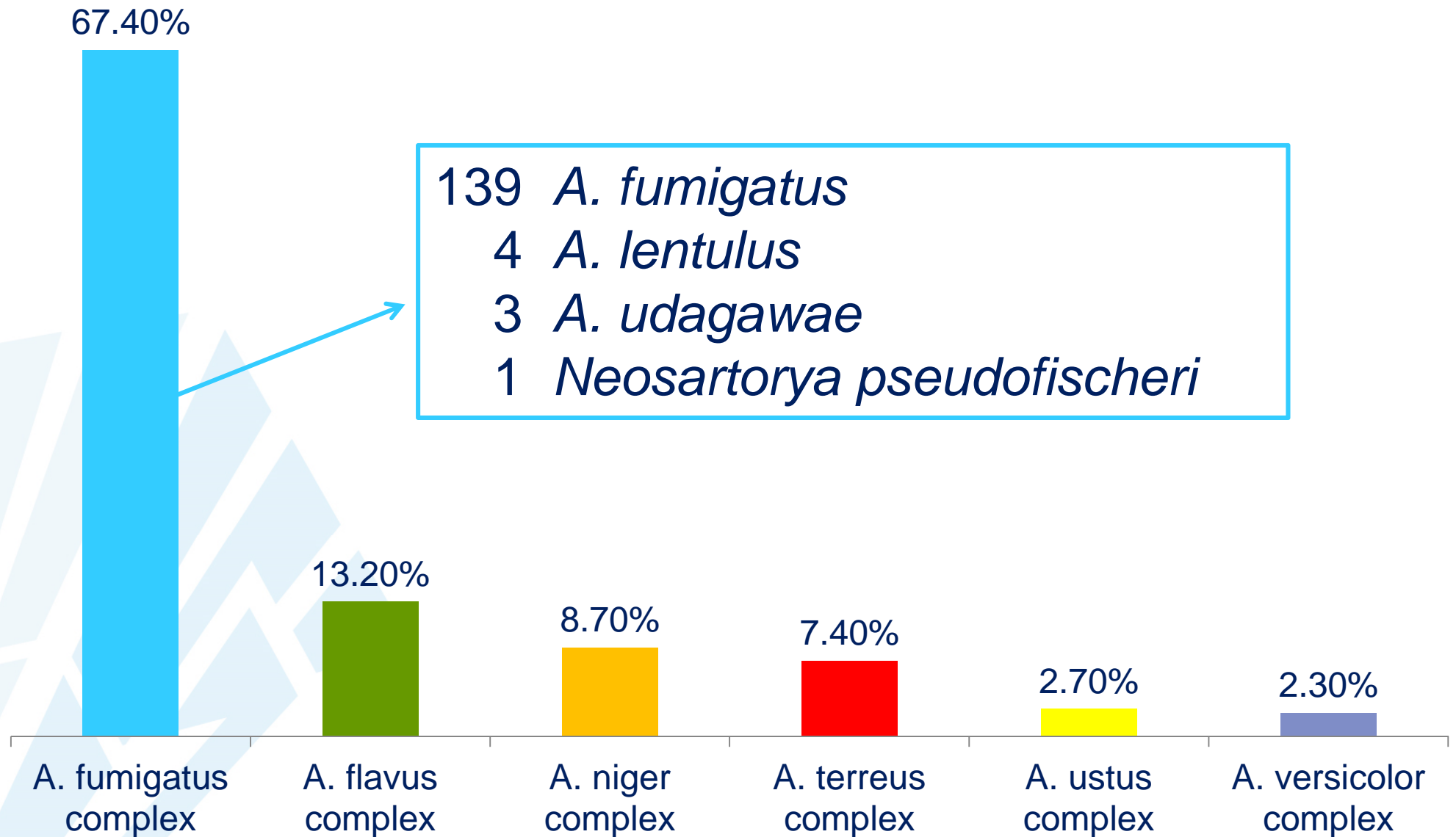


# Transplant-Associated Infection Surveillance Network (TRANSNET)



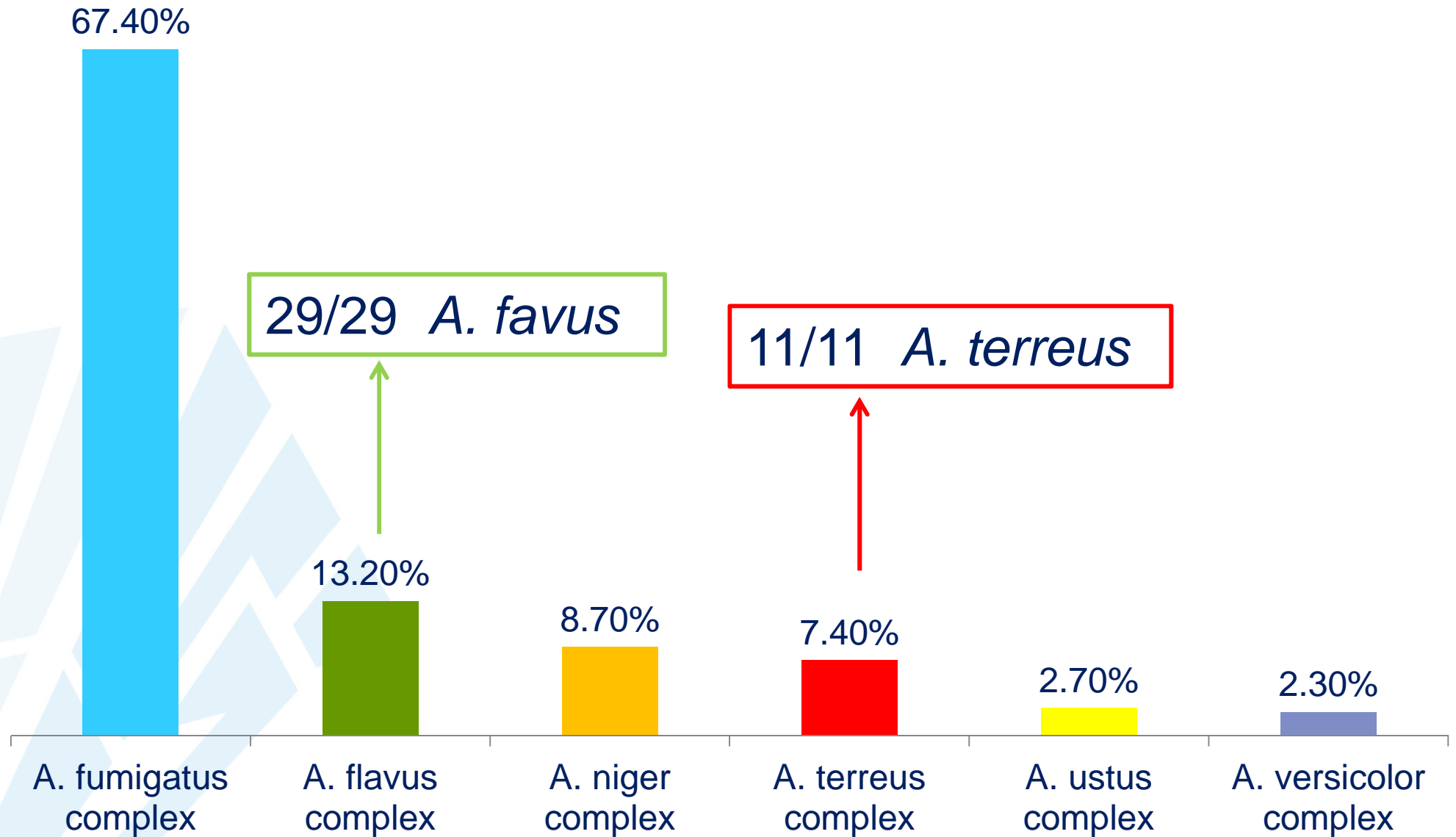


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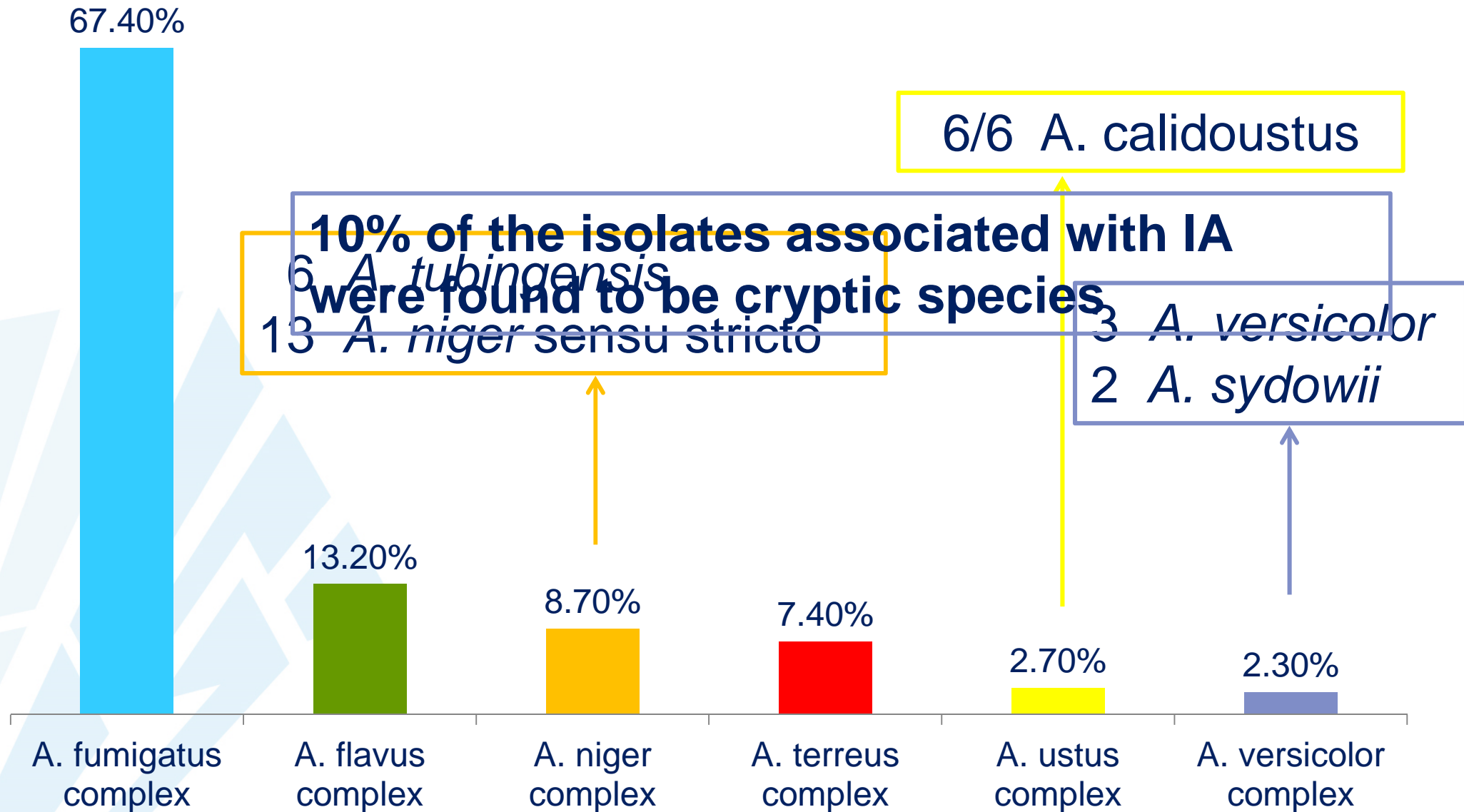


# Transplant-Associated Infection Surveillance Network (TRANSNET)





# Transplant-Associated Infection Surveillance Network (TRANSNET)





# Clinical breakpoints *Aspergillus* spp. EUCAST

(valid from 2013-03-11)

Antifungal agent	MIC breakpoint (mg/L)							
	A. fumigatus		A. flavus		A. niger		A. terreus	
	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >
Amphotericin B	1	<b>2</b>	IE	IE	1	2	-	-
Itraconazole	1	<b>2</b>	1	2	IE	IE	1	2
Voriconazole	1	<b>2</b>	IP	IP	IE	IE	IE	IE
Posaconazole	0.12	<b>0.25</b>	IE	IE	IE	IE	0.12	0.25



# TRANSNET: susceptibility testing

MIC  $\mu\text{g/ml}$

	AMB	ITR	VRZ	POS
<i>A. calidoustus</i> (n= 6)	0.5 - 1	>8	4-8	>8
<i>A. tubingensis</i> ( n=6)	0.125 - 0.25	0.25 - 1	0.5 - 1	0.06 - 0.5
<i>A. lentulus</i> ( n=4)	0.5 - 2	0.25 - 0.5	1 - 4	0.25
<i>A. udagawae</i> (n=3)	1 - 2	0.25 - 2	0.5 - 2	0.125 - 0.25

# Emerging azole resistance



Few sporadic resistant isolates in Sweden, Spain France and UK

Isolates with TR<sub>34</sub>/L98H reported from Denmark, Norway, Belgium, UK, Spain, France, Germany, Italy, China, India and Iran

- Series of Dutch patients – including azole naïve patients- with invasive aspergillosis due to pan-azole-resistant strains
- Resistance attributed to one predominant resistance mechanism, TR<sub>34</sub>/L98H

*Verweij, NEJM 2007*

Itraconazole resistance in 2 cases

*Denning, AAC 1997*

- Dramatic increase of azole resistance in patients of a specialized referral center for patients with chronic and allergic aspergillosis (Manchester).
- Variety of different CYP51A-related resistance mechanisms

*Howard, Emerg Infect Dis 2009*

1995

2000

2005

2010

2015



# Two routes of resistance selection

- ‘In-patient’  
In patients with chronic *Aspergillus* disease and long-term azole treatment
- “In the environment”  
Patients inhale azole-resistant *A. fumigatus* spores



# Emerging azole resistance



- New environmental CYP51A-mediated resistance mechanism in the Netherlands, TR<sub>46</sub>/Y121F/T289A associated with voriconazole therapy failure
- High-grade voriconazole resistance, variable itraconazole MICs
- Spread of this new mechanism to Belgium

*Van der Linden, CID 2011; Vermeulen, Eurosurveillance 2012*

Increase of *A. fumigatus* isolates with CYP51A-unrelated azole resistance

*Tashiro AAC 2012; Bader, AAC 2013,*

1995

2000

2005

2010

2015



**Eurosurveillance, Volume 17, Issue 48, 29 November 2012**

**Rapid communications**

**AZOLE-RESISTANT ASPERGILLUS FUMIGATUS DUE TO TR46/Y121F/T289A MUTATION  
EMERGING IN BELGIUM, JULY 2012**

E Vermeulen<sup>1</sup>, J Maertens<sup>2</sup>, H Schoemans<sup>2</sup>, K Lagrou ( [katrien.lagrou@uzleuven.be](mailto:katrien.lagrou@uzleuven.be) )<sup>1,3</sup>

1. Catholic University of Leuven, Department of Microbiology and Immunology, Leuven, Belgium
2. University Hospitals Leuven, Department of Hematology, Leuven, Belgium
3. University Hospitals Leuven, Department of Laboratory Medicine, Leuven, Belgium

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Citation style for this article: Vermeulen E, Maertens J, Schoemans H, Lagrou K. Azole-resistant *Aspergillus fumigatus* due to TR46/Y121F/T289A mutation emerging in Belgium, July 2012. *Euro Surveill.* 2012;17(48):pii=20326. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20326>

Date of submission: 16 November 2012

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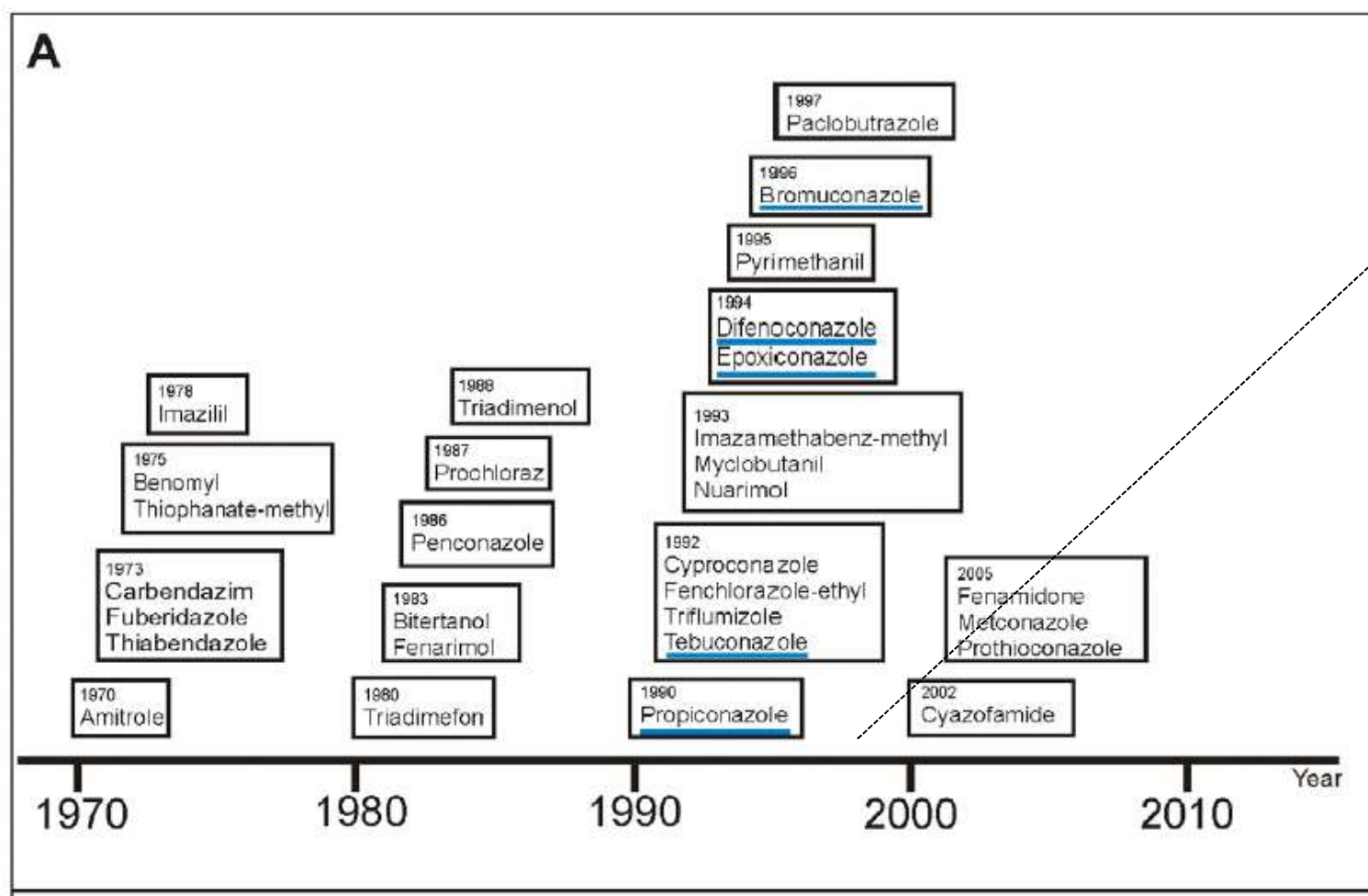
**A new azole resistance mechanism in *Aspergillus fumigatus* consisting of a TR46/Y121F/T289A alteration in the *cyp51A* gene was recently described in the Netherlands. Strains containing these mutations are associated with invasive infection and therapy failure. This communication describes the first case of fatal invasive aspergillosis caused by TR46/Y121F/T289A outside the Netherlands, in the neighboring country of Belgium, suggesting geographical spread. TR46/Y121F/T289A leads to a recognisable phenotypic susceptibility pattern which should trigger *cyp51A* genotyping to monitor further spread.**

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# Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in *Aspergillus fumigatus*

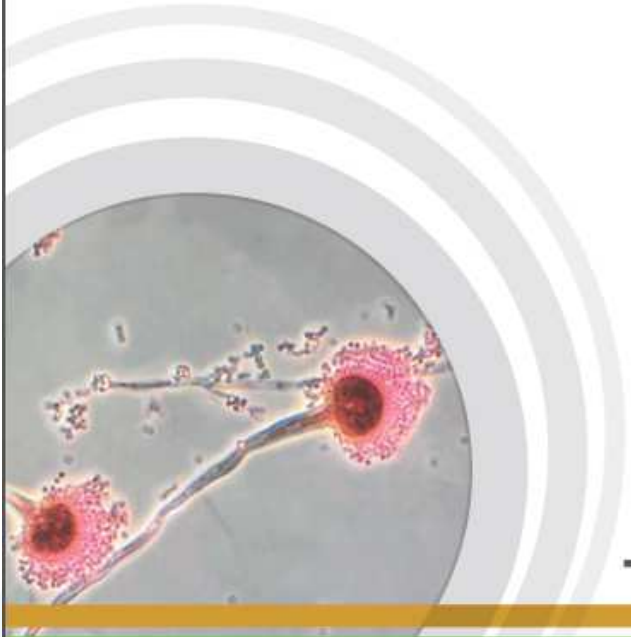
Eveline Snelders<sup>1,3,9</sup>, Simone M. T. Camps<sup>1,3,9</sup>, Anna Karawajczyk<sup>2,†</sup>, Gijs Schaftenaar<sup>2</sup>, Gert H. J. Kema<sup>5</sup>, Henrich A. van der Lee<sup>1,3</sup>, Corné H. Klaassen<sup>4</sup>, Willem J. G. Melchers<sup>1,3</sup>, Paul E. Verweij<sup>1,3\*</sup>

Most identical docking with medical triazoles



1998: first TR<sub>34</sub>/L98H isolate cultured

Evolution analysis of microsatellite genotypes: TR<sub>34</sub>/L98H developed from a single ancestor that emerged around 1997



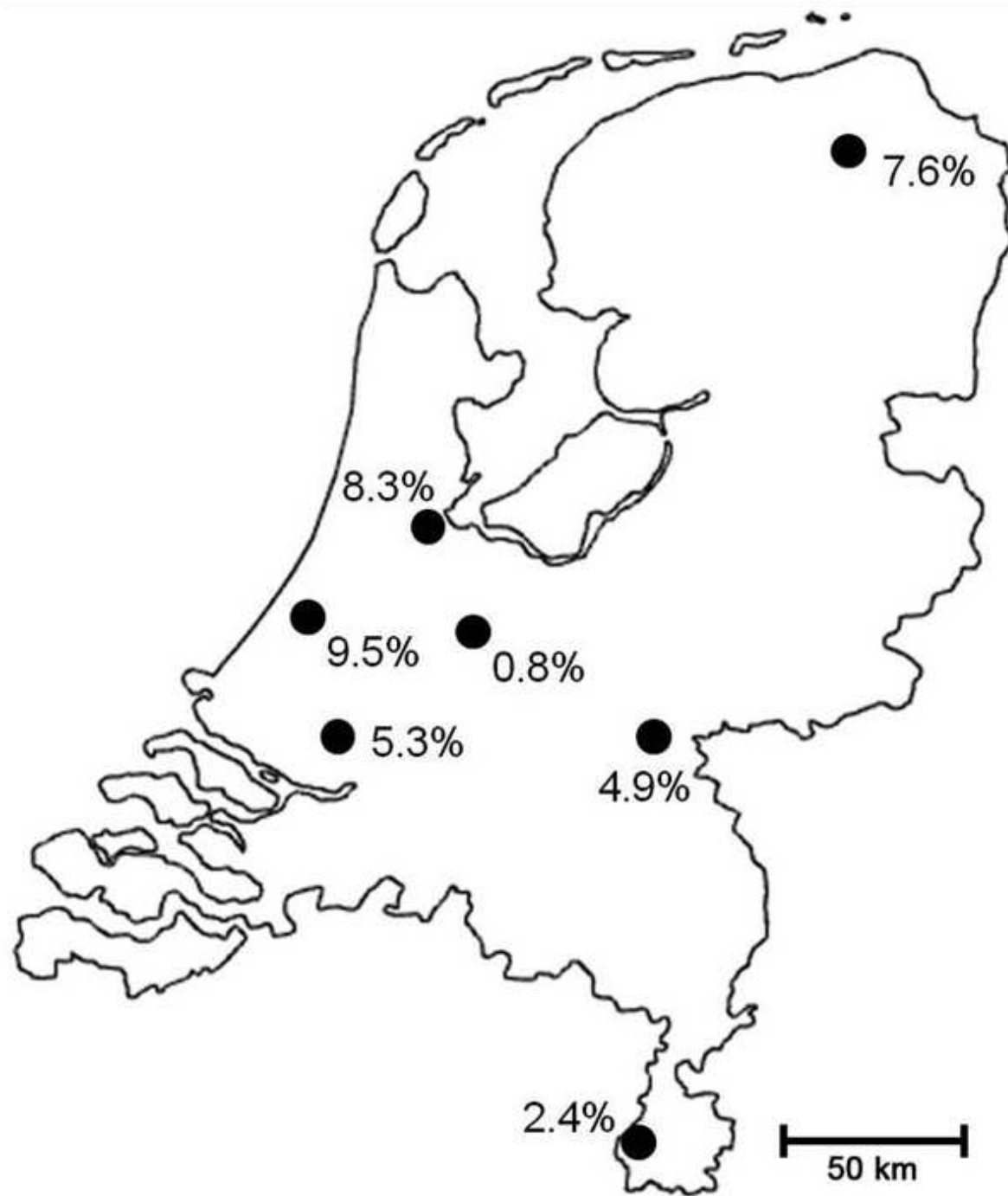
## TECHNICAL REPORT

# Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in *Aspergillus* species

Stockholm, February 2013

[www.ecdc.europa.eu](http://www.ecdc.europa.eu)





Prospective nationwide multicenter study

2062 isolates from 1385 patients

Prevalence itraconazole resistance in *A. fumigatus* = 5,3 %

Patients with hematologic or oncologic disease more likely to harbor azole resistant isolate

64% azole naive

**Case fatality rate 88%**

**Figure 2.** Prevalence (%) of azole-resistant *Aspergillus fumigatus* infections in university medical centers, the Netherlands, 2007–2009.



# UZ Leuven study August 2006 - May 2007

240 *A. fumigatus* isolates (one isolate per patient) cultured from clinical samples

No. isolate	Sex (age)	Underlying disease	Infection/Colonisation	Azole exposure prior to isolation	MIC ( $\mu\text{g/mL}$ )			<i>cyp51A</i> alterations	
					ITR	VOR	POS	TR promotor	AA substitution
ASFU 24	F (24)	Cystic fibrosis (ABPA)	Colonisation	ITR (since 2003)	4	4	0.5	-	-
ASFU 177	M (41)	Cystic fibrosis	Colonisation	no	$\geq 32$	8	1	+	L98H
ASFU 198	F (39)	LTx	Colonisation	VOR	1	4	0.5	-	D225E, M248T, M172V, E427K

Prevalence of reduced triazole susceptibility was 1.25% (3/240)  
Resistant isolates showed variable phenotypic and genetic profiles

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

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



# CONCLUSIONS



- Azole resistance in *A. fumigatus* is a growing public health concern with global dimensions.
- Prevalence of azole resistance in *A. fumigatus* recovered from Belgian patients is still low (< 10%).
- Susceptibility testing of clinically relevant *A. fumigatus* isolates is indicated but no isolate is available in up to 50% of patients with invasive aspergillosis.
- Sensitivity of PCR for detection of azole resistance directly in samples inadequate. Increasing diversity of CYP51A-mediated resistance mechanisms.
- Epidemiological characteristics continue to evolve: surveillance studies of both clinical and environmental isolates are important!