Μετεκπαιδευτικό Σεμινάριο Λοιμώξεων

«Ενιαία υγεία και λοιμώξεις στη Λεκάνη της Μεσογείου / Οστικό Έλλειμμα και Λοίμωξη»

Aspergillus resistant to azoles: what do we know about the Mediterranean area?

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Aspergillus lung infections

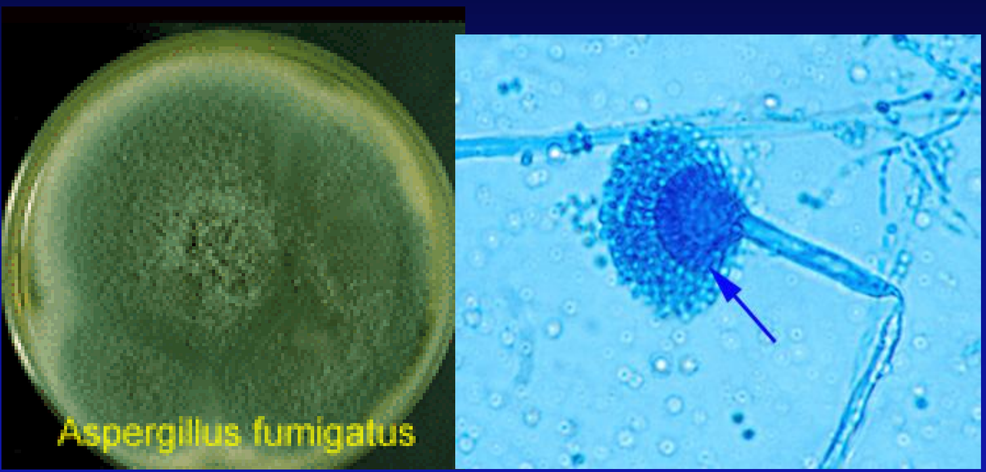
- **Allergic aspergillosis**
  - allergic bronchopulmonary aspergillosis (ABPA)
  - severe asthma with fungal sensitization
- **Aspergillus bronchitis**
  - cystic fibrosis or bronchiectasis, lung transplant recipients, and those receiving mechanical ventilation
- **Chronic pulmonary aspergillosis (CPA)**
  - immunocompetent patients with underlying lung disease
  - cavitary CPA, fibrosing CPA, nodule and single aspergilloma
- **Invasive aspergillosis (IA)**
  - hematopoietic stem cell (HSCT) or solid organ transplant recipients
  - Under chemotherapy or corticosteroids

Epidemiology

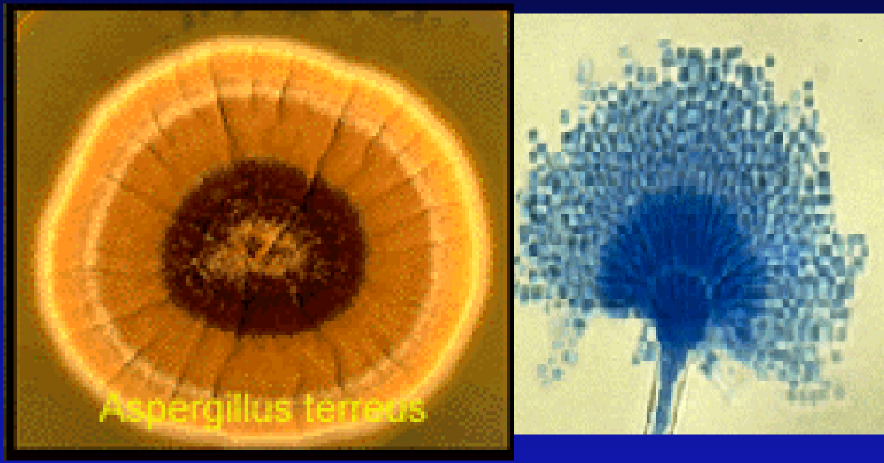
- >10 million cases with allergic manifestations annually
- >1.2 million have chronic pulmonary aspergillosis
- >200.000 cases of invasive aspergillosis (IA) annually
- Triazoles have been the mainstay of therapy
- Mortality rates associated with IA remain high 30-50%
- *Aspergillus fumigatus* remains the most common species in all pulmonary syndromes
- *Aspergillus flavus* is a more common cause of allergic rhinosinusitis, postoperative aspergillosis, and fungal keratitis.

Common *Aspergillus* species

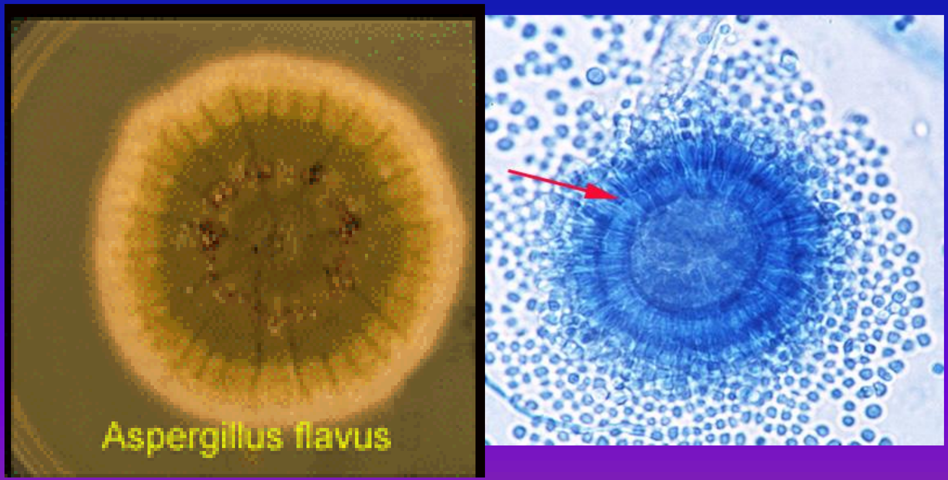
70-85%



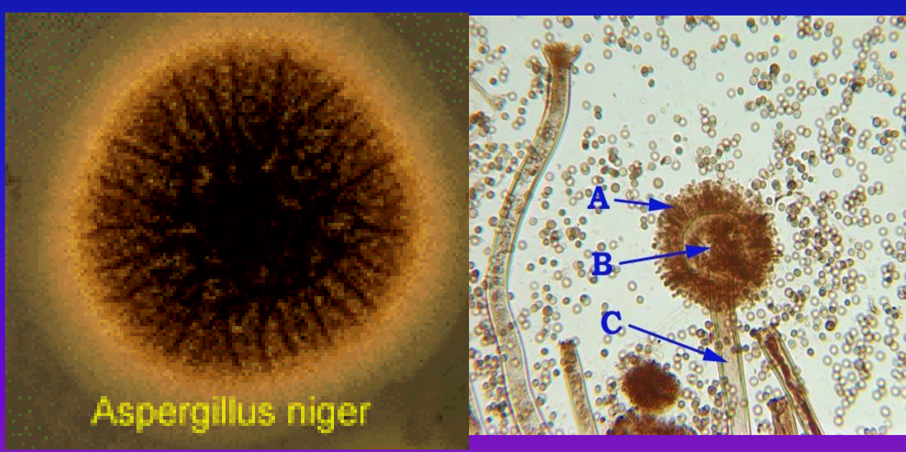
2-10%



5-10%

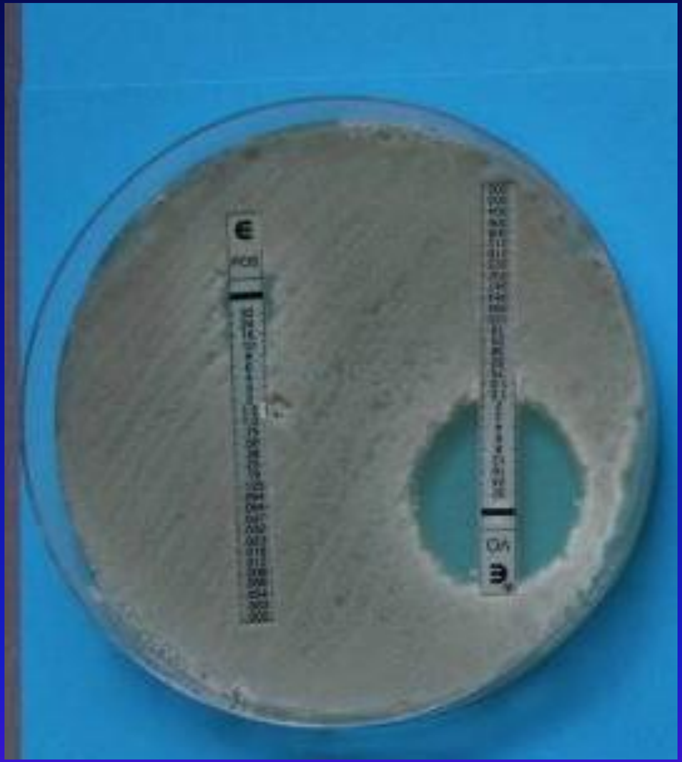


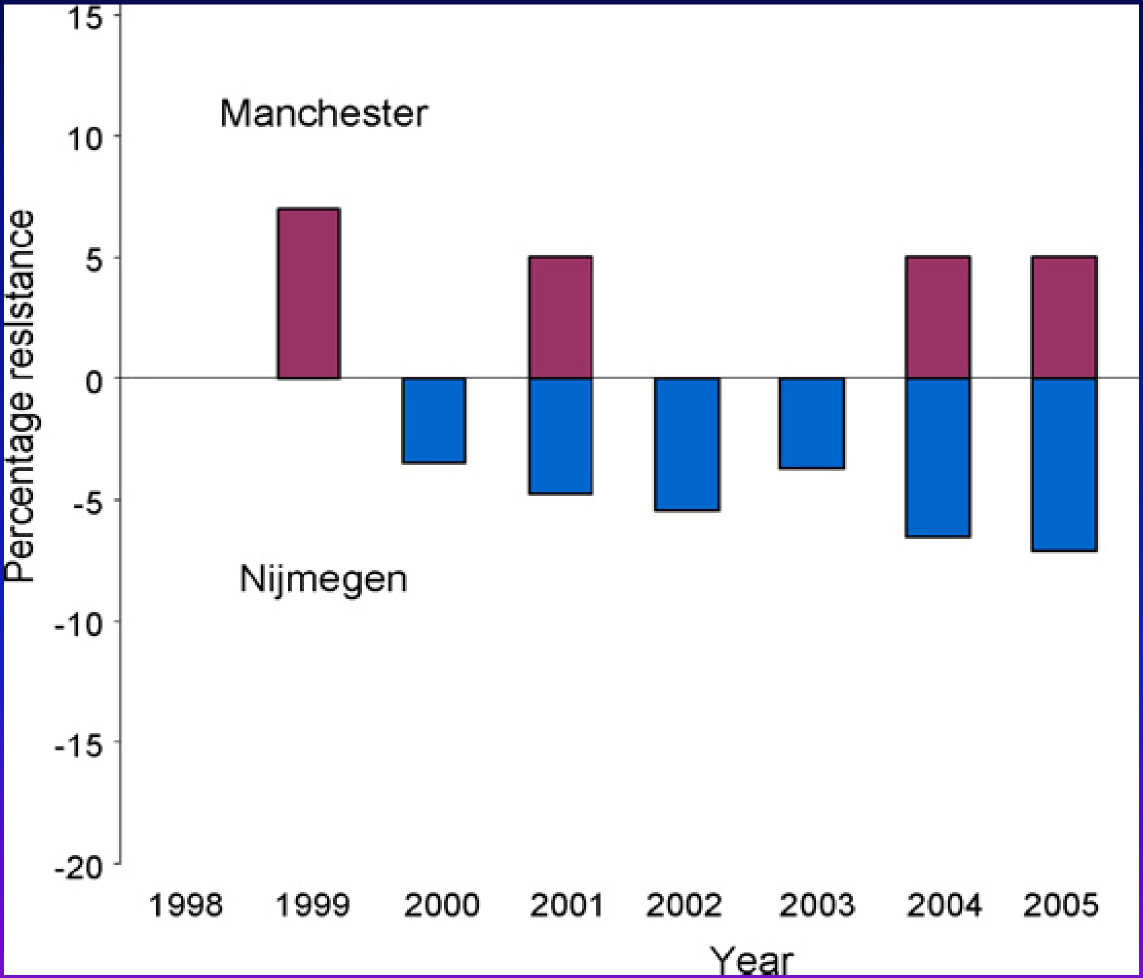
2-3%



10-15% cryptic species

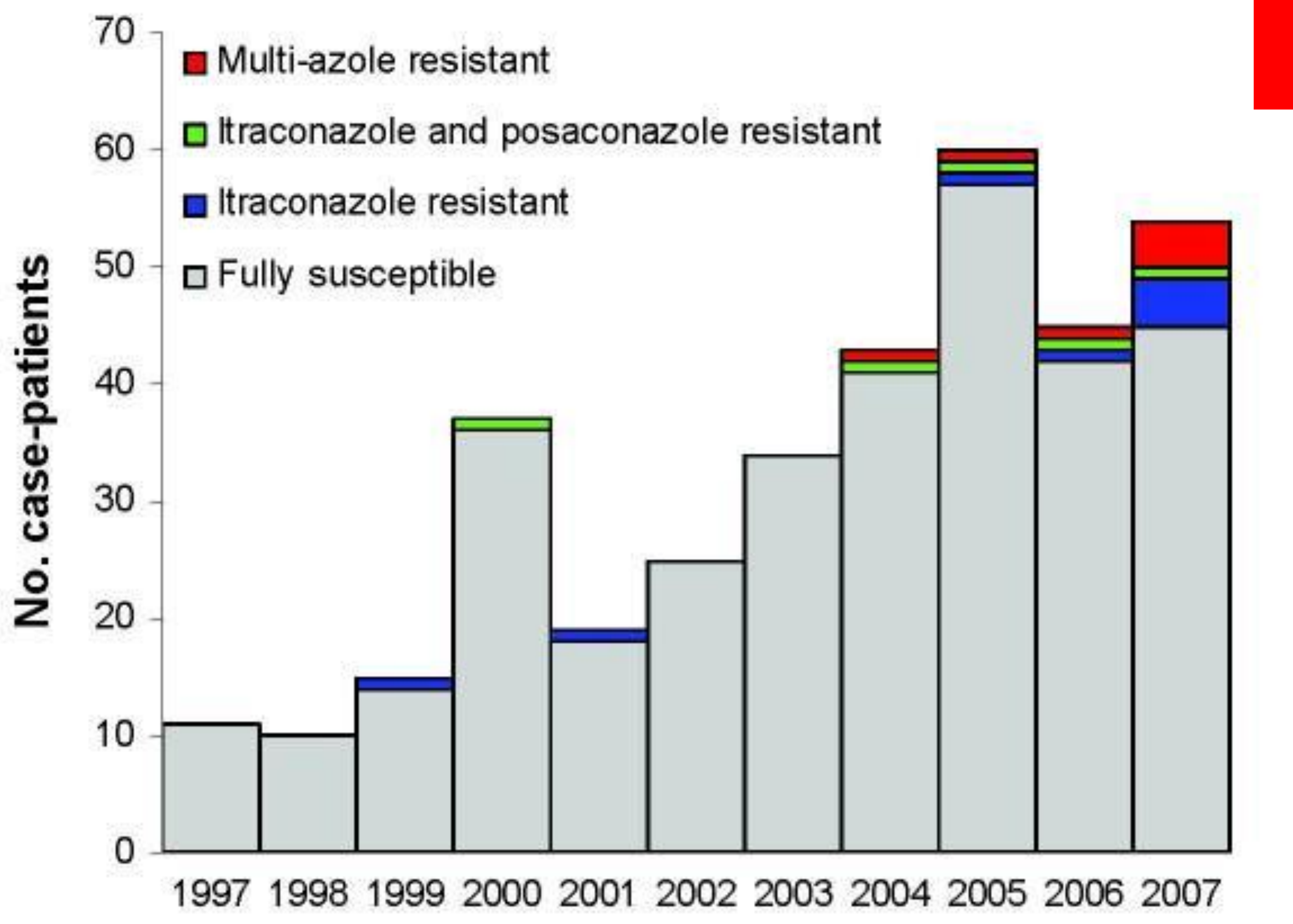
Epidemiological change in A. *fumigatus*



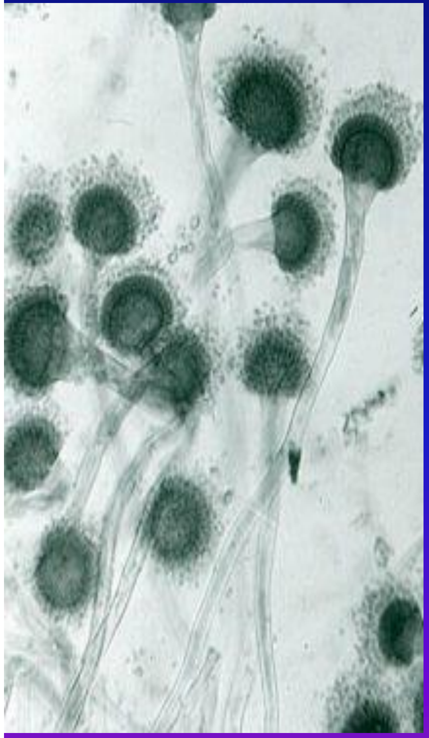


Verweij Drug Resistance Updates 2009

Azole-resistance phenotypes of A. *fumigatus*



88-100% mortality rates



Howard et al EID 2009

CYP51A mutations

Nijmegen cases

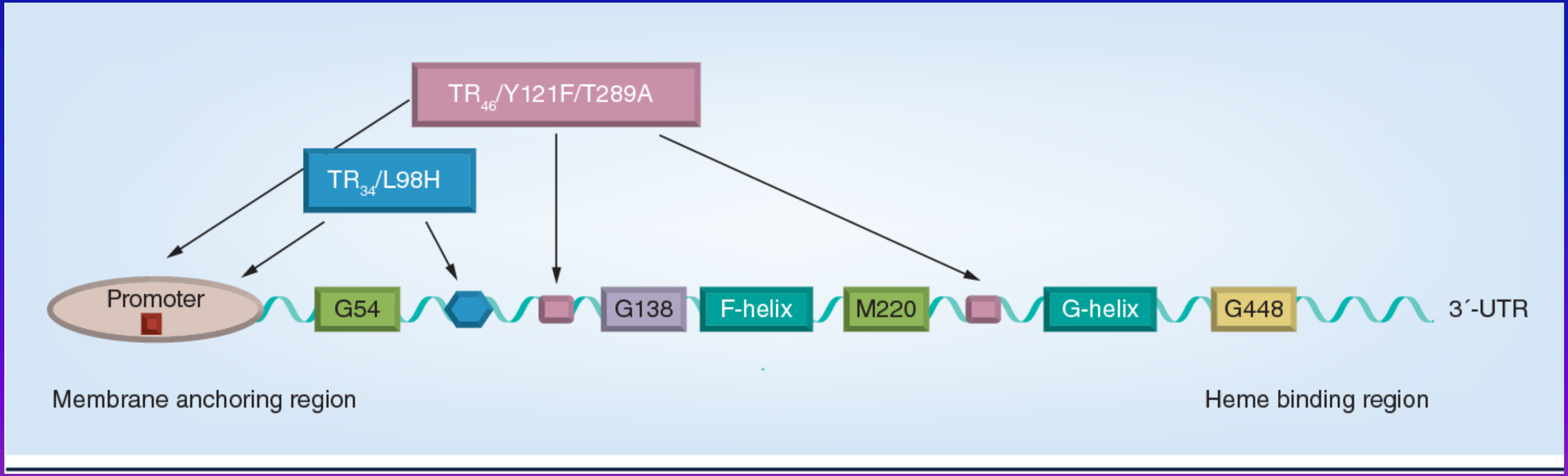
Tandem repeats in promoter region

- TR34/L98H (70-90%)→gene overexpression
- TR46/Y121F/T289A (10-30%)→high VRC R
- TR53 → ITC and VRC resistance
- Environmental isolates (>95%)
- Soil samples with azole fungicides
- Intercountry transfer
- Clinical strains from azole naïve patients

Manchester cases

Point Mutations

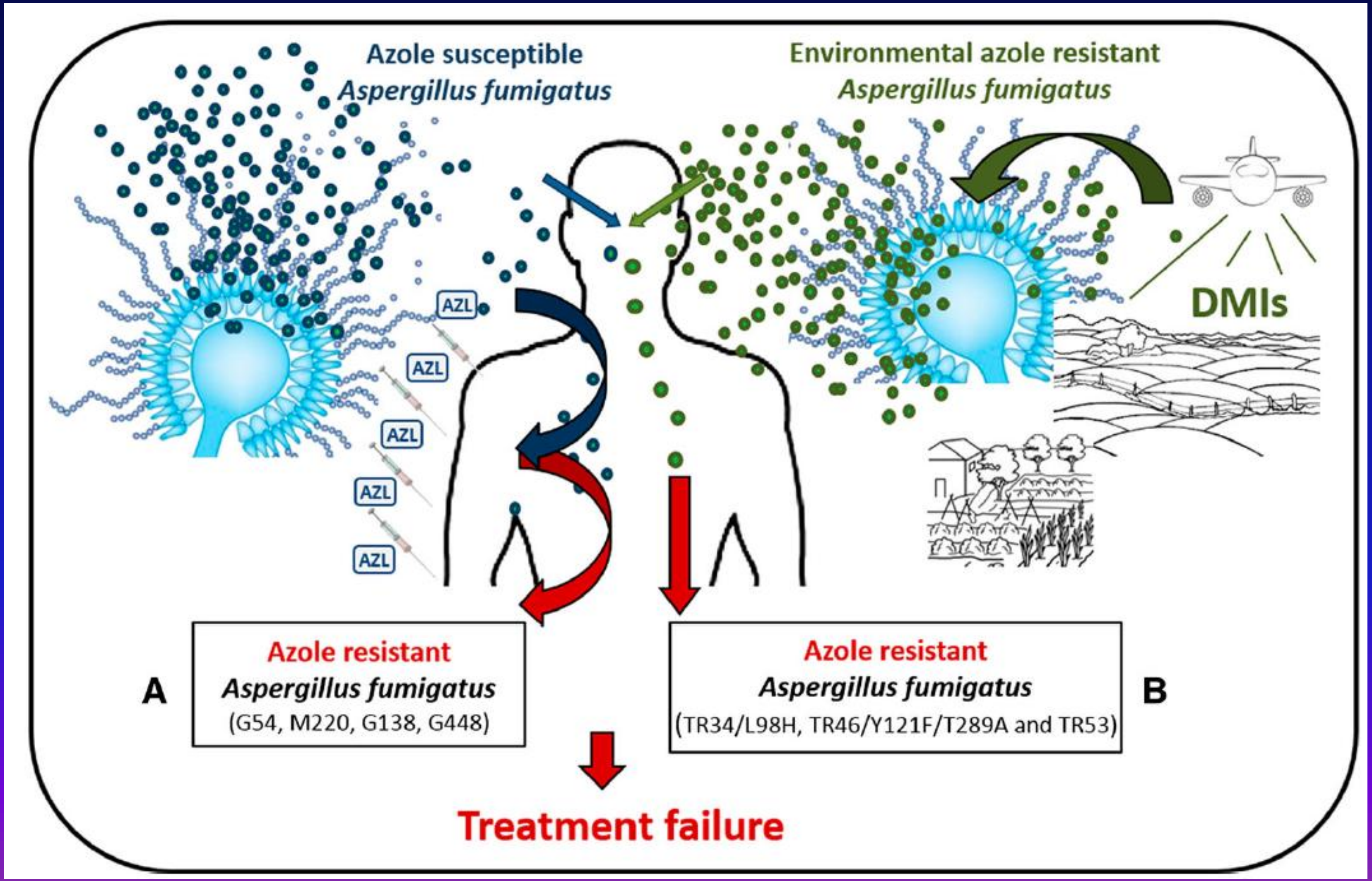
- G54 E/R/W → ITC & POS R
- G138 C/S → ITC & VOR R
- G448 S → VRC R
- M220 I/V/T/K/R → different R profiles
- Other mutations (P216L,F219C etc)
- Clinical isolates from CPA patients after 4mo (3w-23mo) azole therapy
- Some environmental isolates with G54



Verweij, Drug Resist Updat 2009

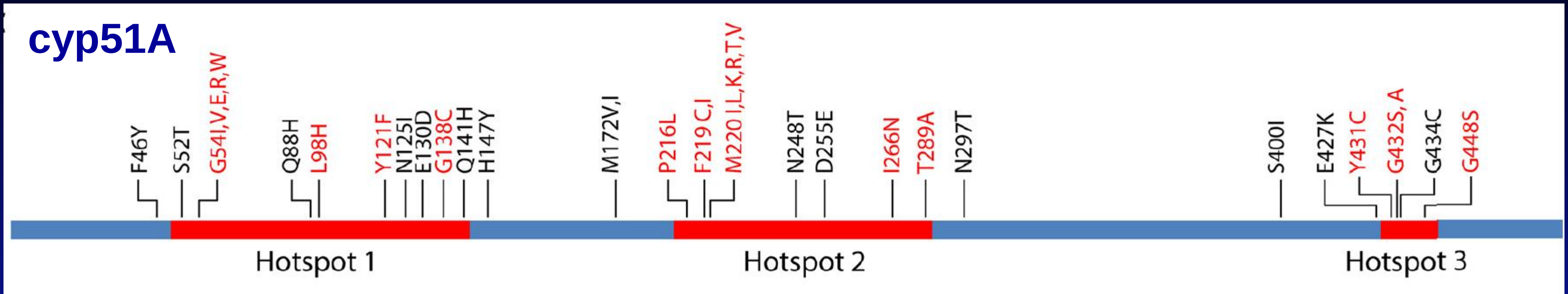
Development of Azole Resistance

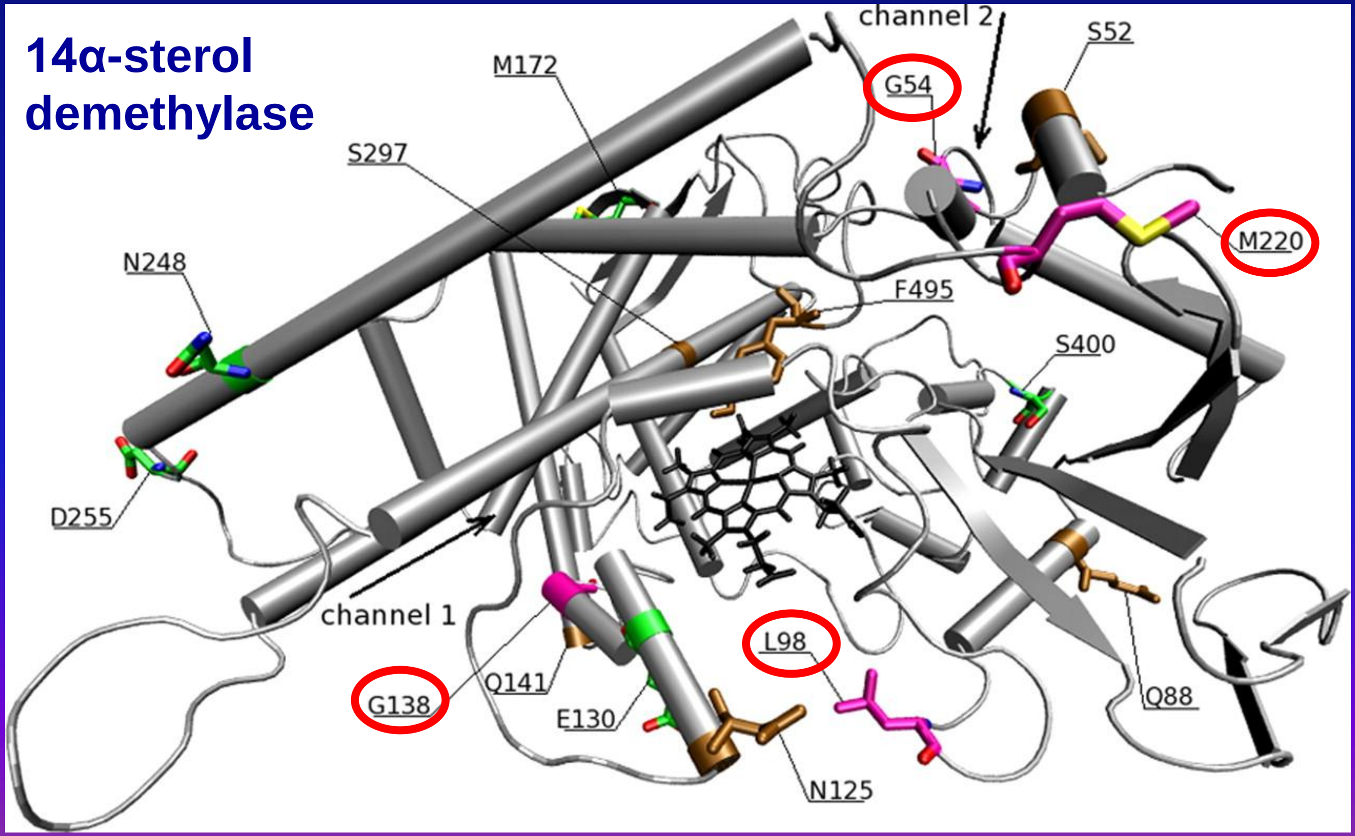
Patient route | **Environmental route**



Garcia-Rubio Drugs 2017

Mapping of Cyp51A mutations





G54W
→ ITC&POS R
G54E
→ ITC R

M220R/I/V
→ITC R
M220K
→ ITC & POS R

G138C→pan-azole resistance

Snelders AAC 2010

Non-CYP51 mutations

- Up to 50% without mutations in cyp51A or promoter duplications
- CYP51B overexpression
- Activation of efflux pumps
  - ABC and MFS transporters
- CCAAT-binding transcription factor complex
  - Mutation P88L in HapE subunit
- Oxidative stress tolerance
  - mutations in genes Yap1 and AldA
- loss of algA (calcium-dependent protein encoding gene)
- R243Q mutation in farnesyltransferase (AfCox10) gene
- Cholesterol import to compensate for ergosterol depletion
  - SrbA (a transcriptional regulator of the sterol regulatory element binding protein)

Cyp51 substitutions in non-*A. fumigatus*

TABLE 3 Cyp51 substitutions in non-*A. fumigatus* organisms and their correspondence to the reference positions used

	Reference position[a]			Observed substitution	
Organism	Gene	Position[b]	Reference	Gene	Substitution
A. flavus	*Afcyp51A*	Y68	29	*cyp51A*	Y132N
	Afcyp51A	K133	29	*cyp51A*	K197N
	Afcyp51A	NA		*cyp51A*	A205T
	Afcyp51A	D280	29	*cyp51A*	D282E
	Afcyp51A	M286	29	*cyp51A*	M288L
	Afcyp51A	T470	29	*cyp51A*	T469S
	Ztcyp51B	H430	29	*cyp51B*	H399P
	Ztcyp51B	A453	29	*cyp51B*	D411N
	Ztcyp51B	T496	29	*cyp51B*	T454P
	Ztcyp51B	NA		*cyp51B*	T486P
	Afcyp51C	M54	Proposed here	*cyp51C*	M54T
	Afcyp51C	S196	Proposed here	*cyp51C*	S196F
	Afcyp51C	S240	Proposed here	*cyp51C*	S240A
	Afcyp51C	D254	Proposed here	*cyp51C*	D254N
	Afcyp51C	I285	Proposed here	*cyp51C*	I285V
	Afcyp51C	Y319	Proposed here	*cyp51C*	Y319H
	Afcyp51C	A324	Proposed here	*cyp51C*	A324P
	Afcyp51C	N423	Proposed here	*cyp51C*	N423D
	Afcyp51C	V465	Proposed here	*cyp51C*	V465 M
A. terreus	*Afcyp51A*	M220	29	*cyp51A*	M217I
A. niger	*Afcyp51A*	K230	29	*cyp51A*	R228Q
A. tubingensis	*Afcyp51A*	L21	29	*cyp51A*	L21F

Dudakova CMR 2017

Global distribution

SCARE multicenter prospective study

3,788 *Aspergillus* isolates were screened from 2009-2011 from 21 centers

60 azole-resistant *A. fumigatus* SC isolates in 11/19 centers (58%)

Prevalence of resistance = 3.2% (0-26%)

47 *A. fumigatus* sensu stricto isolates

85% CYP51A mutations

- 55% TR_{34}/L98H, TR_{46}/Y121G/T289A
- 30% single point mutations (M220, G54)

15% non-CYP51A

13 *A. fumigatus* sibling species

7 A. lentulus
4 N. pseudofisheri
2 N. udagawae

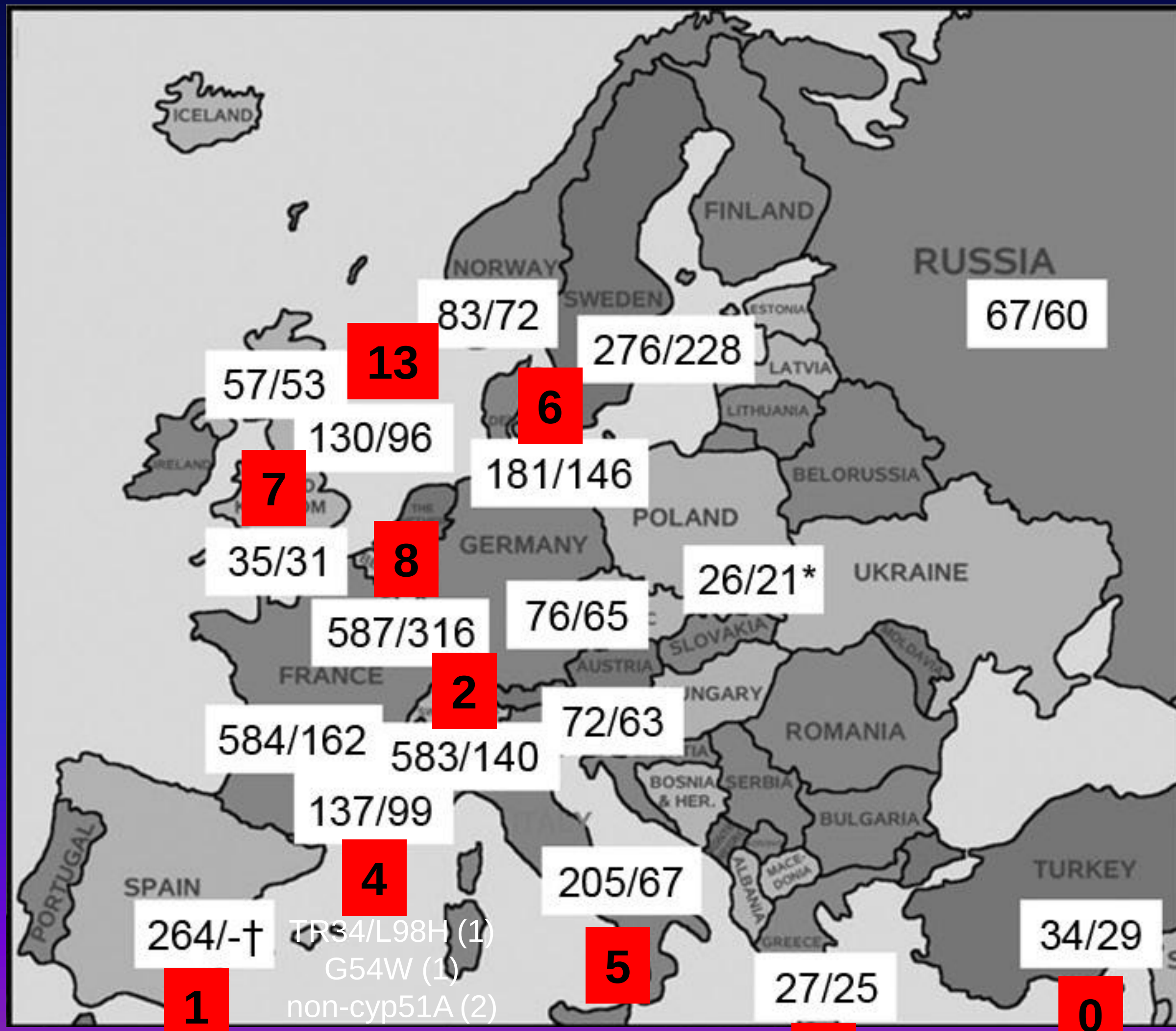
A. fumigatus:	
ITZ-resistant (>2 mg/L)	100%
VCZ-resistant (>2 mg/L)	60%
PCZ-resistant (>0.5 mg/L)	58%

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

van der Linden, Emerging Infectious Diseases 2015

Azole-resistant *A. fumigatus in* Europe

(number of isolates/patients screened)



F46Y/M172G (1)

TR34/L98H (5)

0

Linden, Emerging Infectious Diseases 2015

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Italy

Azole Resistance in *Aspergillus fumigatus* Clinical Isolates from an Italian Culture Collection

Cristina Lazzarini,[a] Maria Carmela Esposto,[a] Anna Prigitano,[a] Massimo Cogliati,[a] Gabriella De Lorenzis,[b] Anna Maria Tortorano[a]

Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy[a]; Department of Agricultural and Environmental Sciences-Production, Landscape, Agroenergy, Università degli Studi di Milano, Milan, Italy[b]

TABLE 1 Itraconazole resistance according to year of isolation

Year	No. of tested isolates	No. of patients	No. (%) of itraconazole-resistant isolates[a]	No. (%) of patients harboring resistant isolates
1995	33	24	0	0
1996	21	15	0	0
1997	31	23	0	0
1998	41	26	5 (12.2)	5 (19.2)
1999	37	32	4 (10.8)	2 (6.3)
2000	49	48	3 (6.1)	3 (6.3)
2001	57	57	4 (7.0)	4 (7.0)
2002	60	51	4 (6.7)	3 (5.9)
2003	81	58	8 (9.9)	4 (6.9)
2004	62	54	1 (1.6)	1 (1.9)
2005	37	36	0	0
2006	24	17	2 (8.3)	2 (11.8)

[a] MIC of >2 mg/liter.

70% TR34/L98H

30% G54E

Turkey

J Infect Chemother. 2015 Aug;21(8):581-6. doi: 10.1016/j.jiac.2015.04.012. Epub 2015 May 14.

First determination of azole resistance in Aspergillus fumigatus strains carrying the TR34/L98H mutations in Turkey.

Özmerdiven GE[1], Ak S[2], Ener B[3], Ağca H[1], Cilo BD[1], Tunca B[2], Akalın H[4].

Author information

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2 Uludağ University, Faculty of Medicine, Department of Medical Biology, Görükle, 16059 Bursa, Turkey.
3 Uludağ University, Faculty of Medicine, Department of Medical Microbiology, Görükle, 16059 Bursa, Turkey. Electronic address: bener@uludag.edu.tr.
4 Uludağ University, Faculty of Medicine, Department of Clinical Microbiology and Infectious Diseases, Görükle, 16059 Bursa, Turkey.

- Culture collection of A. fumigatus isolates collected between 1999 and 2012 from clinical specimens.
- 746 *A. fumigatus* isolates from 419 patients.
- 10.2% Itraconazole resistance
- From 2000 onwards, patients were observed annually with an itraconazole-resistant isolate.
- Presence of TR34/L98H in 86.8% (n = 66) of isolates

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Spain

Molecular Identification and Susceptibility Testing of Molds Isolated in a Prospective Surveillance of Triazole Resistance in Spain (FILPOP2 Study)

Ana Alastruey-Izquierdo,[a,n] Laura Alcazar-Fuoli,[a,n] Olga Rivero-Menéndez,[a] Josefina Ayats,[b,n] Carmen Castro,[c] Julio García-Rodríguez,[d] Lidia Goterris-Bonet,[e] Elisa Ibáñez-Martínez,[f] María José Linares-Sicilia,[g] M. Teresa Martin-Gomez,[e] Estrella Martín-Mazuelos,[c] Teresa Pelaez,[h] Javier Peman,[f] Antonio Rezusta,[i] Susana Rojo,[j] Rocio Tejero,[g] Diego Vicente Anza,[k,l,m] Jesús Viñuelas,[i] Maria Soledad Zapico,[k] Manuel Cuenca-Estrella,[a] on behalf of the FILPOP2 Project from GEMICOMED (SEIMC) and REIPI

Group	Total no. of isolates	No. of isolates resistant to:			
		AMB at >2 mg/liter	ITZ at >2 mg/liter	VCZ at >2 mg/liter	PCZ at >0.25 mg/liter
A. fumigatus	260	2	3	2	3
A. lentulus	6	3		4	
A. fumigatiaffinis	2	2			
Aspergillus felis	1			1	
A. niger	26		4		2
A. tubingensis	16		1		1
A. flavus	25	1			
A. alliaceus	10	10			
Aspergillus tamarii	1				
A. terreus	23	2	1	1	3
A. citrinoterreus	2				
A. nidulans	6				
A. quadrilineatus	5	1	1	1	1
Aspergillus delacroxii	1	1	1		
Aspergillus spinulosporus	1				
A. calidoustus	5	1	5	5	4
A. puniceus	1	1	1	1	1
A. sydowii	4				
Aspergillus chevalieri	1				
Total	396	24	17	15	15

frontiers in Microbiology

Portugal

Aspergillus Species and Antifungals Susceptibility in Clinical Setting in the North of Portugal: Cryptic Species and Emerging Azoles Resistance in *A. fumigatus*

Eugénia Pinto[1,2], Carolina Monteiro[1], Marta Maia[1], Miguel A. Faria[3], Virgínia Lopes[4], Catarina Lameiras[5] and Dolores Pinheiro[6]*

- 227 clinical isolates, mainly from the respiratory tract (92.1%)
- 86.7% *Aspergillus fumigatus sensu stricto*, 7.5% cryptic species
- 5 *A. fumigatus sensu stricto* pan-azole resistance

  -1 TR46/Y121F/T289A, 2 TR34/L98H mutation
- Amongst cryptic species, 47%, 82% and 100% were resistant to voriconazole, posaconazole and isavuconazole, respectively.

France

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Medical Mycolog
doi
Advance Access Publication
Original Article

Original Article

Prospective evaluation of azole resistance in *Aspergillus fumigatus* clinical isolates in France

F. Choukri[1,2], F. Botterel[1,2], E. Sitterlé[1,2], L. Bassinet[3], F. Foulet[1,2], J. Guillot[2,4], J. M. Costa[2,5], N. Fauchet[6] and E. Dannaoui[2,7,*]

165 *A. fumigatus* isolates were recovered from 134 patients.

3 (1.8%) isolates recovered from three patients were found resistant

All had the TR_{34}/L98H mutation,

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2011, p. 3025–3030 Vol. 55, No. 6
0066-4804/11/$12.00 doi:10.1128/AAC.01491-10

Polyphasic Identification and Susceptibility to Seven Antifungals of 102 *Aspergillus* Isolates Recovered from Immunocompromised Hosts in Greece▽

Michael Arabatzis,[1]* Manousos Kambouris,[2] Miltiades Kyprianou,[1] Aikat
Maria Foustoukou,[4] Maria Kanellopoulou,[5] Lydia Kondyli,[6] Georgia
Chrysa Koutsia-Karouzou,[8] Evangelia Lebessi,[4] Anastasia Pan
Efthimia Petinaki,[9] Ageliki Stathi,[3] Eleftheria Trikka-Graphakos,
Erriketi Vartzioti,[6] Aliki Vogiatzi,[11] Timoleon-Achilleas Vyzantiadis,[12]
Loukia Zerva,[13] and Aristea Velegraki[1]

Greece

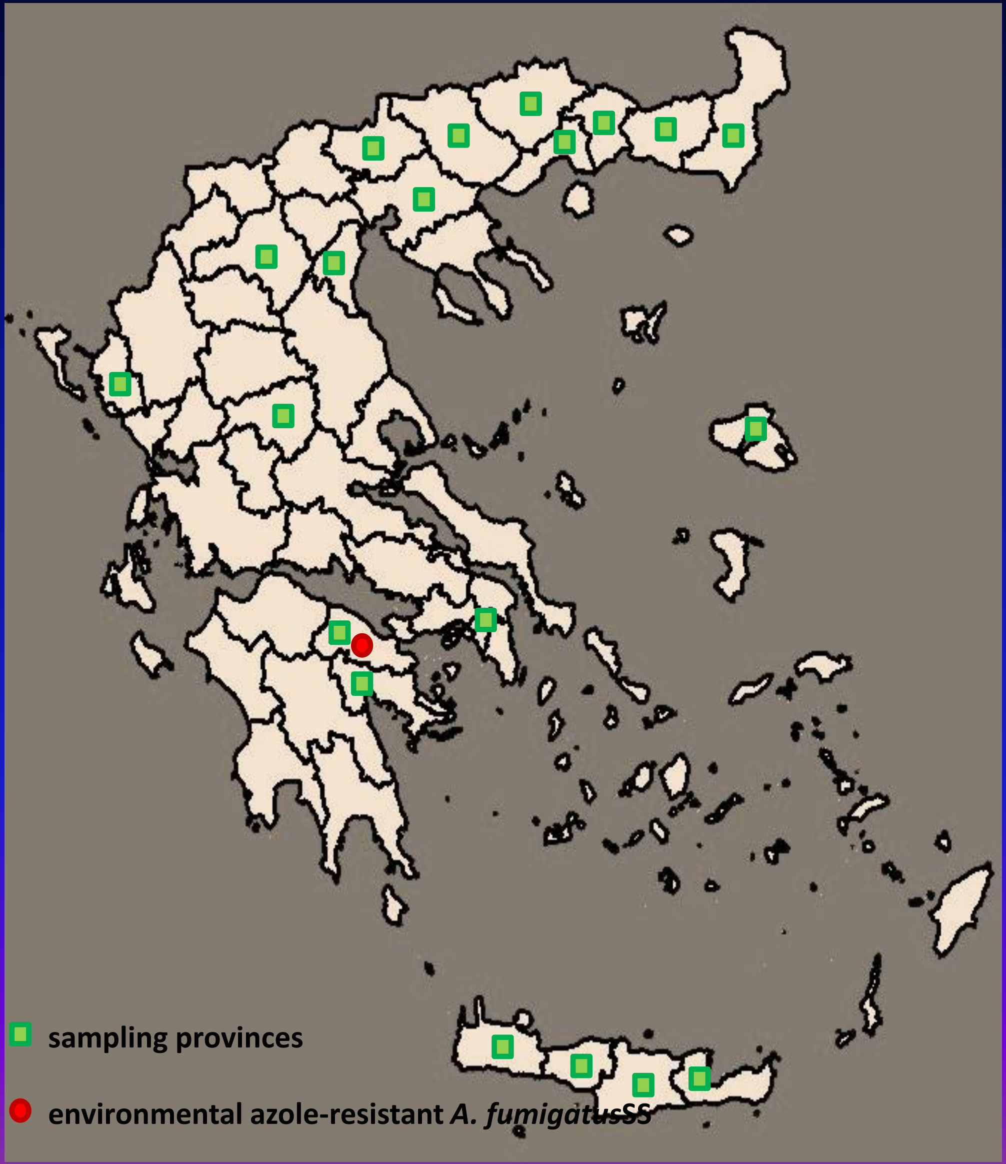
Aspergillus section (no. of isolates)	*Aspergillus* or *Emericella* species (no. of isolates)[b]	Antifungal agent[c]	MIC or MEC[d] (µg/ml) range	GM MIC or MEC[d] (µg/ml) (95% CI)[e]
Fumigati (42)	*A. fumigatiaffinis* (3)	AMB	1–4	0.04
		ITZ	0.5	0.5
		POS	0.064–0.5	0.2
		VOR	0.25–1	0.5
		AND	0.5–1	0.63
		CAS	0.125–0.5	0.31
		MCF	0.5	
	A. fumigatus (37)	AMB	0.016–16	0.28 (0.19–0.41)
		ITZ	0.032–32	0.52 (0.35–0.77)
		POS	0.032–32	0.34
		VOR	0.064–1	0.28
		AND	0.032–2	0.16
		CAS	0.032–32	0.74
		MCF	0.032–1	0.26 (0.20–0.35)
	N. hiratsukae (2)	AMB	1	1
		ITZ	0.125–2	0.5
		POS	0.25	0.25
		VOR	0.032–1	0.18
		AND	0.125–0.25	0.18
		CAS	0.5	0.5
		MCF	2	2

13% (5/37)

Arabatzis et al AAC 2011

First detection of environmental azole resistant *A. fumigatus* in Greece

Greece



710 soil samples (10/2016-10/2017)

494 (72%) *Aspergillus* spp.
- 95 (19%) *A. fumigatus*SC
- 120 (24%) *A. terreusSC*
- 453 (92%) *A. nigerSC*
- 32 (7%) *A. flavusSC*

Azole-resistant

1/95 (1%) *A. fumigatus sensu stricto*

organically grown raisin grapes lubricated with compost

ITRA>8 mg/l VOR>8 mg/l,
POSA=1 mg/l ISAV>8 mg/l

TR_{46}/Y121F/T289A resistance mechanism

Siopi et al, ECCMID19

Azole resistant *A. fumigatus* in cystic fribrosis

Turkey

- 31 isolates of *A. fumigatus*,14 different genotypes, 6 CF patients.
- 1 pan-resistant genotypes
- No mutations were detected in the Cyp51A gene

Gungur O, Mycopathologia 2018

Portugal

- 59 isolates of *A. fumigatus* collected from cystic fibrosis (CF) patients receiving azole antifungal therapy
- No resistant isolates

Amorim A, Int J Antimicrob Agents 2010

Italy

- 423 isolates (220 CF patients) in 2 centers
- 0% and 8.2% resistant isolates were found in the two centers
- 7 isolates with TR_{34}/L98H

Prigitano A, J Cyst Fibros. 2017

France

- 6.5% (231/355) isolates and 6.8% (6/88) patients with azole-resistance
- 50% TR34/L98H, 50% without cyp51A mutations

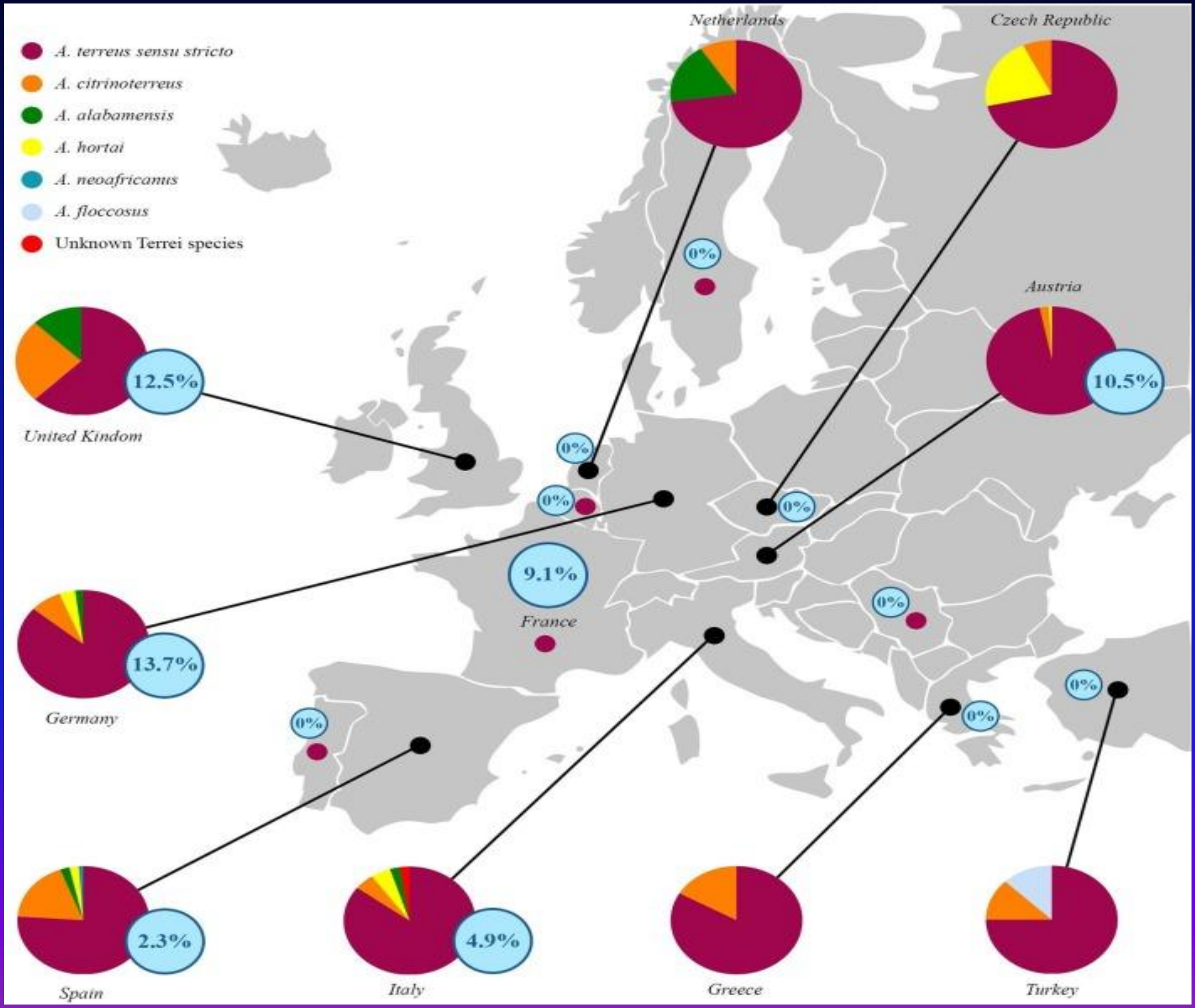
Lavergne RAJ Antimicrob Chemother. 2019

Azole-resistant *A. terreus in* Europe

~500 *A. terreus* SC
86% *A. terreus s.s.*

5.4% azole-R

Most common cyp51A mutation M217T/V



Zoran Front Microbiol. 2018

Take-home messages

- ✓ Azole resistance in *A. fumigatus* was detected in most European countries both in environmental and clinical isolates
  - - less common in other species and mainly in *A. flavus*.
  - - major problem in some centers in *A. fumigatus* cryptic species
- ✓ Overall prevalence of azole resistance was 3.2%
  - - higher prevalence (30%) observed in some centers in north Europe
- ✓ Azole resistance in Mediterranean area is low (<5%)
- ✓ TR34/L98H was the predominant mechanism of resistance (~50%) in patients with invasive aspergillosis
  - - most common in Mediterranean area
- ✓ Single point mutations at G54, G138 and M220 codons are the predominant mechanisms of resistance in centers with chronic aspergillosis patients
  - - 15-50% with no cyp51A mutation
- ✓ Azole resistance is associated with worsened outcome
  - - A rapid and convenient screening method for resistance
  - - Avoid azole monotherapy in centers with high (>10%) prevalence

Thank you

University General Hospital Attikon, Athens, Greece

Azole resistance prevalence in *A. fumigatus*

Continent/Country	% Resistance	Source of the Isolates	References
		Europe	
Belgium	5.7	C	[76]
France	0.85–10.6	C	[30,48,50]
Germany	1.1–12	C and E	[32,47,60]
Netherlands	2.1–20	C and E	[20,53,67,74]
Poland	2.25	C	[69]
Spain	1.8	C	[63]
Turkey	10.2	C	[71]
United Kingdom	6.6–28	C	[17,33]
		Other continents	
Asia *	1.9–11.1	C and E	[55,77,78,80–86,121]
Africa (Tanzania)	13.9	E	[90]
America (USA)	0.6–11.8	C	[58,122]
Oceania (Australia)	2.6	C	[59]
		International surveillance studies	
America-Asia-Australia-Europe	1.4–5.8	C and E	[52,70,123,124]

C = clinical strains, E = environmental strains; * including China, India, Iran, Japan, Kuwait and Pakistan.

Rivero-Menendez, JoF 2016

Distribution of azole resistance mechanisms in *A. fumigatus*