

A BATTLE WITH FUNGI

A battle round two: Case *Aspergillus*

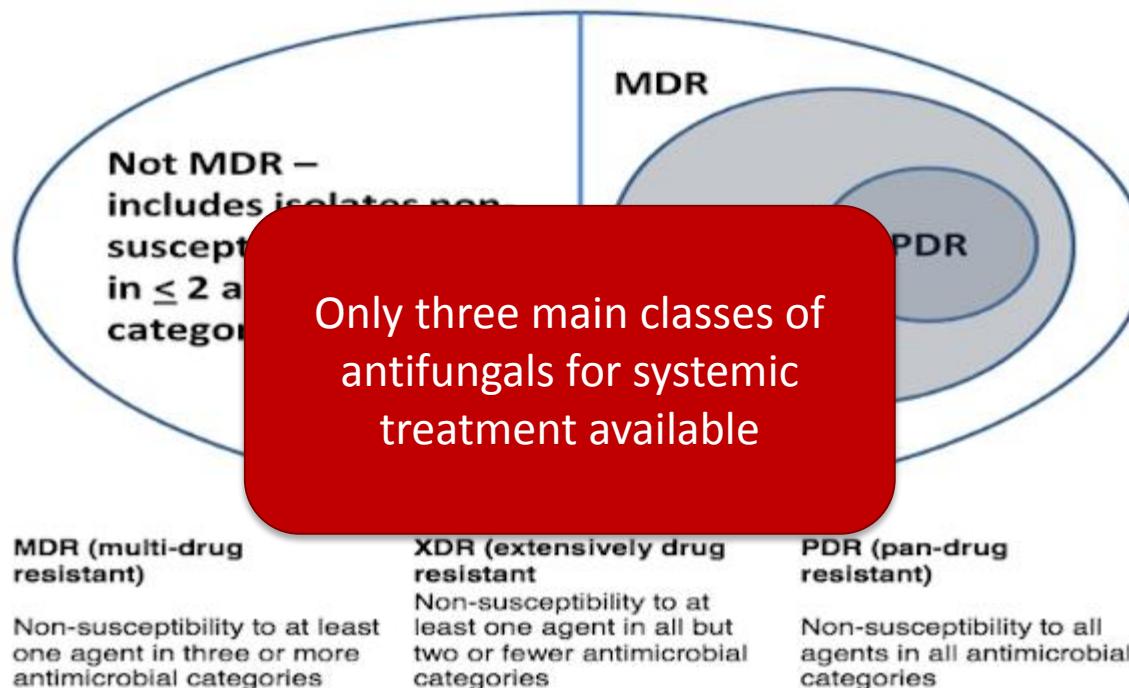
MDR *Aspergillus*



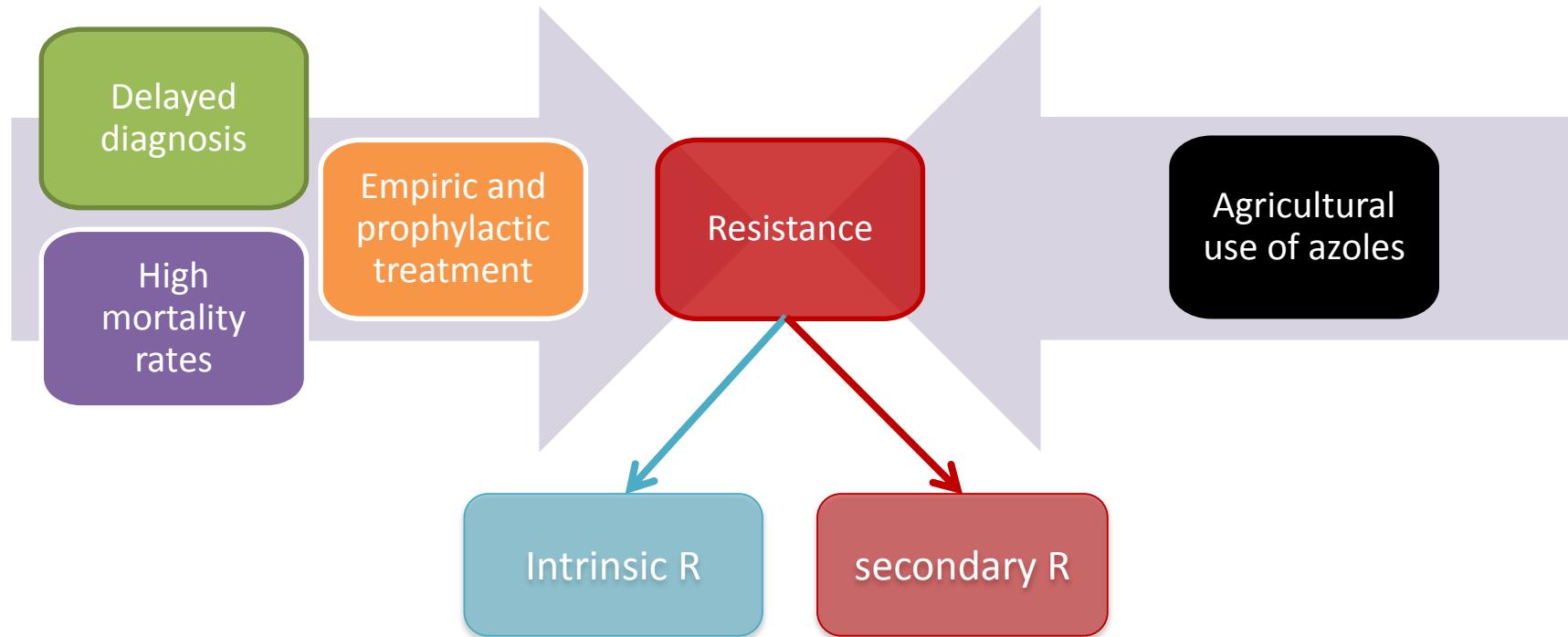
Ana Alastruey Izquierdo

Mycology Reference Lab Spain
Instituto de Salud Carlos III

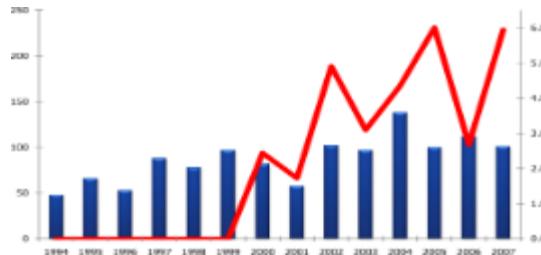
Multidrug resistance



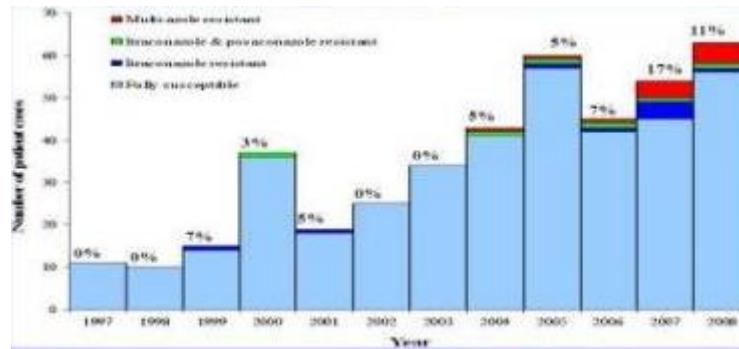
AF resistance



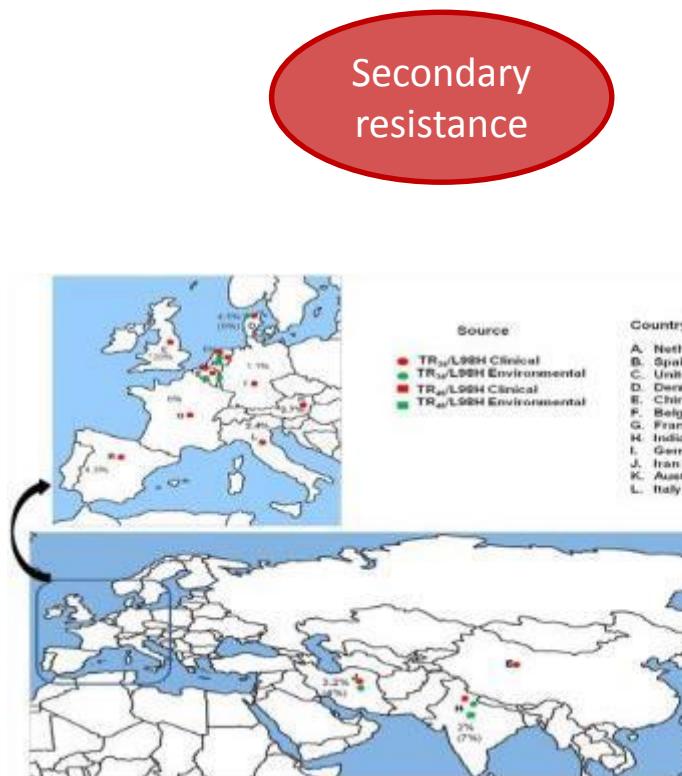
Secondary resistance



Snelders et al., PLOS 2008 Vol. 5:11



Howard et al., EID 2009, Vol. 15, No. 7



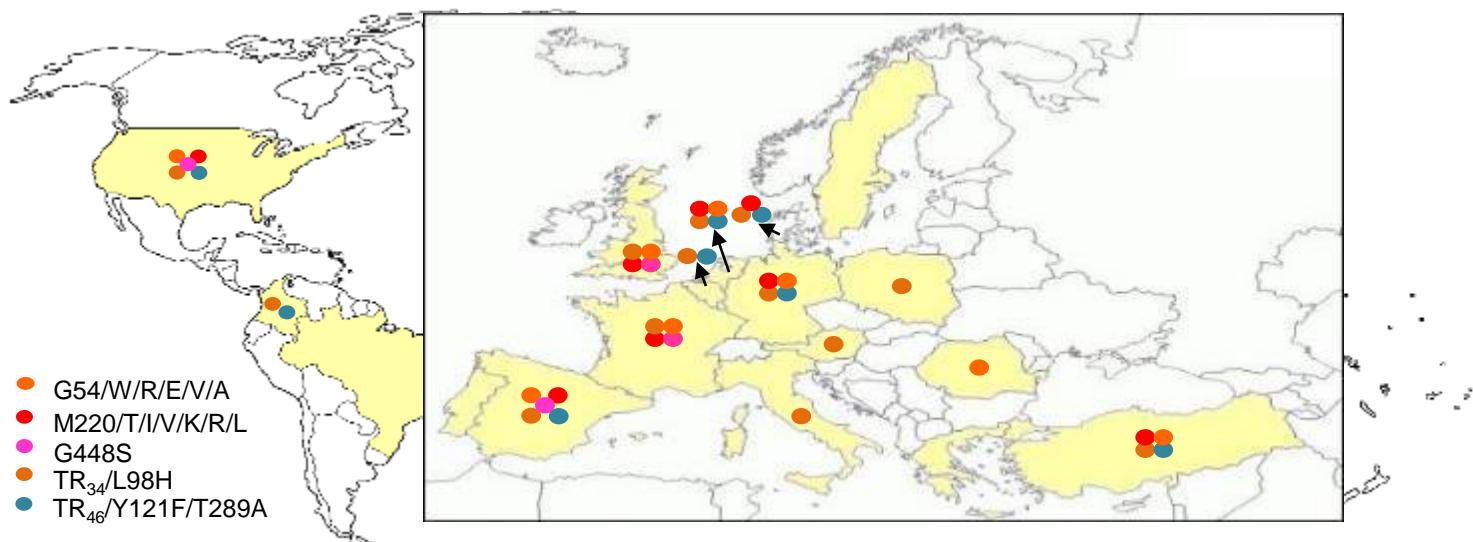
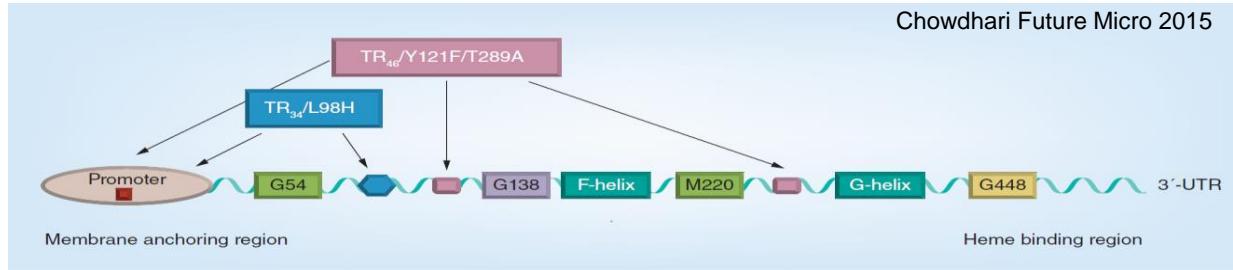
Chowdhary et al. PLoS Pathog. Oct 2013; 9(10)

Secondary resistance



Figure 1. Shaded areas show countries that have reported the TR₃₄/L98H and TR₄₅/Y121F/T289A resistance mechanism in clinical or environmental *Aspergillus fumigatus* isolates.

Secondary resistance



Secondary resistance

Table 2. Azole resistance prevalence in *A. fumigatus* by continent and/or country. Only significant publications with more than 50 isolates tested are reported.

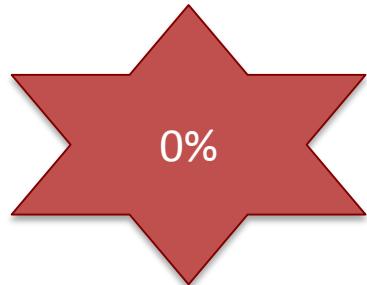
Continent/Country	% Resistance	Source of the Isolates	References
Europe			
Belgium	5.7	C	[76]
France	0.85–10.6	C	[30,48,50]
Germany	1.1–12	C and E	[32,47,60]
Netherlands	2.1–20	C and E	[20,53,67,74]
Poland	2.25	C	[69]
Spain	1.8	C	[63]
Turkey	10.2	C	[71]
United Kingdom	6.6–28	C	[17,33]
Other continents			
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.

FILPOP Study: Epidemiological survey Spain

FILPOP

- 30 Spanish Centers
- 2010-2011 (two months)
- 156 *A. fumigatus* senso stricto

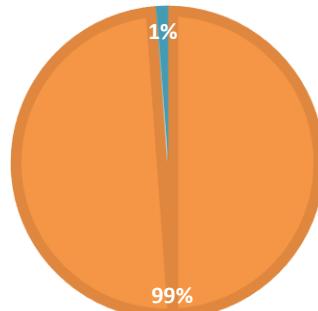


Alastruey-Izquierdo AAC 2013

FILPOP 2

- 10 Centers
- 2015
- 260 *A. fumigatus* senso stricto

■ Susceptible ■ Resistant



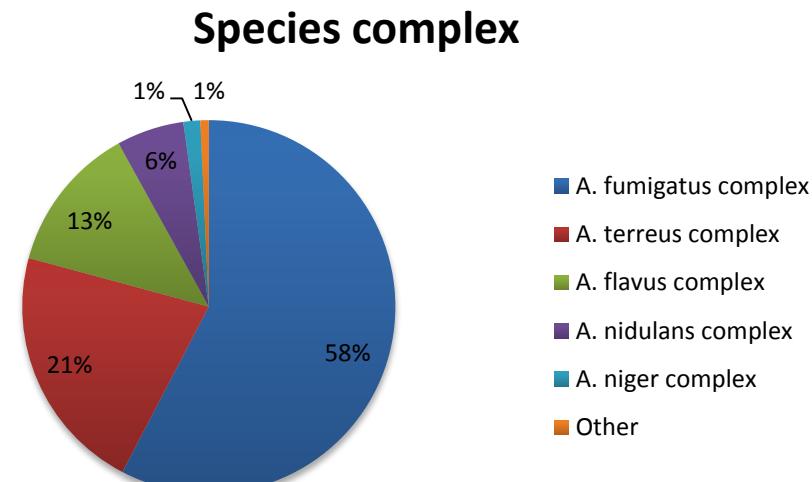
Cyp51A	AMB	ICZ	VCZ	PCZ	TB	CPF	MCF	AND
TR34/L98H	0,25	16	2	0,25	2	0,25	0,015	0,007
WT	0,5	16	2	0,5	4	0,25	0,03	0,007
TR34/L98H	0,5	16	2	0,5	2	0,5	0,007	0,015

Alastruey-Izquierdo AAC 2018

Aspergillus resistance surveillance program

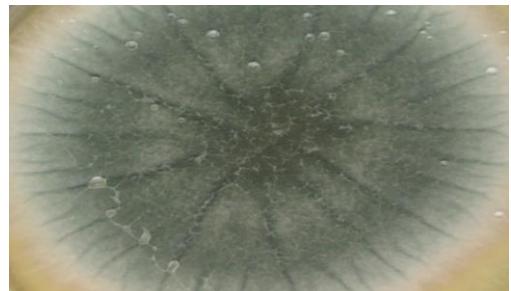
- July 2014- December 2018
- 274 samples from 45 centers
- EUCAST AFST

Aspergillus complex	No. of strains resistant to (with MIC values above their established EUCAST breakpoint):				
	AMB	ITZ	VRZ	PCZ	ISV
<i>A. fumigatus</i> complex (158)	19	17	16	21	28
<i>A. terreus</i> complex (59)	NA	2	NA	3	13
<i>A. flavus</i> complex (35)	NA	2	NA	NA	NA
<i>A. nidulans</i> complex (15)	NA	1	NA	NA	6



Aspergillus fumigatus

Strain	ICZ	VCZ	PCZ	ISAV	Mutation
CNM CM-7582	16	4	0.5	ND	TR ₃₄ /L98H (cyp51A)
CNM CM-7609	16	16	1	ND	TR ₃₄ /L98H (cyp51A)
CNM CM-9399	16	4	0.5	8	TR ₃₄ /L98H (cyp51A)
CNM CM-9114	16	0.5	2	0.5	G54R (cyp51A)
CNM CM-9501	16	0.5	2	0.5	G54R (cyp51A)
CNM CM-8057	16	16	1	16	TR ₄₆ /Y121F/T289A (cyp51A)



- 17 strains WT for Cyp51A
- 7 resistant to more than one azole
- 10 ISAV = 2 mg/L

Aspergillus terreus

Strain	ICZ	VCZ	PCZ	ISAV	Mutation
CNM CM-9079	0.25	2	0.06	2	D344N (cyp51A)
CNM CM-9280	0.5	2	0.12	2	D344N (cyp51A)
CNM CM-9490	4	8	0.5	8	D344N (cyp51A)
CNM CM-7846	16	16	1	ND	M217I; D344N
CNM CM-9284	0.5	1	0.12	2	A249G (cyp51A)



Strain	ICZ	VCZ	PCZ	ISAV	Mutation
CNM CM-8056	0.5	2	0.06	2	WT
CNM CM-8671	0.5	2	0.25	2	WT
CNM CM-8852	2	2	0.25	2	WT
CNM CM-8952	0.5	8	0.25	8	WT
CNM CM-8981	0.25	1	0.06	2	WT

Aspergillus flavus

Strain	ICZ	VCZ	PCZ	ISAV	Mutation
CNM CM-7668	16	0.25	1	ND	P220L (cyp51A)
CNM CM-9326	16	16	2	16	H349R (cyp51C)



Clinical outcome

Table 4. Characteristics of patients with azole-resistant invasive aspergillosis, the Netherlands, 2007–2009*

Patient age, y/ sex	Underlying disease	Disease	No. positive cultures†	Resistance mechanism	VCZ MIC, mg/L	Prior azole treatment (duration)‡	Treatments§	Outcome at 12 wk		
66/M	Lung carcinoma	Proven pulmonary aspergillosis	1	TR/L98H	4	None	VCZ	Died		
59/M	Hematologic malignancy, allo-SCT, GvHD	Proven pulmonary aspergillosis	4	TR/L98H	8	VCZ (>1 mo)	VCZ	Died		
54/M	Acute myeloid leukemia, relapse, allo-SCT	Aspergillosis						VCZ	Died	
50/M	Non-Hodgkin lymphoma, CNS, allo-SCT, GvHD, cavity	Susceptible strains			Resistant strains				VCZ	Died
36/F	Breast carcinoma, metastasis	Mortality						VCZ	Died	
13/F	Non-Hodgkin lymphoma	30-50%						Z, CAS, AMB	Died	
58/M	Liver transplantation for hepatic failure after methotrexate treatment for arteritis	CNS aspergillosis Proven pulmonary and CNS aspergillosis	5	TR/L98H	2	None	AMB, VCZ	Survived		
60/M	Acute myeloid leukemia, allo-SCT, GvHD	Proven pulmonary and CNS aspergillosis	3	TR/L98H	4	FCZ (1–2 wk)	VCZ, CAS, AMB, POS	Survived		

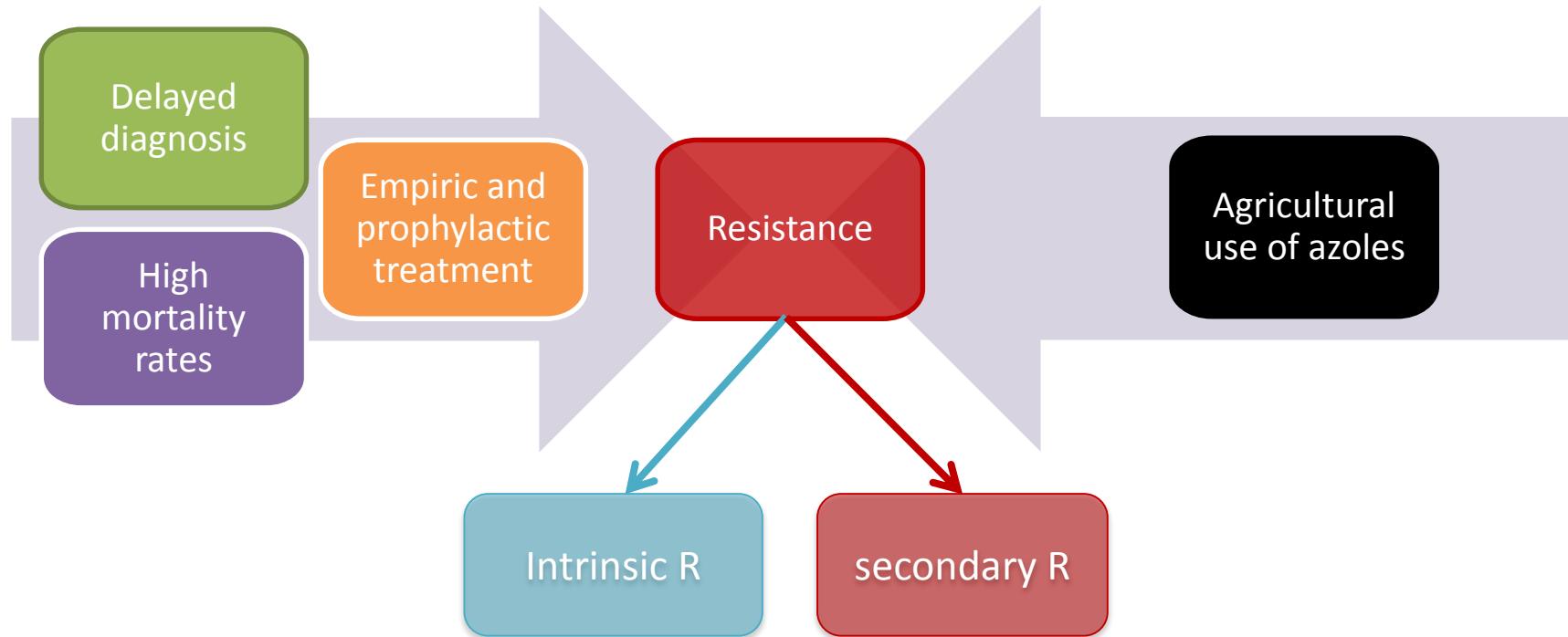
*VCZ, voriconazole; allo-SCT, allogeneic hematopoietic stem cell transplantation; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; ITZ, itraconazole; CNS, central nervous system; CAS, caspofungin; AMB, amphotericin B; FCZ, fluconazole; POS, posaconazole.

† All cultures were *Aspergillus fumigatus*.

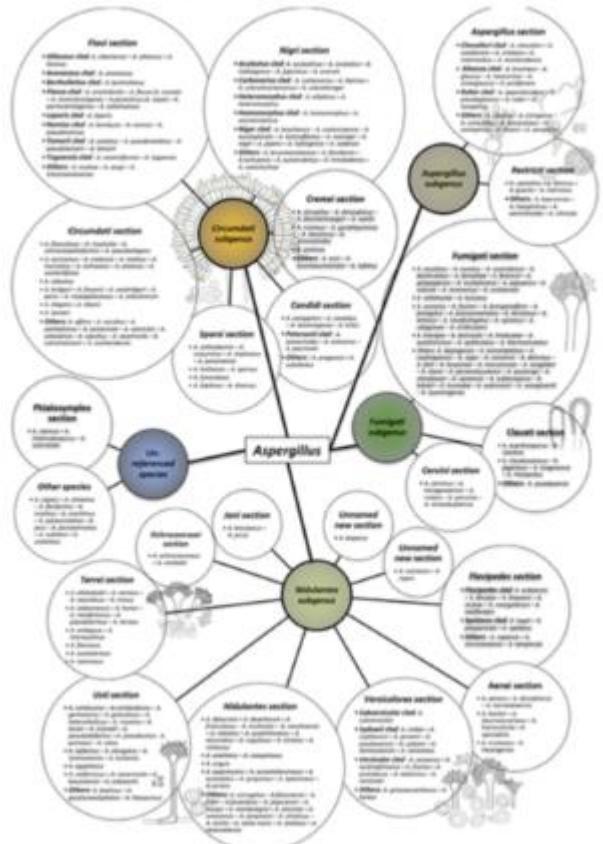
‡Azole treatment <12 wk before the first culturing of an azole-resistant isolate.

§Azole treatment after first culturing of resistant isolate.

AF resistance



Species complex



Fumigati section

- *A. assulatus* – *A. auratus* – *A. australensis* – *A. denticulatus* – *A. fennelliae* – *A. ferenczii* – *A. galapagensis* – *A. multiplicatus* – *A. papuensis* – *A. solicola* – *A. stramenius* – *A. unilateralis*
 - *A. nishimurae* – *A. turcosus*
 - *A. aureolus* – *A. fischeri* – *A. fumigatiaffinis* – *A. fumigatus* – *A. fumisynnematus* – *A. laciniatus* – *A. lentulus* – *A. novofumigatus* – *A. spinosus* – *A. udagawae* – *A. viridinutans*
 - *A. brevipes* – *A. duricaulis* – *A. hiratsukae* – *A. quadricinctus* – *A. spathulatus* – *A. thermomutatus*
 - Others: *A. beijingensis* – *A. brevistipitatus* – *A. caatingaensis* – *A. cepii* – *A. conversis* – *A. delicatus* – *A. felis* – *A. huiyaniae* – *A. marvanovae* – *A. neoglaber* – *A. otanii* – *A. pernambucoensis* – *A. qizutongii* – *A. shendawei* – *A. siamensis* – *A. sublevisporus* – *A. takakii* – *A. tsunodae* – *A. waksmanii* – *A. wangduanlii* – *A. wyomingensis*



Fig. 1. Classification according to their subgenus and section of the 310 currently valid species in the *Aquilegia* genus.

Cryptic species

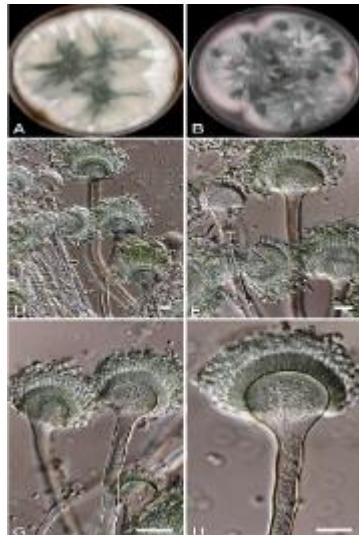
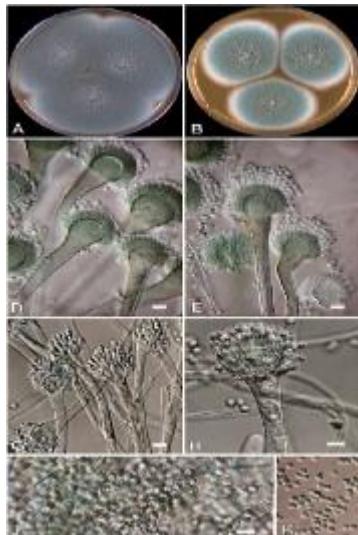
EUKARYOTIC CELL, Mar. 2005, p. 625–632
1535-9776/05/0300-00+0 doi:10.1128/EUK.4.3.625–632.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Vol. 4, No. 3

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

S. Arunmozhi Balajee,¹ Jennifer L. Gribskov,¹ Edward Hanley,¹ David Nickle,² and Kieren A. Marr^{1,3*}

Program in Infectious Diseases, Fred Hutchinson Cancer Research Center,¹ and Department of Microbiology² and Department of Medicine,³ University of Washington, Seattle, Washington



Cryptic species

11% cryptic species

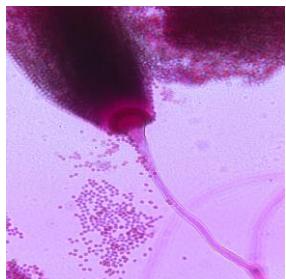
Table 1. *Aspergillus* species distribution according to epidemiological surveys from Spain and the U. S.²²

Species	Section	Transnet		FILPOP	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>N. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	0	0.0
<i>A. terreus</i>	Terrei	11	5.0	0	0.0
<i>A. carneus</i>	Terrei	0	0.0	0	0.0
<i>A. tubingensis</i>	Nigri	6	2.7	0	0.0
<i>A. niger</i>	Nigri	13	5.9	0	0.0
<i>A. calidoustus</i>	Usti	6	2.6	4	1.4
<i>A. insuetus</i>	Usti	0	0.0	1	0.4
<i>A. keveii</i>	Usti	0	0.0	1	0.4
<i>A. sydowii</i>	Versicolores	2	0.9	1	0.4
<i>A. versicolor</i>	Versicolores	3	1.4	0	0.0
<i>E. quadrilineata</i>	Nidulantes	1	0.5	0	0.0
<i>A. nidulans</i>	Nidulantes	0	0.0	8	2.9
<i>A. westerdijkiae</i>	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

Chowdhary et al. (2016)
16.6% cryptic species

Balajee et al, JCM 2009

Alastruey-Izquierdo et al. AAC 2013



14% cryptic species

Brazil

133 patients with suspected aspergillosis

12 medical centers in Brazil

TABLE 2 Cryptic and rare *Aspergillus* species distributions, *in vitro* susceptibility profiles, and sources of infection.

Section (no. of isolates)	Species identification (no. of isolates)	Source (no. of isolates)	MIC data ($\mu\text{g/ml}$) for:					
			Itraconazole		Voriconazole		Posaconazole	
			Range	GM ^a	Range	GM	Range	GM
<i>Clavati</i> (1)	<i>A. clavatus</i> (1)	Respiratory tract biopsy specimen (1)	1.0	1.0	1.0	0.5	0.5	0.5
<i>Circumdati</i> (1)	<i>A. ochraceus</i> (1)	Respiratory secretion (1)	4.0	1.0	1.0	0.5	0.5	0.5
<i>Flavi</i> (8)	<i>A. arachidicola</i> (1)	Respiratory tract biopsy specimen (1)	0.5	1.0	1.0	0.125	0.125	0.125
	<i>Aspergillus</i> sp. 1 (3)	Respiratory secretion (2), respiratory tract biopsy specimen (1)	0.5–1.0	0.63	0.5–1.0	0.63	0.125–0.25	0.16
	<i>A. tamarii</i> (2)	Tissue biopsy specimen (1), respiratory tract biopsy specimen (1)	0.25–0.5	0.35	0.125–0.5	0.25	0.03–0.125	0.06
	<i>A. transmontanensis</i> (1)	Respiratory tract biopsy specimen (1)	0.25		1.0		0.125	
<i>Flavipedes</i> (1)	<i>A. nomius</i> (1)	Respiratory secretion (1)	0.5		1.0		0.25	
	<i>A. flavipes</i> (1)	Tissue biopsy specimen (1)	0.125		0.125		0.25	
<i>Fumigati</i> (1)	<i>A. thermomutatus</i> (1)	Tissue biopsy specimen (1)	2.0		16.0		0.5	
<i>Nidulantes</i> (5)	<i>A. temeseensis</i> (3)	Respiratory tract secretion (2), skin biopsy specimen (1)	0.5–1.0	0.63	0.5–1.0	0.63	0.25	0.25
	<i>A. tabacinus</i> (1)	Respiratory secretion (1)	1.0		1.0		0.25	
	<i>A. creber</i> (1)	Respiratory secretion (1)	0.5		0.25		0.5	
	<i>A. awamori</i> (3)	Respiratory secretion (2), skin biopsy specimen (1)	0.25–2.0	0.5	0.25–0.5	0.4	0.125–0.25	0.16
	<i>Aspergillus</i> sp. 2 (4)	Respiratory secretion (3), ear secretion (1)	0.5–1.0	0.84	0.25–1.0	0.6	0.25–0.5	0.3
	<i>Aspergillus</i> sp. 3 (1)	Respiratory secretion (1)	1.0		1.0		0.25	
	<i>A. foetidus</i> (1)	Respiratory secretion (1)	2.0		0.25		0.25	
<i>Terrei</i> (2)	<i>A. terreus</i> (2)	Tissue biopsy specimen (1), ear secretion (1)	0.25–0.5	0.35	0.25–1.0	0.5	0.25	0.25
<i>Usti</i> (1)	<i>A. calidoustus</i> (1)	Tissue biopsy specimen (1)	>32		4.0		4.0	

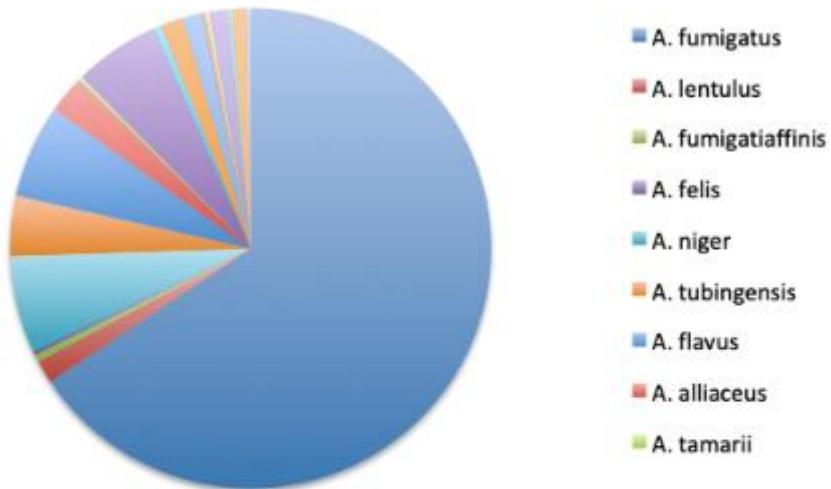
^a GM, geometric mean.

Portugal



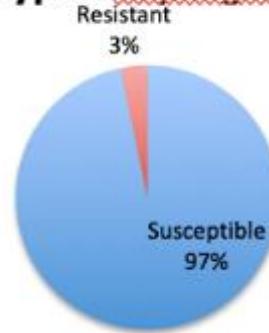
57 *Aspergillus*
10 health institutions
A. awamorii
A. brasiliensis
A. fructus
A. lentulus
A. sydowii
A. tubingensis
Emericella echinulata

FILPOP2 Study

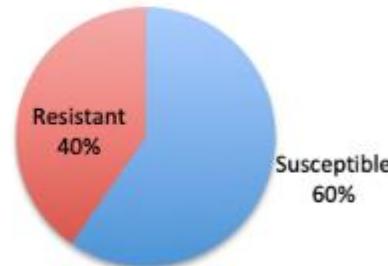


15.7% cryptic species

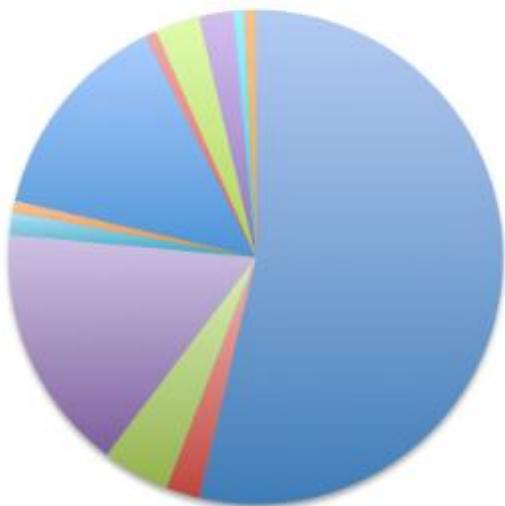
Non cryptic *Aspergillus spp.*



Cryptic *Aspergillus spp.*



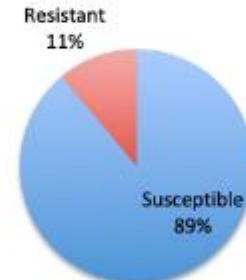
Aspergillus resistance surveillance program



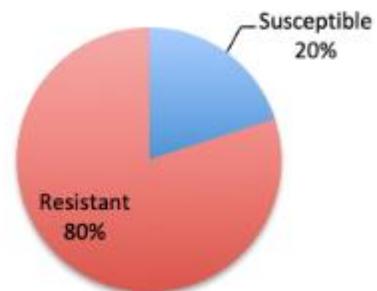
11% cryptic species

- *A. fumigatus*
- *A. udagawae*
- *A. lentulus*
- *A. terreus*
- *A. citrinoterreus*
- *A. hortai*
- *A. flavus*
- *A. nomius*
- *A. nidulans*

Non Cryptic *Aspergillus* spp.



Cryptic *Aspergillus* spp.



Cryptic species

	n	AMB	ICZ	VCZ	PCZ	CPF	MCF	ANF
<i>A. lentulus</i>	26	3	2.3	3.4	0.23	1.6	0.1	0.1
<i>N. hiratsukae</i>	9	1.7	0.9	1.1	0.16	0.11	0.03	0.03
<i>N. pseudofischerii</i>	6	0.25	4	2.51	0.22	0.86	0.03	0.03
<i>A. fumigatiaffinis</i>	6	4.8	5	3.1	0.4	0.22	0.03	0.03
<i>N. udagawae</i>	5	2	0.6	2.3	0.25	0.3	0.03	0.03
<i>A. viridinutans</i>	3	0,7	16	4	0.25	5.66	0,06	0,09
<i>A. tubingensis</i>	22	0.11	0.42	0.76	0.09	0.3	0.05	0.03
<i>A. calidoustus</i>	19	0.9	8.6	6.2	6.8	0.5	0.04	0.04
<i>A. insuetus</i>	2	0.7	11.3	8	2.8	5.6	1.4	0.9
<i>A. keveii</i>	1	0,25	16	16	16	16	16	16
<i>A. alliaceus</i>	30	19.25	0.2	0.5	0.11	12.15	3.8	1.9

Data from Mycology Reference Lab. Spain

Cryptic species

	n	AMB	ICZ	VCZ	PCZ	CPF	MCF	ANF
<i>A. lentulus</i>	26	3	2.3	3.4	0.23	1.6	0.1	0.1
<i>N. hiratsukae</i>	9	1.7	0.9	1.1	0.16	0.11	0.03	0.03
<i>N. pseudofischerii</i>	6	0.25	4	2.51	0.22	0.86	0.03	0.03
<i>A. fumigatiaffinis</i>	6	4.8	5	3.1	0.4	0.22	0.03	0.03
<i>N. udagawae</i>	5	2	0.6	2.3	0.25	0.3	0.03	0.03
<i>A. viridinutans</i>	3	0,7	16	4	0.25	5.66	0.06	0.09
<i>A. tubingensis</i>	22	0.11	0.42	0.76	0.09	0.3	0.05	0.03
<i>A. calidoustus</i>	19	0.9	8.6	6.2	6.8	0.5	0.04	0.04
<i>A. insuetus</i>	2	0.7	11.3	8	2.8	5.6	1.4	0.9
<i>A. keveii</i>	1	0,25	16	16	16	16	16	16
<i>A. alliaceus</i>	30	19.25	0.2	0.5	0.11	12.15	3.8	1.9

Data from Mycology Reference Lab. Spain

A. lentulus

Summary on published clinical cases caused by *Aspergillus lentulus*

Year (Reference)	Number of patients	Underlying disease/ procedure	Source of isolate	Disease classification	Species identification	Susceptibility pattern	Treatment	Outcome
2004 (10)	4	Stem cell transplantation	Lung, mouth, kidney	Proven or probable IA	Genotypic	MIC Itra 0.5–2 mg/L; Vor 2–4 mg/L, AmB 1–4 mg/L; MEC Cas 4>32 mg/L	NR	Died
2008 (11)	1	Chronic obstructive lung disease	Lung	Probable IA, Co-infection with <i>A. fumigatus</i>	Genotypic	MIC AmB 4 mg/L; Itra 3 mg/L; Vor 1.5 mg/L; MEC Cas >32 mg/L	Vor	Died
2009 (12)	1	Kidney transplantation	Lung	Probable IA, Co-infection with <i>A. fumigatus</i>	Genotypic	MIC Vor 2 mg/L, AmB 2 mg/L; MEC Cas >16 mg/L	L-AmB	Died
2009 (4)	4	Stem cell transplantation	Lung	Proven or probable IA	Genotypic	MIC AmB 0.5–2 mg/L, Itra 0.25–0.5 mg/L, Vor 1–4 mg/L, Pos 0.25 mg/L	NR	NR
2010 (13)	1	Cystic fibrosis	Lung	Colonization	Genotypic	MIC AmB 4 mg/L; Itra 2 mg/L; Vor 8 mg/L	NR	NR
2011 (14)	1	Cystic fibrosis	Lung	Colonization	Genotypic	MIC AmB 2 mg/L; Itra 2 mg/L; Vor 2 mg/L, Pos 0.5 mg/L	NR	NR
2012 (current report)	1	Heart transplantation	Lung	Proven IA	Genotypic	MIC AmB 2.0 mg/L; Itra 0.25 mg/L, Vor 1.0 mg/L, Pos 0.125 mg/L; MEC Cas 16 mg/L	Vor, Cas, AmB	Died

A. fumigatus complex

TABLE 1 | Prevalence of *Aspergillus* spp. of section *Fumigati* other than *Aspergillus fumigatus* in clinical specimens.



Country	Aspergillus section <i>Fumigati</i> N strains	Strains other than <i>A. fumigatus</i> N strains (%)	Species (N)	Reference
United States	147	8 (5.4%)	<i>A. lentulus</i> (4) <i>A. udagawae</i> (3) <i>A. thermomutatus</i> ¹ (1)	Balajee et al., 2009b
Spain	362	19 (5.2%)	<i>A. lentulus</i> (9) <i>A. novofumigatus</i> (6) <i>A. udagawae</i> (2) <i>A. viridinutans</i> (2)	Escribano et al., 2013
Spain	162	6 (3.7%)	<i>A. lentulus</i> (3) <i>A. viridinutans</i> (1) <i>A. thermomutatus</i> ¹ (1) <i>A. fumigatiaffinis</i> (1)	Alastruey- Izquierdo et al., 2013
Portugal	29	1 (3.4%)	<i>A. lentulus</i> (1)	Sabino et al., 2014

¹ Teleomorph: *Neosartorva pseudofischeri*.

A. fumigatus complex

TABLE 2 | Phenotypic cha

Species	Antifungal susceptibility ⁴			ection <i>Fumigati</i> other than <i>A. fumigatus</i> .		
	AMB	TAZ	ECH	Outcome		Reference
				Success (1)	Failure (4)	
<i>A. fumigatus</i>	++	++	++	Success (1)	Failure (4)	Zbinden et al., 2012; Escribano et al., 2013; Gurcan et al., 2013; Bastos et al., 2015; Yoshida et al., 2015
						Vinh et al., 2009b; Posterao et al., 2011; Gyotoku et al., 2012
						Gerber et al., 1973; Coriglione
<i>A. lentulus</i>	+/-	+/- ⁵	+/- ⁶	Success (3)	Failure (3)	++ (MIC \leq 1 μ g/ml or MEC \leq 0.25 μ g/ml); + (MIC 1–4 μ g/ml or MEC 0.25–2 μ g/ml), – (MIC > 4 μ g/ml or MEC > 2 μ g/ml).
<i>A. udagawae</i>	+	+ ⁵	+ ⁶	Success (2)	Failure (2)	Katz et al., 2005; Vinh et al., 2009a; Coelho et al., 2011; Shigeyasu et al., 2012
<i>A. viridinutans/felis</i>	+//+	+/- ⁵	+ ⁶	Success (1)	Failure (2)	Guarro et al., 2002; Predari et al., 2007; Kouroutsos et al., 2010
<i>A. fischeri/thermomutatus</i> ¹	++	+ / - 5	+ ⁶	Success (1)	Failure (7)	Alhambra et al., 2008; Montenegro et al., 2009; Escribano et al., 2013; Pelaez et al., 2013

Aspergillus terreus complex

Table 1

Distribution of amphotericin B MICs against *Aspergillus terreus* species complex isolates collected during the study period and tested according to EUCAST methodology

Aspergillus species	Amphotericin B MICs, mg/L								
	0.125	0.25	0.5	1	2	4	8	16	32
<i>A. terreus</i> sensu stricto	3	7	10	14	36	81	86	55	23
<i>A. citrinotterreus</i>				3	13	8	7	5	
<i>A. hortai</i>				1	2	5	2		
<i>A. alabamensis</i>				2	3	1			
<i>A. floccosus</i>					1				
<i>A. neoaficanus</i>									
Potential new species									

Risingler et al TerrNet CMI 2017

A. terreus sensu stricto (*n* = 432)

Etest® (<i>n</i> = 315)	<0.002–0.500	0.032	0.125	0.008–4.000	0.064	0.250	0.016–2.000	0.125	0.250
EUCAST (<i>n</i> = 117)	0.125–0.500	0.250	0.500	0.125–1.000	0.500	0.500	0.250–1.000	0.500	0.500

Cryptic species (*n* = 66)

Etest® (<i>n</i> = 55)	<0.002–0.190	0.032	0.064	0.012–4.000	0.064	0.500	0.003–0.380	0.064	0.250
EUCAST (<i>n</i> = 11)	0.125–0.250	NA	NA	0.125–2.000	NA	NA	0.125–0.250	NA	NA

Minimum inhibitory concentrations (MICs) of posaconazole, voriconazole, and itraconazole were obtained by ETest® and EUCAST method.

MIC, minimum inhibitory concentration; MIC₅₀ and MIC₉₀, MIC for 50 and 90% of tested population; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; EUCAST, European Committee for Antimicrobial Susceptibility Testing; NA, not applicable; N, number of tested isolates.

Cryptic species

	n	AMB	ICZ	VCZ	PCZ	CPF	MCF	ANF
<i>A. lentulus</i>	26	3	2.3	3.4	0.23	1.6	0.1	0.1
<i>N. hiratsukae</i>	9	1.7	0.9	1.1	0.16	0.11	0.03	0.03
<i>N. pseudofischerii</i>	6	0.25	4	2.51	0.22	0.86	0.03	0.03
<i>A. fumigatiaffinis</i>	6	4.8	5	3.1	0.4	0.22	0.03	0.03
<i>N. udagawae</i>	5	2	0.6	2.3	0.25	0.3	0.03	0.03
<i>A. viridinutans</i>	3	0,7	16	4	0,25	5.66	0.06	0.09
<i>A. tubingensis</i>	22	0.11	0.42	0.76	0.09	0.3	0.05	0.03
<i>A. calidoustus</i>	19	0.9	8.6	6.2	6.8	0.5	0.04	0.04
<i>A. insuetus</i>	2	0.7	11.3	8	2.8	5.6	1.4	0.9
<i>A. keveii</i>	1	0,25	16	16	16	16	16	16
<i>A. alliaceus</i>	30	19.25	0.2	0.5	0.11	12.15	3.8	1.9

Data from Mycology Reference Lab. Spain

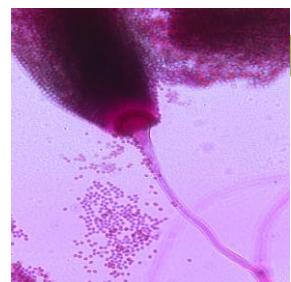
Cryptic species

Table 1. *Aspergillus* species distribution according to epidemiological surveys from Spain and the U. S.²²

Species	Section	Transnet		FILPOP	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>N. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	3	1.1
<i>A. terreus</i>	Terrei	11	5.0	26	9.4
<i>A. carneus</i>	Terrei	0	0.0	1	0.4
<i>A. tubingensis</i>	Nigri	6	2.8	22	7.9
<i>A. niger</i>	Nigri	13	6.0	21	7.6
<i>A. calidoustus</i>	Usti	6	2.8	4	1.4
<i>A. insuetus</i>	Usti	0	0.0	1	0.4
<i>A. keveii</i>	Usti	0	0.0	1	0.4
<i>A. sydowii</i>	Versicolores	2	0.9	1	0.4
<i>A. versicolor</i>	Versicolores	3	1.4	0	0.0
<i>E. quadrilineata</i>	Nidulantes	1	0.5	0	0.0
<i>A. nidulans</i>	Nidulantes	0	0.0	8	2.9
<i>A. westerdijkiae</i>	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

Balajee et al, JCM 2009

Alastruey-Izquierdo et al. AAC 2013

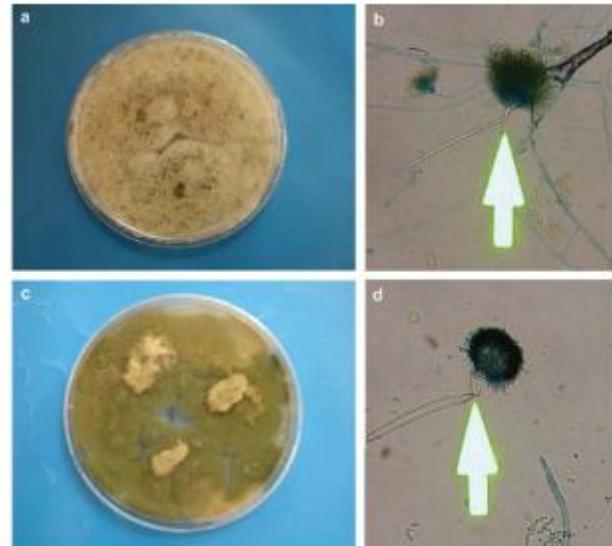


Case Report

***Aspergillus alliaceus* and *Aspergillus flavus* co-infection in an acute myeloid leukemia patient**

BETIL OZHAK-BAYSAN*, ANA ALASTRUEY-IZQUIERDO†, RABIN SABA‡, DILARA OGUNC*,
GOZDE ONGUT*, AYSEN TIMURAGAOGLU§, GOKHAN ARSLAN#, MANUEL CUENCA-ESTRELLA†
& JUAN LUIS RODRIGUEZ-TUDELA†

- Leukemia patient
- AMB
- *A. flavus* + *A. alliaceus* (Sputum and BAL)
- Voriconazole
- Died





Nonsporulating Clinical Isolate Identified as *Petromyces alliaceus* (Anamorph *Aspergillus alliaceus*) by Morphological and Sequence-Based Methods[†]

S. Arunmozhi Balajee,^{1*} Mark D. Lindsley,¹ Naureen Iqbal,¹ James Ito,²
Peter G. Pappas,³ and Mary E. Brandt¹

- Hematological patient
- AMB (Prophylactic) ---> ICZ
- Bronchoscopy--> fungal hyphae
- IPA
- AMB+CPF
- BAL → Non sporulating mould
- Died

AMB = 8
ICZ = 0.12
VCZ = 1
CPF > 32

Cryptic species

	n	AMB	ICZ	VCZ	PCZ	CPF	MCF	ANF
<i>A. lentulus</i>	26	3	2.3	3.4	0.23	1.6	0.1	0.1
<i>N. hiratsukae</i>	9	1.7	0.9	1.1	0.16	0.11	0.03	0.03
<i>N. pseudofischerii</i>	6	0.25	4	2.51	0.22	0.86	0.03	0.03
<i>A. fumigatiaffinis</i>	6	4.8	5	3.1	0.4	0.22	0.03	0.03
<i>N. udagawae</i>	5	2	0.6	2.3	0.25	0.3	0.03	0.03
<i>A. viridinutans</i>	3	0,7	16	4	0,25	5.66	0.06	0.09
<i>A. tubingensis</i>	22	0.11	0.42	0.76	0.09	0.3	0.05	0.03
<i>A. calidoustus</i>	19	0.9	8.6	6.2	6.8	0.5	0.04	0.04
<i>A. insuetus</i>	2	0.7	11.3	8	2.8	5.6	1.4	0.9
<i>A. keveii</i>	1	0,25	16	16	16	16	16	16
<i>A. alliaceus</i>	30	19.25	0.2	0.5	0.11	12.15	3.8	1.9

Data from Mycology Reference Lab. Spain

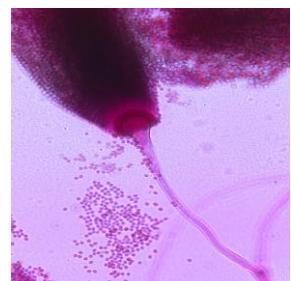
Cryptic species

Table 1. *Aspergillus* species distribution according to epidemiological surveys from Spain and the U. S.²²

Species	Section	Transnet		FILPOP	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>N. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	3	1.1
<i>A. terreus</i>	Terrei	11	5.0	26	9.4
<i>A. carneus</i>	Terrei	0	0.0	1	0.4
<i>A. tubingensis</i>	Nigri	6	2.8	22	7.9
<i>A. niger</i>	Nigri	13	6.0	21	7.6
<i>A. calidoustus</i>	Usti	6	2.8	4	1.4
<i>A. insuetus</i>	Usti	0	0.0	1	0.4
<i>A. keveii</i>	Usti	0	0.0	1	0.4
<i>A. sydowii</i>	Versicolores	2	0.9	1	0.4
<i>A. versicolor</i>	Versicolores	3	1.4	0	0.0
<i>E. quadrilineata</i>	Nidulantes	1	0.5	0	0.0
<i>A. nidulans</i>	Nidulantes	0	0.0	8	2.9
<i>A. westerdijkiae</i>	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

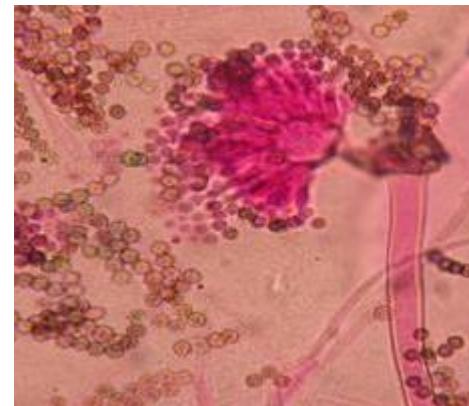
Balajee et al, JCM 2009

Alastruey-Izquierdo et al. AAC 2013



Aspergillus ustus complex

	n	AMB	ICZ	VCZ	PCZ
<i>A. calidoustus</i>	19	0.9	8.6	6.2	6.8
<i>A. insuetus</i>	2	0.7	11.3	8	2.8
<i>A. keveii</i>	1	0.25	16	16	16



A. ustus complex

- 41 cases
- 20 (49%) hematological patients & 13 (32%) SOT

	AMB	ICZ	VCZ	PCZ	TRB	CPF	MCF	ANF
ranges	0.25-4	0.5-8	2-8	1- >16	0.06-2	0.03-8	≤ 0.015 -1	≤ 0.015

- 71% combination therapy (AMB, VCZ, echinocandins)

42% treatment failure
66% mortality (33% attributable)

Conclusions

- ✓ Antifungal resistance is increasing
 - ✓ Variable rates of secondary resistance in *A. fumigatus* but also present in other species
 - ✓ MDR cryptic species(*A. lentulus*, *A. alliaceus*, *A. calidoustus*) present in clinical samples
- ✓ Important to know local epidemiology
- ✓ Perform AFST to any isolate coming from invasive infection If possible
- ✓ If not in available send it to a reference lab

Thanks for your attention

Ana Alastruey-Izquierdo

Mycology Reference Lab Spain

Instituto de Salud Carlos III

anaalastruey@isciii.es