Resistencia a los antifúngicos en *Candida* spp y *A. fumigatus*

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Transparencia

Proyectos de investigación (últimos 3 años): Fondo de Investigación Sanitaria (FIS), Instituto de Investigación Sanitaria Gregorio Marañón, Cidara, Scynexis, F2G, Gilead, Mundipharma

Honorarios por participación en actividades de formación continuada (últimos 3 años): Pfizer, Gilead, MSD, Mundipharma

Tracemos la línea de salida

Conceptos generales en resistencia a antifúngicos

Resistencia en *Candida* y *Aspergillus*. Datos nacionales

Detección en el laboratorio

Clinical Chemistry 68:1
83–90 (2022)

Mini-Review

Emerging Fungal Infections: New Species, New Names, and Antifungal Resistance

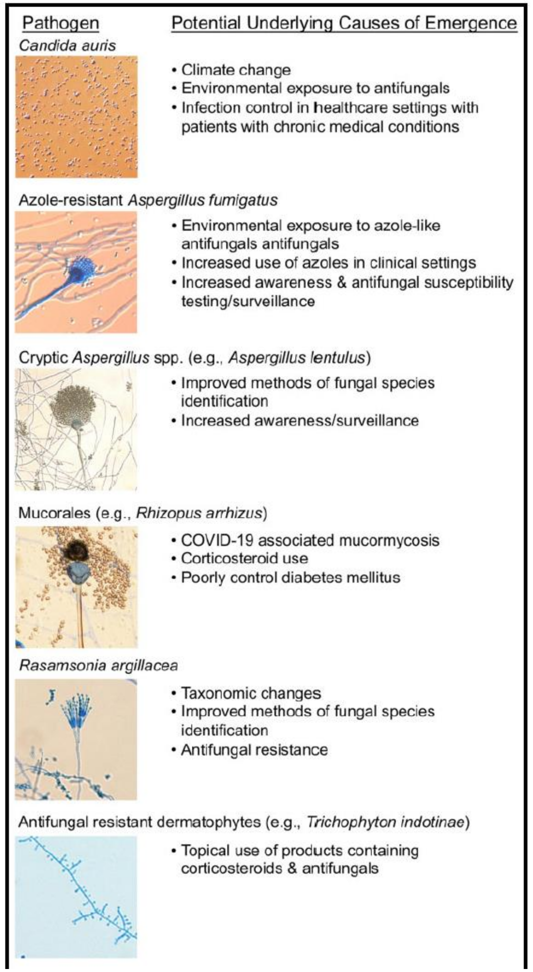
Nathan P. Wiederhold *

1,5 a 5 millones de especies de hongos

300 potencial de causar infecciones a humanos

Retos actuales

- Especies emergentes (nuevas o progreso en taxonomía)
- Resistencia antifúngica



Ref. Wiederhold. Clin Chem 2022

Resistencia a equinocandinas: *C. glabrata*

Table 1. Rates of acquired *FKS* mutations among Candida isolates causing invasive infections

Species	Overall	Any prior echinocandin exposure[a]	Echinocandin breakthrough candidiasis	Echinocandin resistance among *FKS* mutants[b]	Reference(s)
C. albicans	1–3%	5%	50%	Approaching 100%	[18]
C. glabrata	4–18%	15–32%	67–75%	46–81%	[5,18,19■■,20–22,23■■]
C. krusei	Case reports*	NA	NA	NA	[22,24■■,25]
C. tropicalis	Case reports*	NA	NA	NA	[22,26–28]

- **Infrecuente, pero implica a *C. glabrata***
- **Tratamiento prolongado con candinas**
- **Mutaciones puntuales en genes *FKS***

Ref: Shields *et al*. Curr Opin 2015

Ref: Shields *et al*. Antimicrob Agents Chemother 2013

Resistencia a azoles: *A. fumigatus*

J Antimicrob Chemother 2015; **70**: 1522–1526
doi:10.1093/jac/dku566 Advance Access publication 27 January 2015

Journal of Antimicrobial Chemotherapy

Emergence of azole-resistant invasive aspergillosis in HSCT recipients in Germany

J. Steinmann[1]*†, A. Hamprecht[2]†, M. J. G. T. Vehreschild[3,4], O. A. Cornely[3–5], D. Buchheidt[6], B. Spiess[6], M. Koldehoff[7], J. Buer[1], J. F. Meis[8,9] and P.-M. Rath[1]

- *A. fumigatus* aislados en 27 pacientes con TPH
- 7/8 pacientes (88%) cepas resistentes a azoles éxitus
- 7/8 pacientes (88%) cepas resistentes profilaxis con azoles
- TR_{34}/L98H mutación más frecuente (n=5), seguida de TR_{46}/Y121F/T289A (n=2)

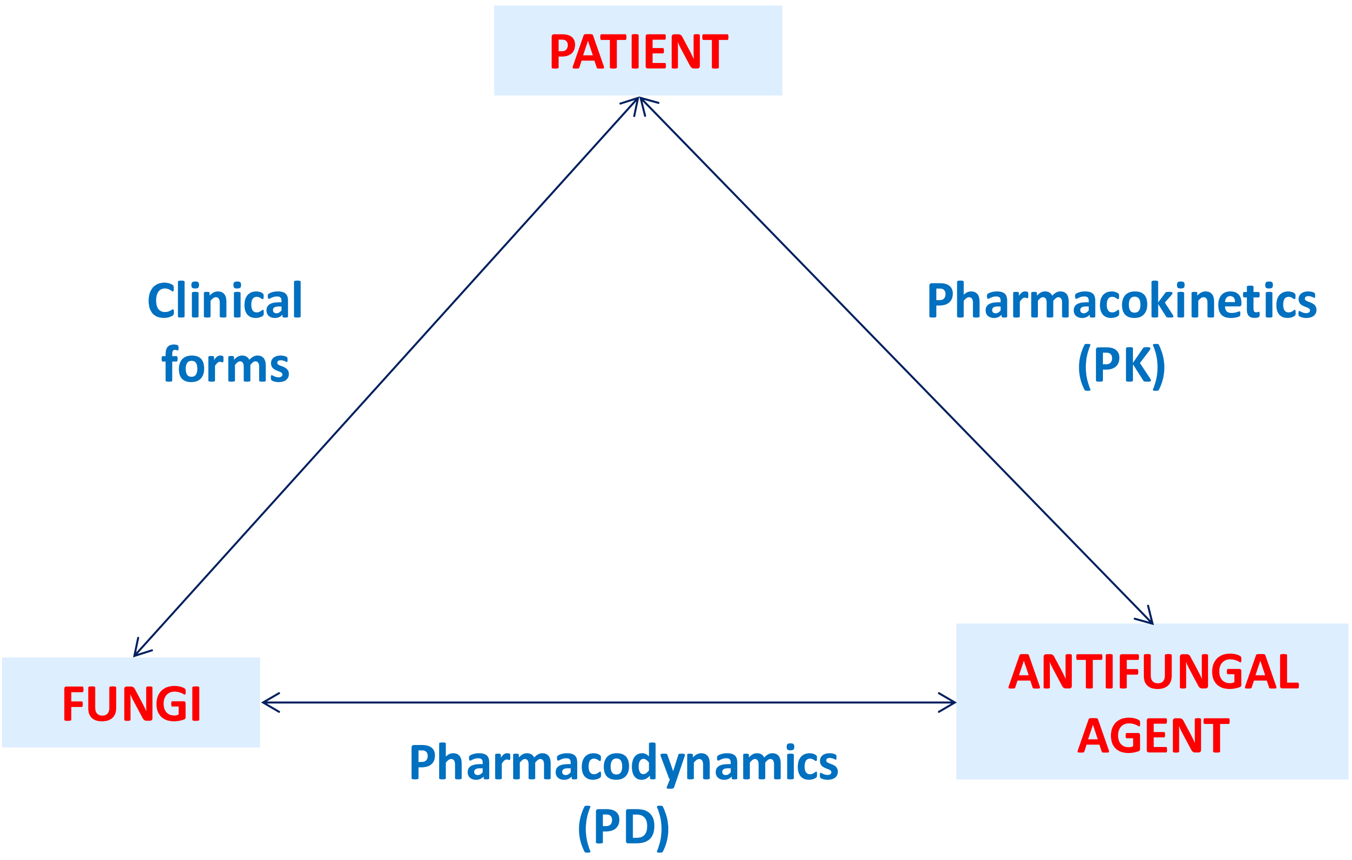
Table 1. Characteristics of HSCT recipients with azole-resistant IA and their corresponding *A. fumigatus* isolates

Patient no.	Sex, age (years)	Underlying disease	aGvHD grade (only if allogeneic Tx)	Date of HSCT	Days after HSCT until ARAF detection (days)	Initial specimen	EORTC/MSG criterion	Antifungal prophylaxis (before detection)	Type of mutation	MIC (mg/L) ITC	MIC (mg/L) VRC	MIC (mg/L) POS	Antifungal treatment	Discharge status (100 days after ARAF detection)	Cause of death
1	M, 46	AML	IV	13.12.2011	140	BAL	probable	CAS	TR_{34}/L98H	>16	2	0.5	CAS	died	sepsis
2	M, 54	AML	II	21.02.2012	112	tracheal secretion	probable	L-AMB	WT	>16	4	0.5	VRC	died	relapse, MOV
3	F, 65	AML	III	22.02.2012	134	sputum	probable	POS	TR_{34}/L98H	>16	4	0.5	POS	died	sepsis, MOV
4	M, 66	Acute biphenotype leukaemia	III	27.06.2012	21	tracheal secretion	probable	POS	TR_{34}/L98H	>16	2	0.5	L-AMB, later VRC	died	sepsis, MOV
5	F, 58	MDS RAEB-II	IV	31.01.2013	137	tracheal secretion	probable	VRC	TR_{34}/L98H	>16	2	0.5	VRC	died	sepsis, MOV
6	F, 38	Plasma cell leukaemia	I	28.03.2013	92	stool	possible	ITC	TR_{34}/L98H	>16	2	0.5	VRC	alive	—
7	M, 43	CLL Binet C	IV	21.12.2012	272	BAL	probable	VRC	TR_{46}/Y121F/ T289A	>16	16	0.5	L-AMB, later VRC	died	GvHD, MOV
8	F, 52	Follicular B-NHL grade IIIa	—	26.06.2012	9	BAL	proven	—	TR_{46}/Y121F/ T289A	1	>16	0.5	L-AMB, later CAS	died	sepsis

Se resaltan en amarillo los pacientes con mala evolución

Ref: Steinmann J *et al.* J Antimicrob Chemother 2015; 70: 1522–1526

It takes three to tango.......



Ref. Guinea J. Adaptado de Rev Esp Quimioter. 2023 Nov;36 Suppl 1(Suppl 1):59-63

https://www.collinsdictionary.com/dictionary/english/resistance

noun

1. the act or an instance of resisting

2. the capacity to withstand something, esp the body's natural capacity to withstand disease

128 64 32 16 8 4 2 1 0.5 0.25 0.125 CC

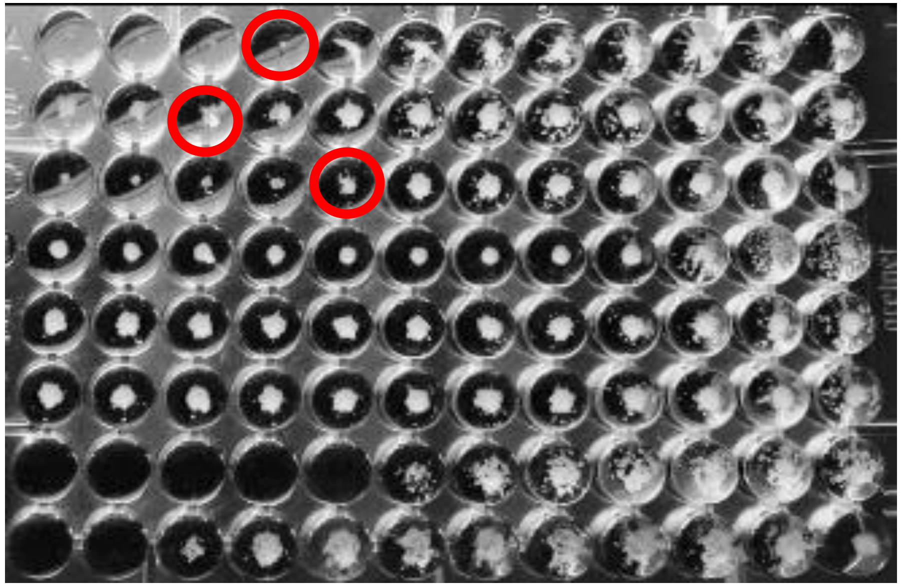


Imagen propia del autor

CMI: Concentración mínima de antifúngico capaz de producir una inhibición parcial o completa del crecimiento del hongo comparado con el control libre de antifúngico

FLUCONAZOLE concentrations (in µg/ml)

64 32 16 8 4 2 1 0.5 0.25 0.125 GC BLANK

Strain	64	32	16	8	4	2	1	0,5	0,25	0,12	CC	B
1	0,0834	0,1122	0,1883	0,3455	0,4524	0,6121	0,7751	0,9493	1,0138	1,1440	1,2798	0,0771
2	0,1003	0,1345	0,2266	0,4249	0,5395	0,7035	0,8001	0,8454	0,8932	0,9094	0,9622	0,0768
3	0,0868	0,0940	0,1235	0,1554	0,1859	0,2142	0,2481	0,2619	0,2845	0,3581	0,3904	0,0770
4	0,0912	0,1087	0,1489	0,1867	0,2142	0,2302	0,2508	0,2688	0,2765	0,3572	0,4078	0,0801
5	0,1728	0,1467	0,1336	0,1224	0,1211	0,1262	0,1197	0,1253	0,1317	0,1736	0,3660	0,0773
6	0,1080	0,0830	0,0890	0,2004	0,4020	0,6883	0,8179	0,8508	0,8997	0,9172	1,0119	0,0765
7	0,1675	0,1581	0,1547	0,1507	0,1505	0,1560	0,1636	0,1727	0,1902	0,2893	0,8268	0,0794
8	0,3001	0,5445	0,6932	0,7657	0,7425	0,7464	0,7932	0,8291	0,8970	0,9459	0,9971	0,0809

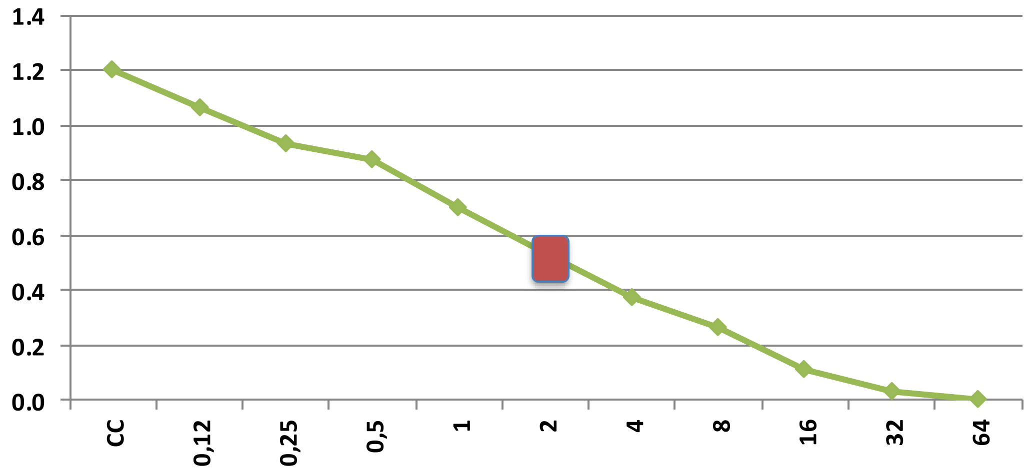
Imagen propia del autor

AmB:
≥90% inhibition

Azoles/candins/flucytosine:
≥50% inhibition

MIC endpoint

C. glabrata



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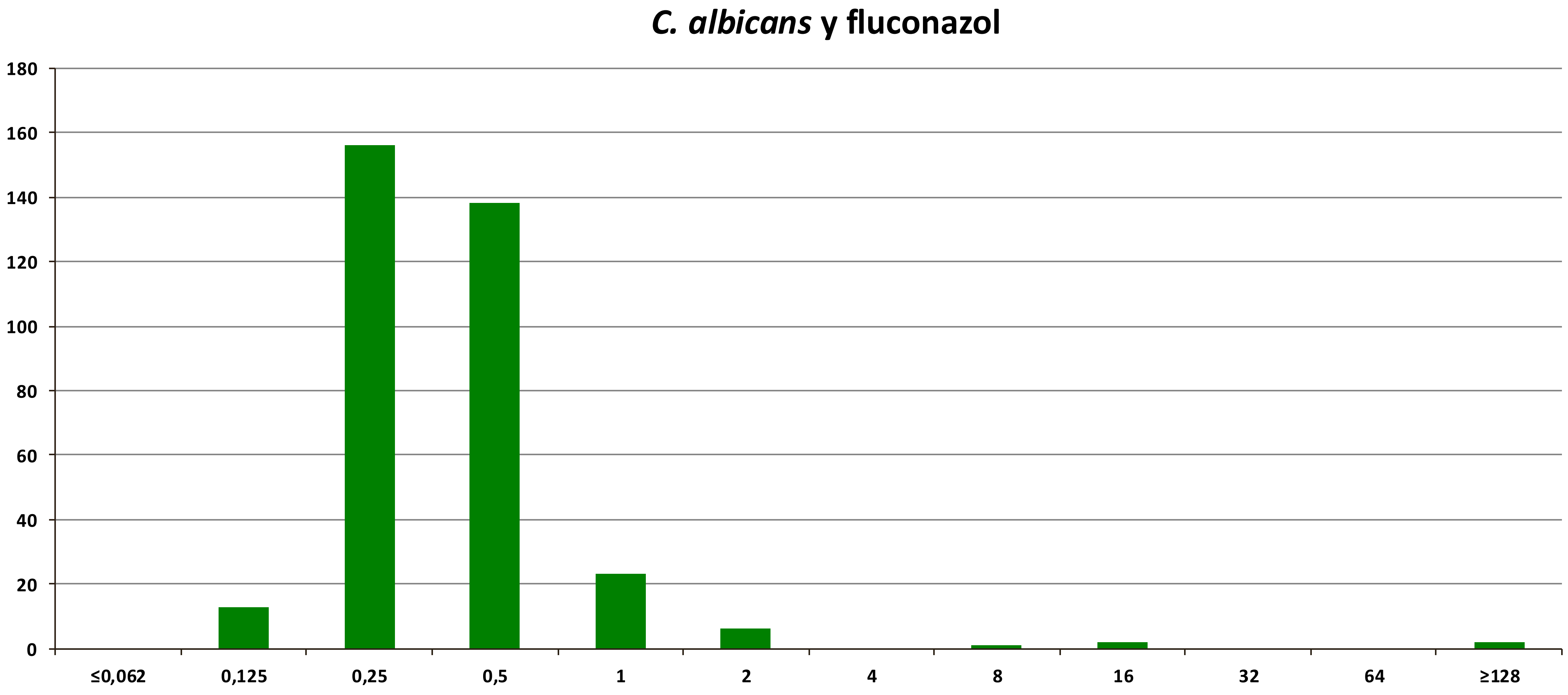
De distribuciones de CMIs a puntos de corte clínicos



Ref: Guinea J *et al*. AAC 2014 (Datos del estudio CANDIPOP)

Distribuciones de CMIs

1. Comités involucrados en desarrollo de puntos de corte clínicos
2. Establecimiento de *epidemiological cut-off values* (ECOFFs)
3. Rangos de CMIS para determinar la distribución salvaje de un microorganismo

Distribuciones aceptables

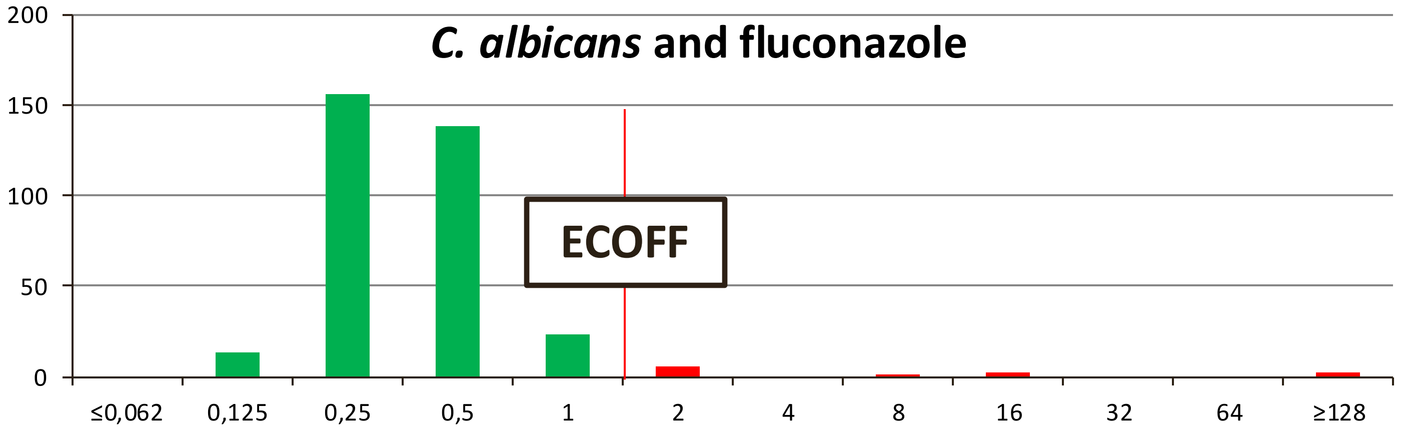
- ID a nivel de complejo
- Métodos de referencia (CMIs válidas, QC, etc)
- Aislados únicos/excluir brotes
- Escalas de CMI diluciones al doble
- Se aceptan aislados resistentes

Ref: Espinel-Ingroff et al. RIAM 2016

http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2017/MIC_and_ECOFF/EUCAST_MIC_and_ECOFF_discussion_document_version_3_20170309.pdf

ECOFF value: highest MIC for organisms devoid of phenotypically detectable acquired resistance mechanisms.

It defines the upper end of the **wild-type MIC distribution** (X mg/L), while the wild type is written as ≤ X mg/L and the non-wild type as > X mg/L.



Ref: Guinea J *et al*. AAC 2014 (Data from CANDIPOP study)

Setting ECOFFs

- **"Visual estimation" 99% isolates in the putative WT**
- **"Statistical approach" ECOFFinder**
- **Both methods should match**

http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2017/MIC_and_ECOFF/EUCAST_MIC_and_ECOFF_discussion_document_version_3_20170309.pdf

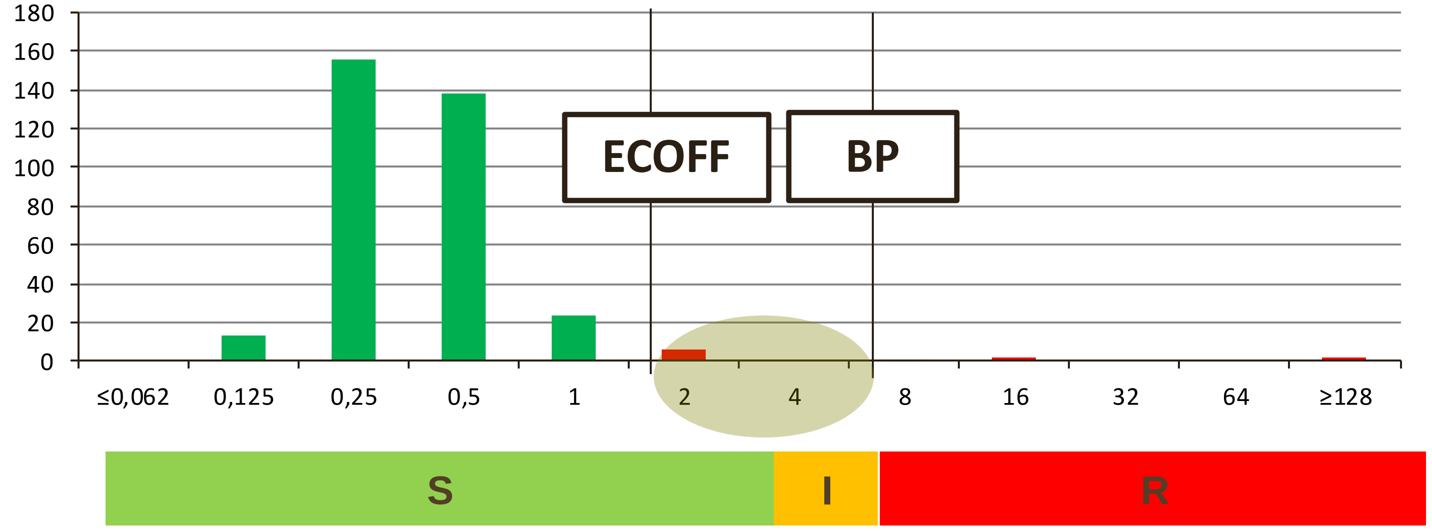
ECOFFs are not breakpoints!!!

ECOFF value: highest MIC for organisms devoid of phenotypically detectable acquired resistance mechanisms. It defines the upper end of the wild-type MIC distribution (X mg/L), while the wild type is written as ≤ X mg/L and the non-wild type as > X mg/L. ***IN VITRO* CONCEPT**

Clinical breakpoint: a micro-organism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success. It is categorized as susceptible (S) by applying the appropriate breakpoint in a defined phenotypic test system. **CLINICAL CONCEPT**

Sometimes ECOFFs used as breakpoints when the latter are lacking

C. albicans and fluconazole



Minimise the risk of setting clinical breakpoints which split wild-type MIC distributions

ECOFFs

Compare rates of acquired resistance in situations where clinical breakpoints differ (e.g. between organisations, between humans and animals), change over time or have not been set

http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2017/MIC_and_ECOFF/EUCAST_MIC_and_ECOFF_discussion_document_version_3_20170309.pdf

De ECOFFs a puntos de corte

Mecanismos resistencia

DISTRIBUTIONS

CMIs (ECOFFs)

Clinical BP

PK

Respuesta clínica

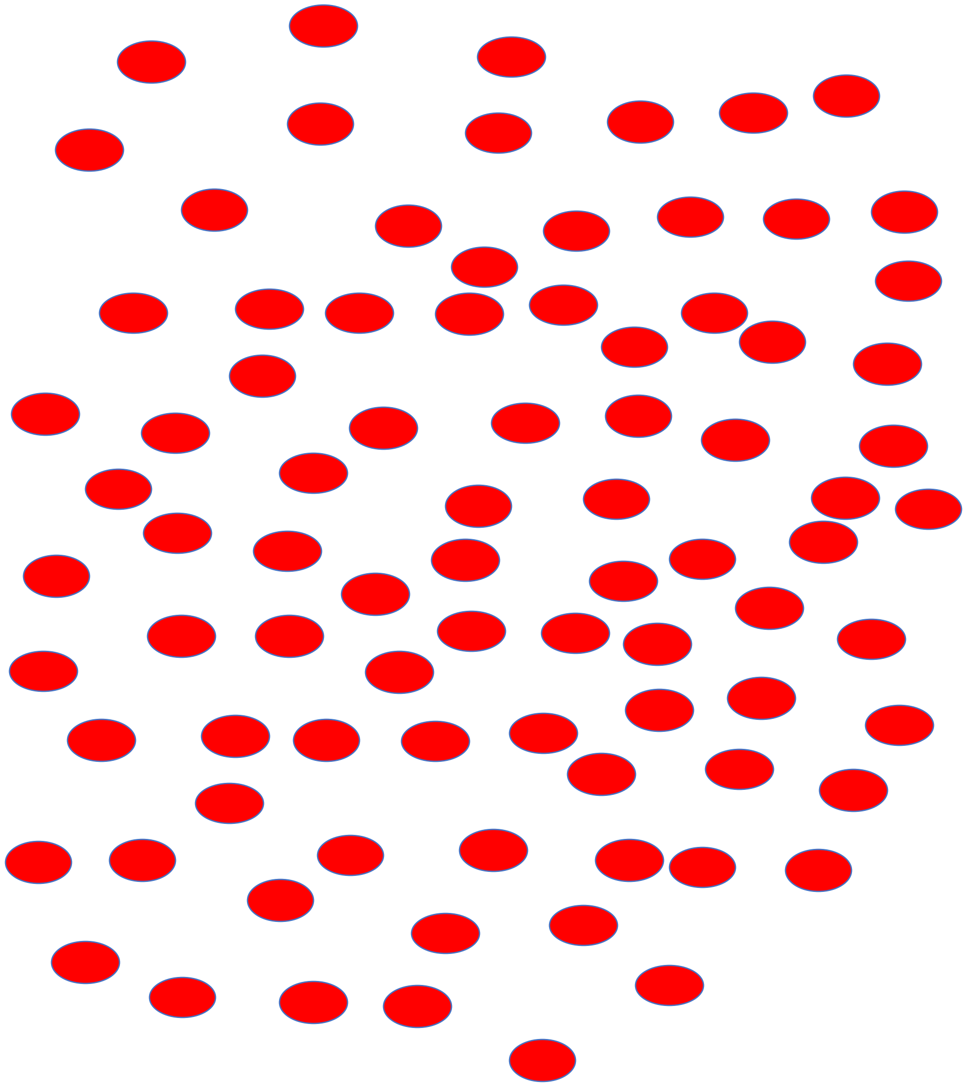
CMI ≤ X mg/L → Susceptible

CMI > X mg/L → Resistant

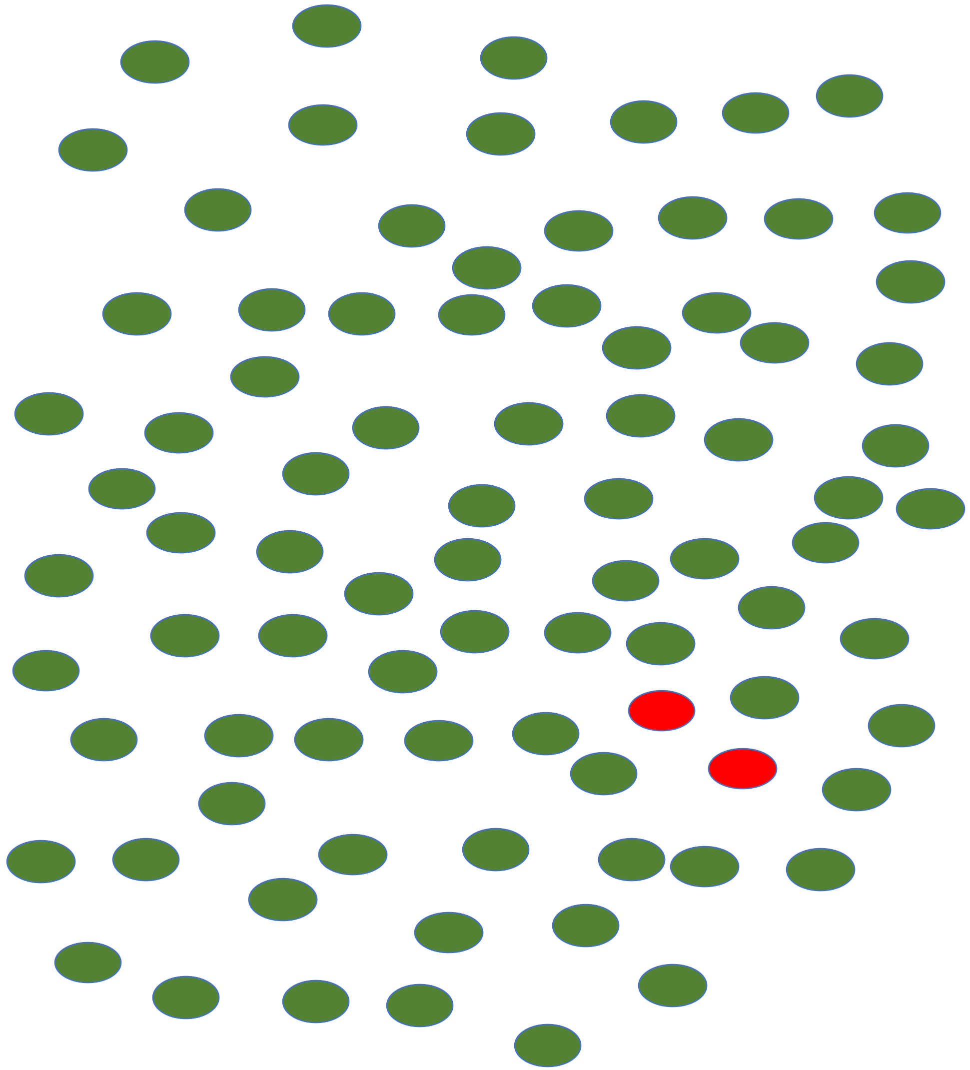
De ECOFFs a puntos de corte

PK/PD integration	Activity concentration	High PAFE	Pharmacodynamic index	
			Best predictor	PDT (Animal models)
Fluconazole	Independent	Yes	AUC/MIC	AUC/MIC >25-50
Voriconazole	Independent	Yes	AUC/MIC	AUC/MIC ≥20 or = 1.7-11
Posaconazole	Independent	Yes	AUC/MIC	AUC/MIC =1.7-11
Isavuconazole	Independent	Yes	AUC/MIC	AUC/MIC >25-50 or = 1.7-11
AmB deoxycholate	Dependent	Yes	C_{max}/MIC	C_{max}/MIC = 2.4 or >4
L-AmB	Dependent	Yes	C_{max}/MIC	
Caspofungin **Anidulafungin** **Micafungin**	Dependent	Yes	C_{max}/MIC or AUC/MIC	C_{max}/MIC >1 or AUC/MIC = 10-20 C_{max}/MIC = 10-20

RESISTENCIA INTRÍNSECA



RESISTENCIA ADQUIRIDA



RESISTENCIA INTRÍNSECA

RESISTENCIA universal inferida tras identificación a nivel de especie

VALOR MARGINAL DE AFST

TODAS las cepas son resistentes

AFST NO IMPACTO EN DECISIONES CLINICAS

Pide a tu micólogo que realice una ID correcta y precisa

RESISTENCIA ADQUIRIDA

SUSCEPTIBILIDAD universal no puede ser inferida tras identificación a nivel de especie

VALOR RELEVANTE DE AFST

ALGUNAS cepas son resistentes

DECISIONES CLINICAS GUIADAS POR AFST

Pide a tu micólogo que además realice AFST

RESISTENCIA INTRÍNSECA

Especies	Antifúngico
Filamentosos	Fluconazol
A. fumigatus especies crípticas	Anfotericina B Azoles
A. terreus *A. nidulans* *A. flavus*	Anfotericina B
A. ustus	Azoles
C. krusei *C. auris*	Fluconazol
Rhodotorula *Trichosporon*	Equinocandinas

ESPECTRO SENSIBILIDAD LEVADURAS

	Anfo B	Fluco	Vori	Posa	Isavu	Candinas	Ibrexafungerp	Olorofima
C. albicans								
C. parapsilosis								
C. glabrata								
C. tropicalis								
C. krusei								
C. lusitaniae								
C. auris								
Otras *Candida* spp								
Otras levaduras								

Anfo B: anfotericina B; **Fluco:** fluconazol; **Vori:** voriconazol; **Posa:** posaconazol; **Isavu:** isavuconazol

Rojo: inactivo; **Naranja:** actividad parcial; **Verde:** actividad *in vitro*

Elaboración propia J Guinea

ESPECTRO SENSIBILIDAD HONGOS FILAMENTOSOS

	Anfotericina B	Fluconazol	Vori	Posa	Isavu	Candinas	Olorofima
Aspergillus fumigatus ss							
***A. fumigatus* especies crípticas**							
A. terreus							
A. flavus							
A. nidulans							
A. ustus							
Mucorales							
Fusarium							Cepa variable
Lomentospora prolificans							
Scedosporium boydii ***Scedosporium apiospermum***							

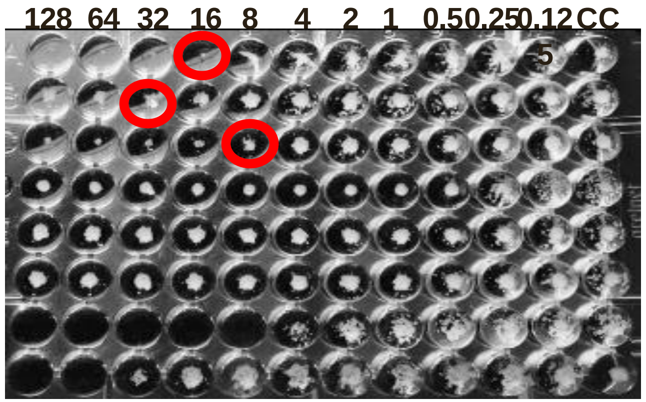
Anfo B: anfotericina B; **Fluco:** fluconazol; **Vori:** voriconazol; **Posa:** posaconazol; **Isavu:** isavuconazol

Rojo: inactivo; **Naranja:** actividad parcial; **Verde:** actividad *in vitro*

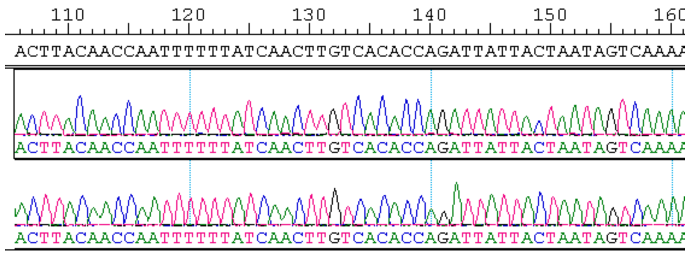
Elaboración propia J Guinea

Combinaciones fármaco-especie marcadas en verde representan aquellas en las que la resistencia secundaria puede aparecer

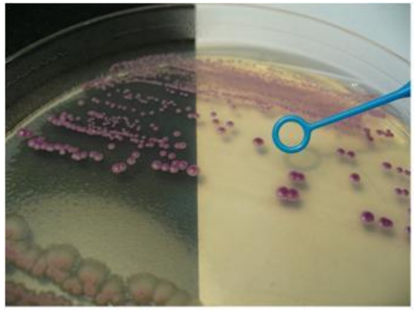
	Anfotericina B	Fluconazol	Vori	Posa	Isavu	Candinas	Olorofima
Aspergillus fumigatus ss							
A. fumigatus especies crípticas							
A. terreus							
A. flavus							
A. nidulans							
A. ustus							
Mucorales							
Fusarium							Cepa variable
Lomentospora prolificans							
Scedosporium boydii *Scedosporium apiospermum*							



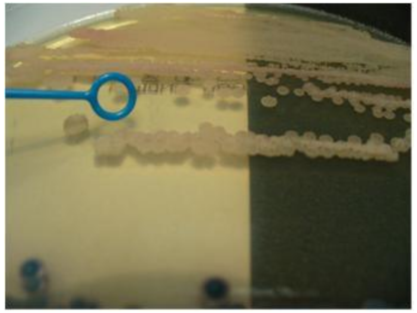
CMI: Concentración mínima de antifúngico capaz de provocar una inhibición (parcial o completa) del crecimiento fúngico respecto al control libre de antifúngico



SNP: Single nucleotide polymorphisms



C. glabrata **resistente a equinocandinas**



C. parapsilosis **resistente a fluconazol**

C. auris

RESISTENCIA EN *CANDIDA*

- **Estudios multicéntricos nacionales**
- **Resistencia variable**
  - Localización geográfica
  - Uso de antifúngicos
  - Dispersión de clones resistentes
  - Distribución de especies
  - ***C. auris***
- **España** (Estudio CANDIPOP)
  - R equinocandinas: 1,8-3,1%
  - R fluconazol: 6%-6,9%

País	Periodo	Número hospitales	% Resistencia		Referencia
			FLZ	ECN	
Islandia	2000-2011	14	2,7	0	(Asmundsdottir et al., 2013)
Noruega	2004-2012	ND	5,2	1,8	(Hesstvedt et al., 2015)
Suecia	2015-2016	ND	0 - 100	3,8	(Klingspor et al., 2018)
Dinamarca	2016-2018	ND	11,1	0,2 - 4,2	(Risum et al., 2021)
Bélgica	2013-2014	30	7,6	0,3	(Trouvé et al., 2017)
Suiza	2004-2018	7	0 - 12	0 - 2	(Adam et al., 2021)
Austria	2007-2016	6	2 - 13	1 - 16	(Beyer et al., 2019)
Grecia	2009-2018	28	10,6	0,5 - 1,3	(Mamali et al., 2022)
España	2010-2011	29	6-6,9	1,8 - 3,1	(Guinea et al., 2014)
Portugal	2011-2012	10	4 - 5	3 - 15	(Faria-Ramos et al., 2014)
Italia	2016-2017	12	1,2-10	0 - 1,9	(Prigitano et al., 2020)
Canadá	2011-2016	18	0,6 - 4,9	0 - 2,5	(Fuller et al., 2019)
Estados unidos	2012-2016	ND	7	1,6	(Toda et al., 2019)
Brasil	2007-2010	16	8,5	0	(Doi et al., 2016)
Colombia	2008-2009	7	17,6	ND	(Cortés et al., 2014)
Perú	2013-2015	3	2,6	0	(Rodriguez et al., 2017)
Chile	2013-2017	26	0-48	0 - 3,7	(Santolaya et al., 2019)
China	2015-2017	77	2,5 - 29,7	0-7	(Xiao et al., 2020)
Kuwait	2018	12	0-100	0-3	(Alobaid et al., 2021)
Corea del Sur	2020-2021	9	2,9	0 - 0,1	(Won et al., 2022)
Australia	2014-2015	29	0,87 - 23,1	0 - 0,8	(Chapman et al., 2017)
Sudáfrica	2009-2010	16	0,2 - 53	0,2 - 2	(Govender et al., 2016)
Argelia	2016-2019	7	0 - 31,5	0	(Megri et al., 2020)

RESISTENCIA EN *CANDIDA* EN NUESTRO ENTORNO

- **Como regla general podemos asumir que….**
  - No hay resistencia a anfotericina B en *Candida*
  - Resistencia a azoles o equinocandinas anecdótica en *C. albicans* o *C. tropicalis*
  - Despistaje de resistencia a equinocandinas y fluconazol en *C. glabrata*
  - Con las levaduras diferentes a *Candida* las cosas se complican

- **Pero tenemos retos actuales….**
  - Resistencia a azoles en *C. parapsilosis*
  - Emergencia de *C. auris*

Bases moleculares resistencia a fluconazol *C. parapsilosis*

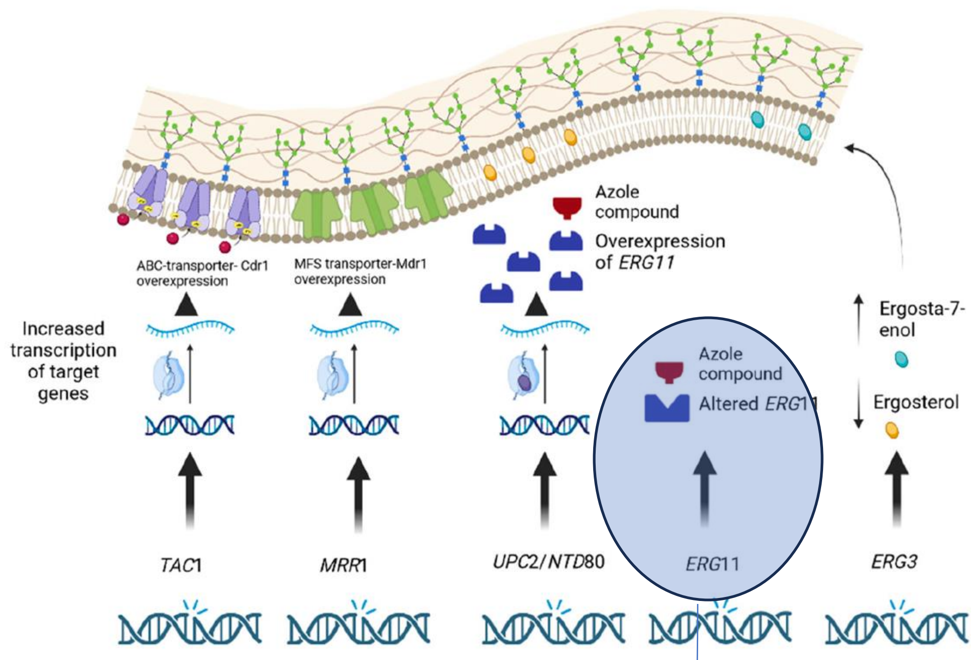


Figure 1. Overview of azole-resistance mechanisms in *C. parapsilosis sensu stricto*.

Ref. Franconi I. *J Fungi* 2023

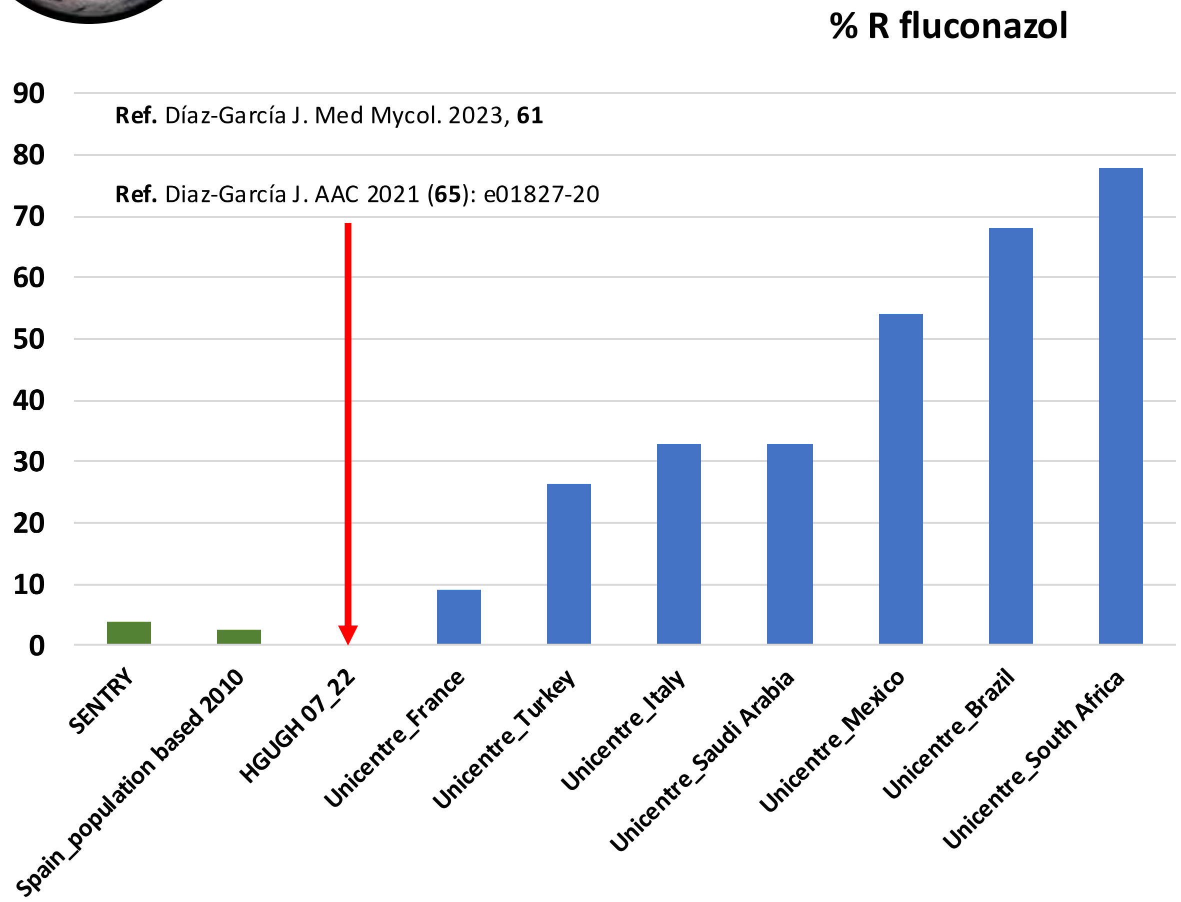
Lanosterol 14-α desmetilasa (ERG11p)

TABLE 1 ERG11p substitutions reported in *C. parapsilosis* isolates and their fluconazole resistance profile.

ERG11p amino acid substitutions	Expected pattern of azole susceptibility		References
	Fluconazole	Voriconazole	
Y132F*	R	R or I	(Grossman et al., 2015; Souza et al., 2015; Asadzadeh et al., 2017; Magobo et al., 2017; Choi et al., 2018; Thomaz et al., 2018; Alobaid and Khan, 2019; Singh et al., 2019, Arastehfar et al., 2020a, Arastehfar et al., 2020b, Castanheira et al., 2020; Magobo et al., 2020; Martini et al., 2020; Arastehfar et al., 2021; Corzo-Leon et al., 2021; Demirci-Duarte et al., 2021; Fekkar et al., 2021; Thomaz et al., 2021; Alcoceba et al., 2022, Diaz-Garcia et al., 2022a, Hare et al., 2022; Thomaz et al., 2022; Zaragoza et al., 2022)
K143R**	R	S	(Singh et al., 2019, Arastehfar et al., 2020a, Castanheira et al., 2020)
G458S***	R	R or I	(Arastehfar et al., 2020a, Demirci-Duarte et al., 2021, Diaz-Garcia et al., 2022a)
K128N	R	S	(Choi et al., 2018)
D133Y	S	S	(Hare et al., 2022)
D247G	S	S	(Arastehfar et al., 2020b)
G89V	S	S	(Arastehfar et al., 2020b)
I302T	S	S	(Hare et al., 2022)
L109F	S	S	(Arastehfar et al., 2020b)
M178T	SDD or S	S	(Grossman et al., 2015; Asadzadeh et al., 2017)
N283Y	SDD or S	S	(Grossman et al., 2015; Asadzadeh et al., 2017)
P406Q	S	S	(Arastehfar et al., 2020b)

Ref. Escribano P. *Frontiers* 2022

C. parapsilosis resistente a fluconazol



Ref. Pfaller M. *Open Forum Infect Dis* 2017

Ref. Magobo. *Mycoses* 2020 63:471-477

Ref. Thomaz DY. *J Fungi* 2021 7(4):259

Ref. Corzo-Leon. *Med Mycol* 2021 59:664-671

Ref. Arashtefar. *Antimicrob Agents Chemother* 2020 64(10):e01001-20

Ref. Mesini. *Mycoses* 2020 63:361-368

Ref. Fekkar. *Antimicrob Agents Chemother* 2021 65(5):e02036-20

Ref. Aladeer. *BMC Infect Dis* 2020 20(1):55

C. parapsilosis resistente a fluconazol

Fluconazole-resistant *Candida parapsilosis*: A new emerging threat in the fungi arena

Pilar Escribano[1,2] and Jesús Guinea[1,2,3]*

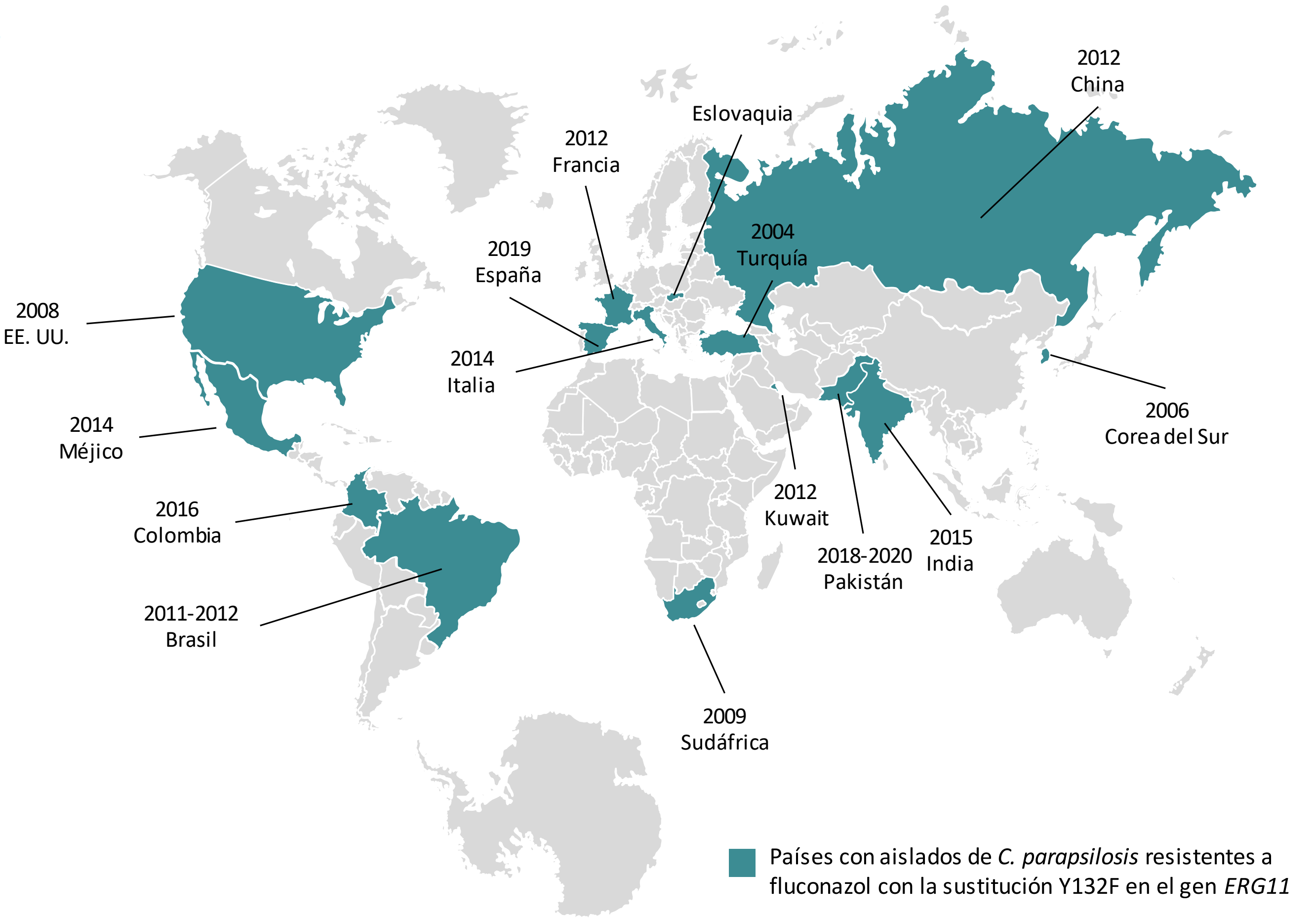
[1]Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain, [2]Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain, [3]CIBER Enfermedades Respiratorias-CIBERES (CB06/06/0058), Madrid, Spain

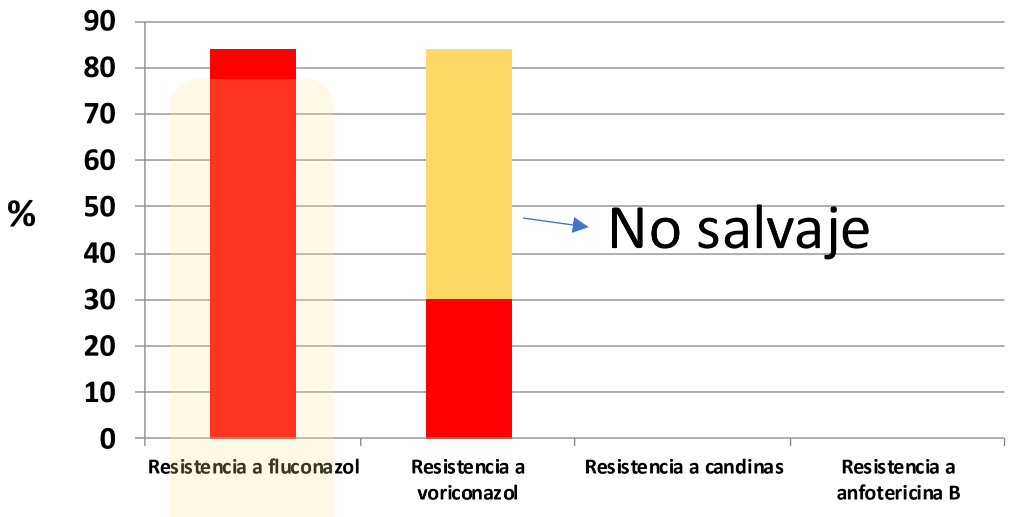
frontiers | Frontiers in Fungal Biology

Ref. Escribano P. Frontiers 2022

País	Sustitución Y132Fp (año de detección)	Referencias
Turquía	2004	Demirci-Duarte et al., 2021
Corea del Sur	2006	Choi et al., 2018
EEUU	2008	Grossman et al., 2015
Sudáfrica	2009	Magobo et al., 2020
Kuwait	2012	Asadzadeh et al., 2017
México	2014	Corzo-Leon et al., 2021
Italia	2014	Martini et al., 2020
India	2015	Singh et al., 2019
Colombia	2016	Ceballos-Garzón et al., 2023
Francia	2017	Castanheira et al., 2020
Brasil	2018	Thomaz et al., 2018
España	**2019**	**Alcoceba et al., 2022**

Modificación Y132F ERG11p mecanismo dominante

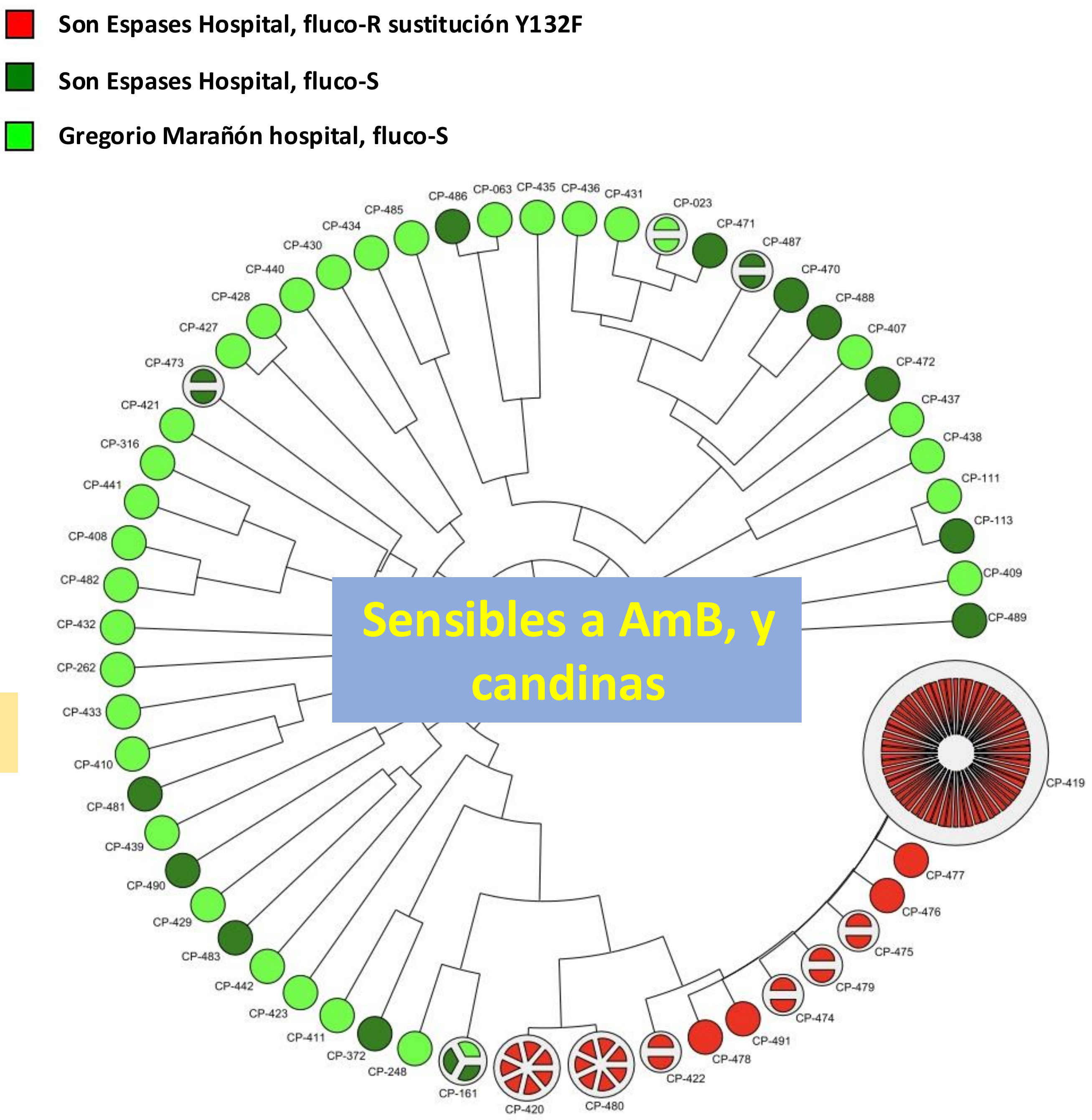




70% Resistencia en sangre

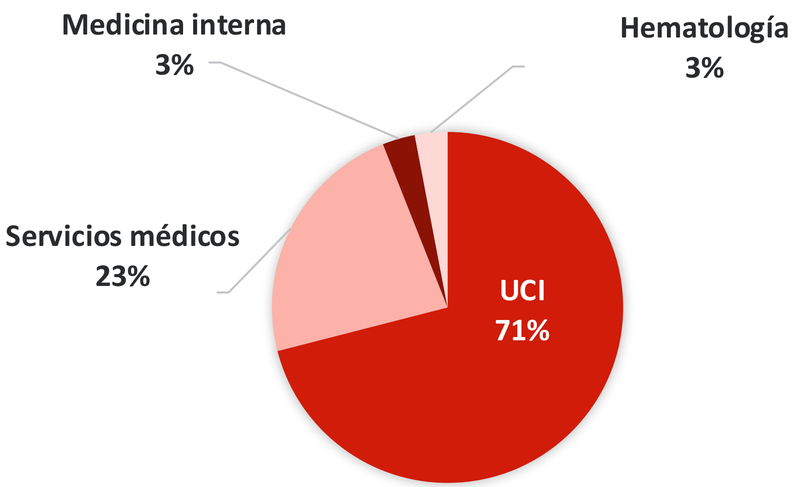
Voriconazol no salvajes

Todas Y132F ERG11p



Ref: Alcoceba E *et al.* Clin Microbiol Infect. 2022

Resistencia a fluconazol en 45 pacientes (78%) infectados



34% mortalidad

Sólo 20% de pacientes previo uso de fluconazol

Ref: Alcoceba E *et al*. Clin Microbiol Infect. 2022

Journal of *Fungi*

MDPI

Article

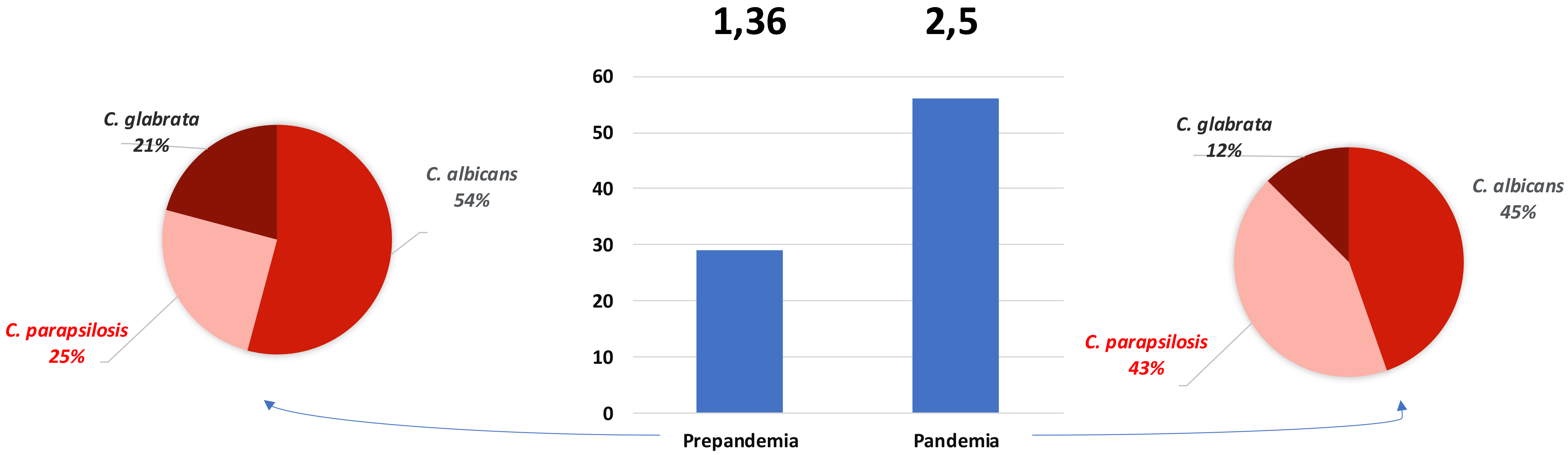
Impact of the COVID-19 Pandemic on the Clinical Profile of Candidemia and the Incidence of Fungemia Due to Fluconazole-Resistant *Candida parapsilosis*

Antonio Ramos-Martínez [1,2,*], Ilduara Pintos-Pascual [3], Jesús Guinea [4,5,6], Andrea Gutiérrez-Villanueva [3], Edith Gutiérrez-Abreu [3], Judith Díaz-García [4,5,6], Ángel Asensio [7], Reyes Iranzo [8], Isabel Sánchez-Romero [9], María Muñoz-Algarra [9], Víctor Moreno-Torres [3], Jorge Calderón-Parra [1], Elena Múñez [1] and Ana Fernández-Cruz [1]

Candidemias detectadas entre Enero 2019/Febrero 2020 y Marzo 2020/Septiembre 2021

Hospital Puerta de Hierro, Madrid

Incidencia (casos / 10.000 pacientes-día)



Ref: Ramos-Martínez A et al. J Fungi 2022

Pacientes sin COVID (prepandemia *vs* pandemia) NO DIFERENCIAS

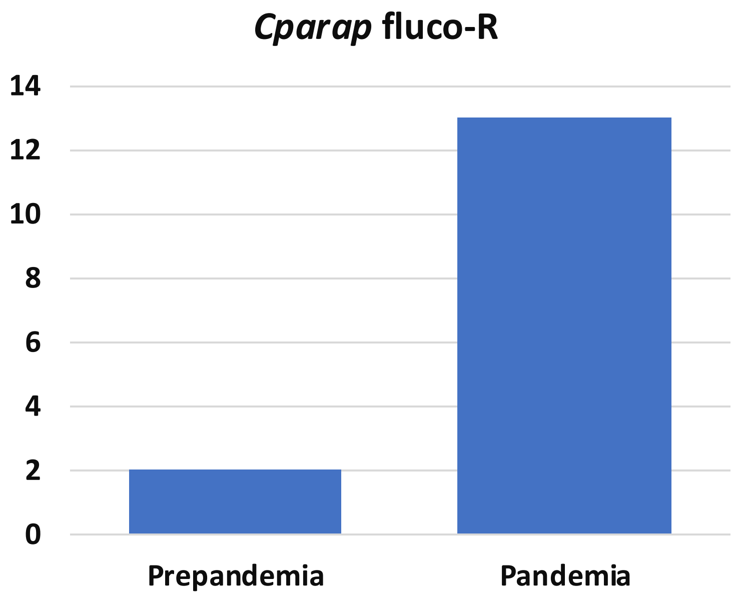
Pacientes con COVID *vs* no-COVID:

> Ingreso en UCI

> Previos corticoides y tocilizumab

> Catéter

< Cirugía abdominal



C. parapsilosis resistentes a fluconazol COVID *vs* no-COVID (44% *vs* 11%)

Mas frecuente en la UCI postquirúrgica

Todas las cepas portadoras Y132F en ERG11p

Ref: Ramos-Martínez A et al. J Fungi 2022

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SUSCEPTIBILITY

Check for updates

Evidence of Fluconazole-Resistant *Candida parapsilosis* Genotypes Spreading across Hospitals Located in Madrid, Spain and Harboring the Y132F ERG11p Substitution

Judith Díaz-García,[a,b] Ana Gómez,[a,b] Luis Alcalá,[a,b,c] Elena Reigadas,[a,b,c] Carlos Sánchez-Carrillo,[a,b,c] Ana Pérez-Ayala,[d] Elia Gómez-García de la Pedrosa,[e] Fernando González-Romo,[f] Paloma Merino-Amador,[f] María Soledad Cuétara,[g] Coral García-Esteban,[h] Inmaculada Quiles-Melero,[i] Nelly Daniela Zurita,[j] María Muñoz-Algarra,[k] Isabel Sánchez-Romero,[k] María Teresa Durán-Valle,[l] Aída Sánchez-García,[m] Eva Alcoceba,[n] Patricia Muñoz,[a,b,c,o] Pilar Escribano,[a,b] Jesús Guinea,[a,b,c] on behalf of the CANDIMAD study group

J Antimicrob Chemother 2022; **77**: 3102–3109
https://doi.org/10.1093/jac/dkac288 Advance Access publication 29 August 2022

Journal of Antimicrobial Chemotherapy

Blood and intra-abdominal *Candida* spp. from a multicentre study conducted in Madrid using EUCAST: emergence of fluconazole resistance in *Candida parapsilosis*, low echinocandin resistance and absence of *Candida auris*

Judith Díaz-García[1,2], Ana Gómez[1,2], Marina Machado[1,2], Luis Alcalá[1,2,3], Elena Reigadas[1,2,3,4], Carlos Sánchez-Carrillo[1,2,3], Ana Pérez-Ayala[5], Elia Gómez-García De La Pedrosa[6], Fernando González-Romo[7], María Soledad Cuétara[8], Coral García-Esteban[9], Inmaculada Quiles-Melero[10], Nelly Daniela Zurita[11], María Muñoz-Algarra[12], María Teresa Durán-Valle[13], Aída Sánchez-García[14], Patricia Muñoz[1,2,3,4], Pilar Escribano[1,2]†, and Jesús Guinea[1,2,3]*† on behalf of the CANDIMAD Study Group‡

Enero 2019 – Dic. 2022

1 aislado por paciente y tipo de muestra

Aislados levaduriformes (n=2.900) de hemocultivos (51,7%) y muestras intraabdominales

Pacientes de 16 hospitales (Madrid)

Identificación molecular

Región ITS1-5.8S-ITS2 o f-ITS2-PCR-CE

Sensibilidad antifúngica EUCAST E.Def 7.3.2

Anfotericina B, azoles, anidulafungina, micafungina, ibrexafungerp

Secuenciación gen *ERG11* en cepas de *C. parapsilosis* resistentes a fluconazol
Secuenciación genes *FKS* en cepas de *Candida* spp resistentes a candinas

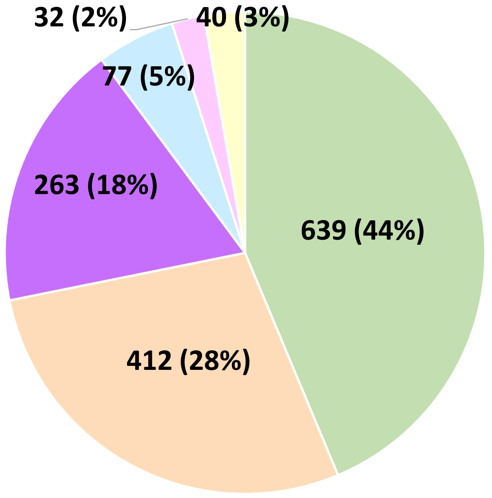
Ref. Díaz-García J. Antimicrob Agents Chemother 2022

Ref. Díaz-García J. J Antimicrob Chemother 2022

Situación de resistencia antifúngica en Madrid

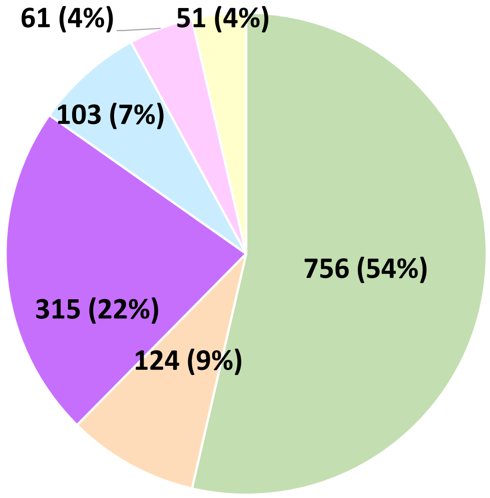
No *C. auris*

Hemocultivos



Mayor probabilidad desarrollar resistencia a fluconazol en sangre

Muestras intraabdominales



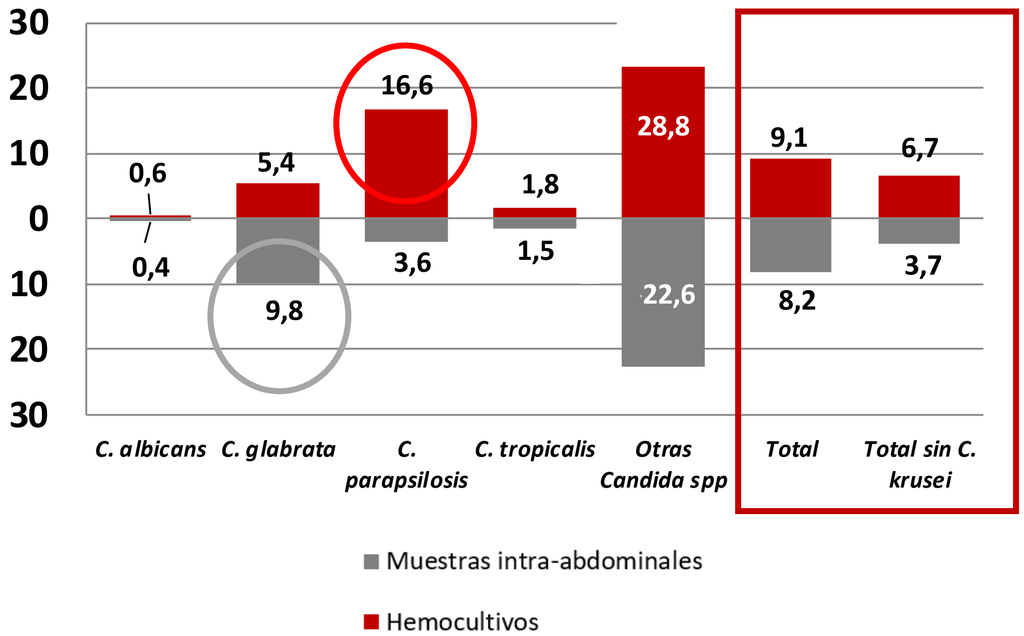
Mayor probabilidad desarrollar resistencia a equinocandinas en abdomen

C. albicans · *C. parapsilosis complex* · *C. glabrata* · *C. tropicalis* · *C. krusei* · Otras Candida spp.

Resistencia a fluconazol CANDIMAD

	Fluconazol
Hemocultivos	9,1%
Muestras intra-abdominales	8,2%

Fluconazol

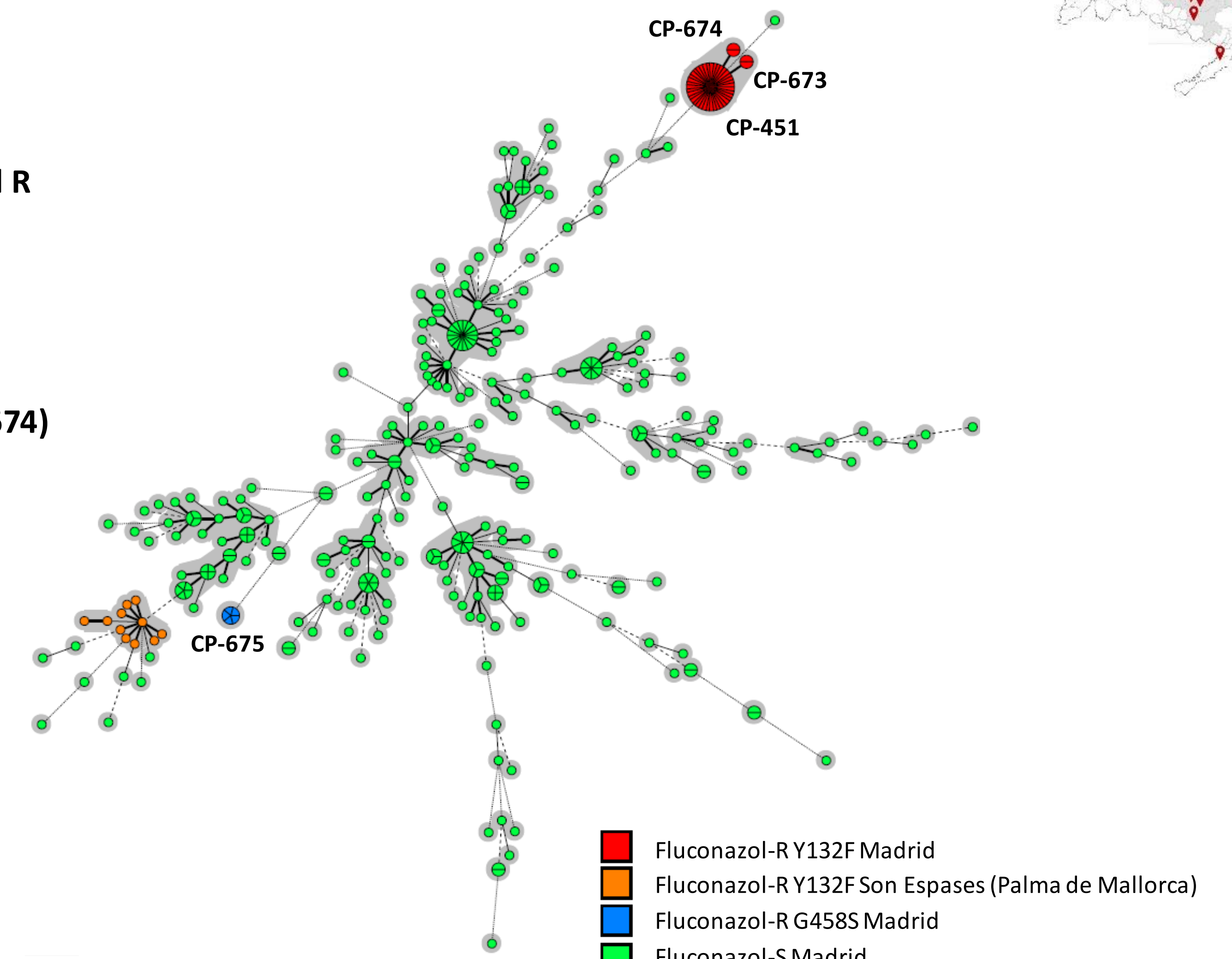


Ref. Díaz-García J. Antimicrob Agents Chemother 2022

Ref. Díaz-García J. J Antimicrob Chemother 2022

- **Y132F descrita en cuatro hospitales de Madrid**
- **G458S descrita por primera vez en España**

- **Dos perfiles de resistencia (*ERG11*)**
  - **Y132F + R398I (89,6%): Fluconazol y voriconazol R**
  - **G458S (10,4%): Panazol R**
- **Dos patrones genotípicos**
  - **Y132F: Complejo clonal (CP-451 + CP-673 + CP-674)**
  - **G458S: CP-675**

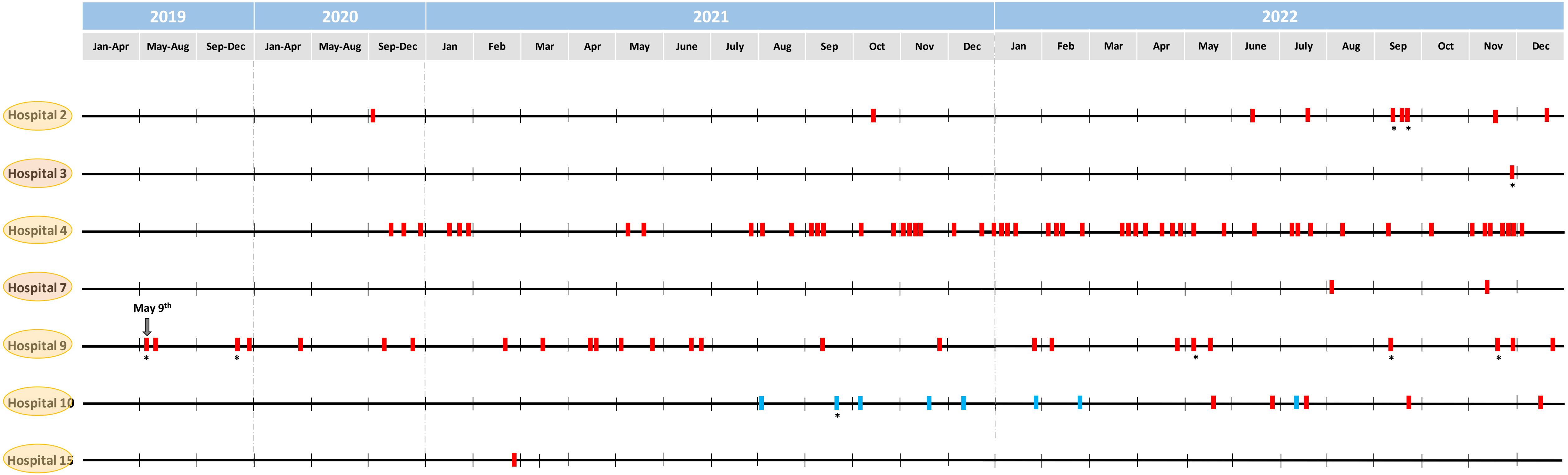


Ref. Díaz-García J. Antimicrob Agents Chemother 2022

Ref. Díaz-García J. J Antimicrob Chemother 2022

7 hospitales afectados en Madrid

PRECOVID



Símbolos en rojo cepas con Y132F

Símbolos en azul cepas con G458S

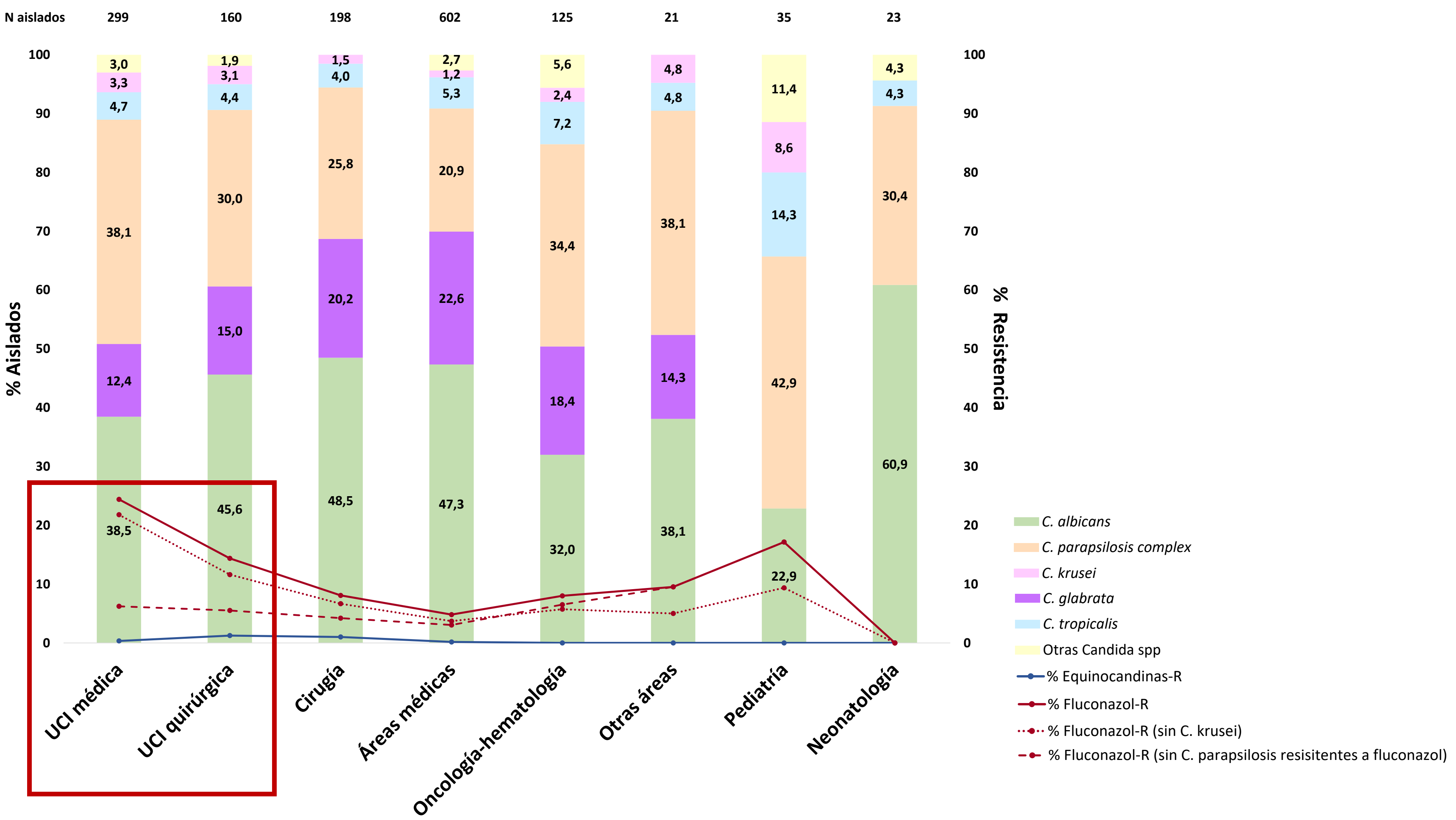
* Muestras intra-abdominales

Ref. Díaz-García J. Antimicrob Agents Chemother 2022

Ref. Díaz-García J. J Antimicrob Chemother 2022

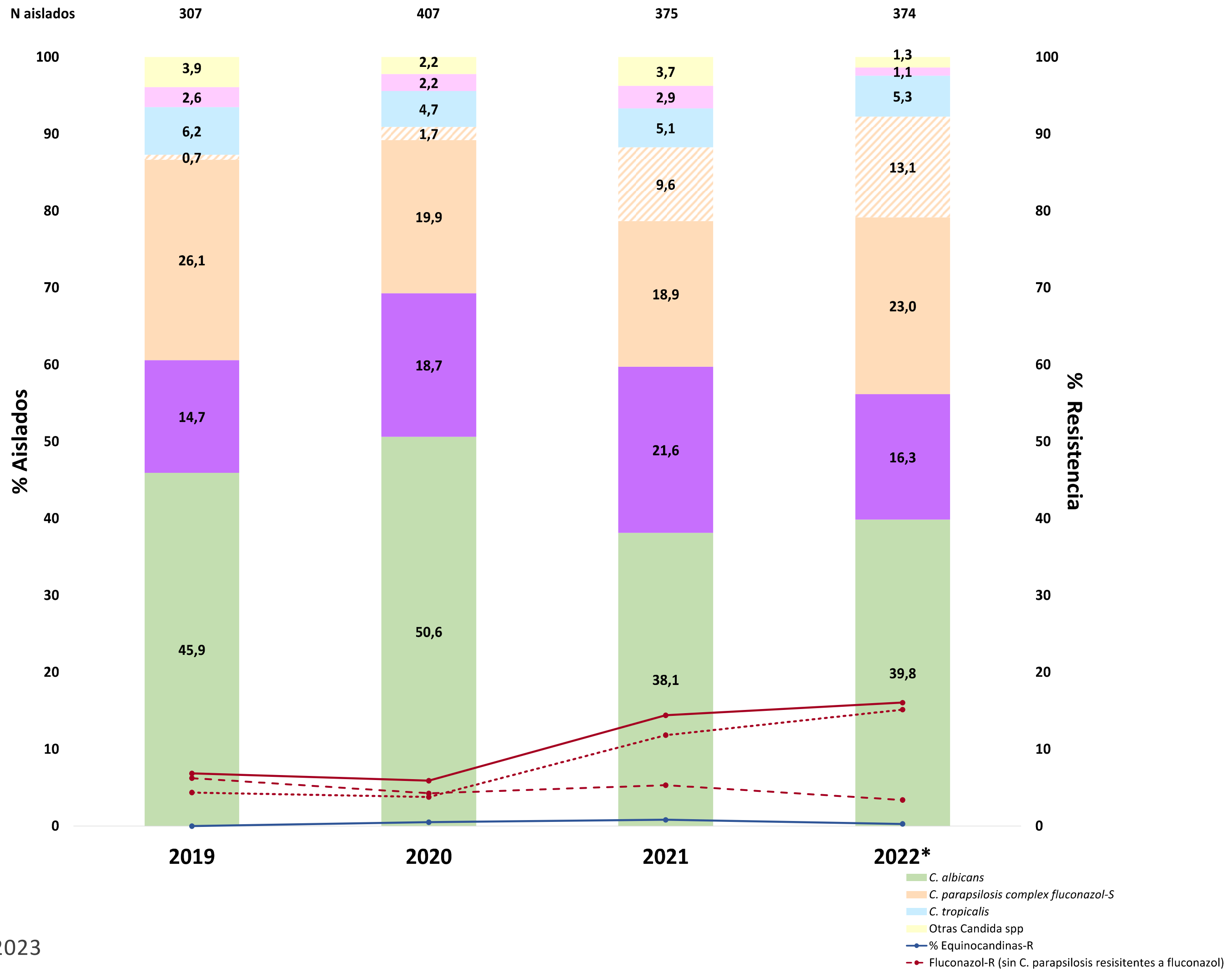
Ref. Díaz-García J. Antimicrob Agents Chemother 2023

Resistencia a fluconazol CANDIMAD



Resistencia a fluconazol CANDIMAD

Hemocultivos 2019-2022



Ref. Díaz-García J. Antimicrob Agents Chemother 2023

Journal of *Fungi*

MDPI

Article

Candida Genotyping of Blood Culture Isolates from Patients Admitted to 16 Hospitals in Madrid: Genotype Spreading during the COVID-19 Pandemic Driven by Fluconazole-Resistant C. parapsilosis

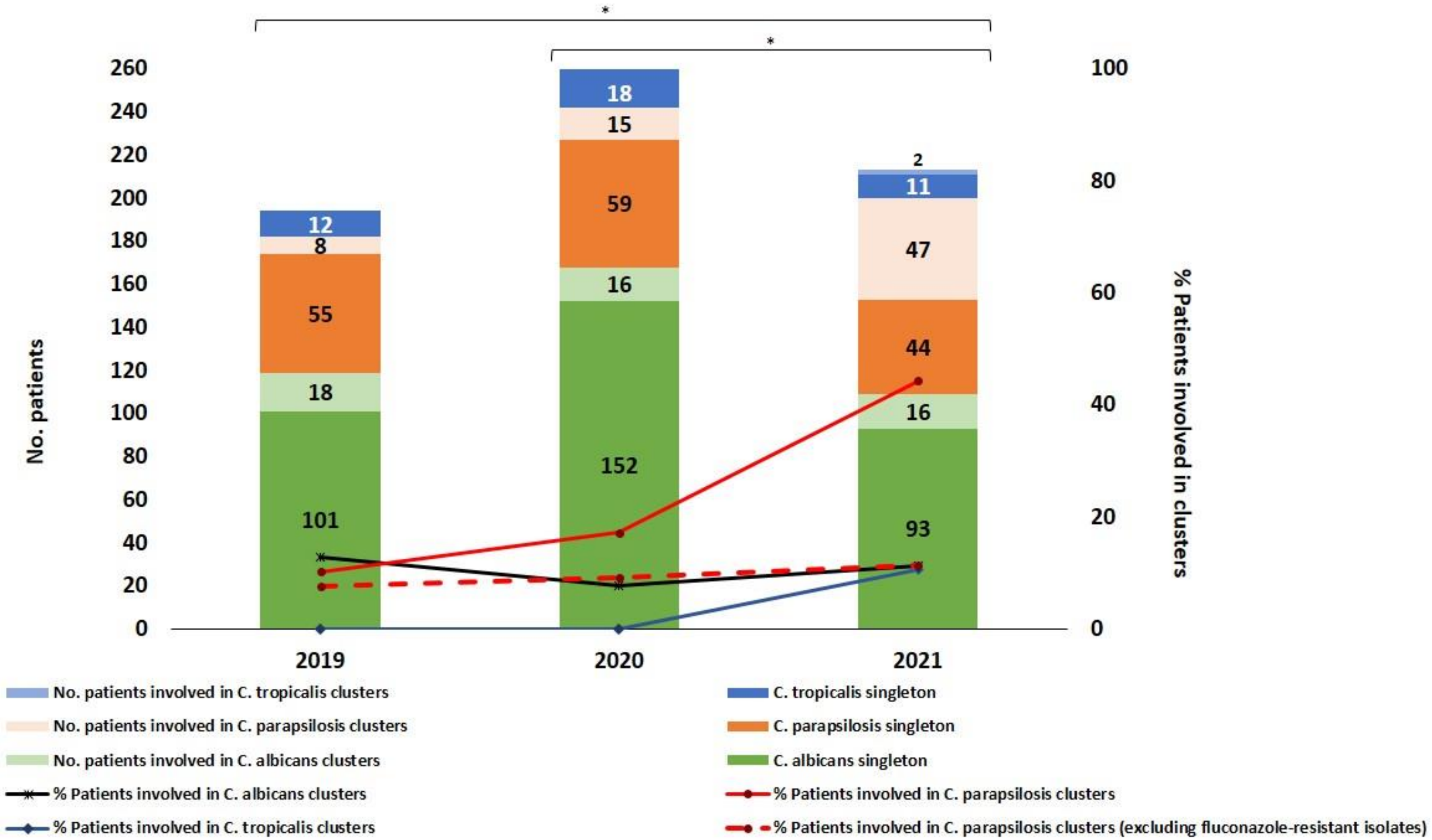
Judith Díaz-García [1,2], Ana Gómez [1,2], Marina Machado [1,2], Luis Alcalá [1,2,3], Elena Reigadas [1,2,3], Carlos Sánchez-Carrillo [1,2,3], Ana Pérez-Ayala [4,5], Elia Gómez-García de la Pedrosa [6,7,8], Fernando González-Romo [9,10], María Soledad Cuétara [11], Coral García-Esteban [12], Inmaculada Quiles-Melero [13], Nelly Daniela Zurita [14], María Muñoz Algarra [15], María Teresa Durán-Valle [16], Aída Sánchez-García [17], Patricia Muñoz [1,2,3,18], Pilar Escribano [1,2,†], Jesús Guinea [1,2,3,*,†] and on behalf of the CANDIMAD Study Group [‡]

> Nº *clusters* pandemia

> Nº de pacientes en *cluster* 2019/20/21

10,3%/17,2%/44,3%

Dominado por el clon fluco-R de *C. parapsilosis*



Ref. Díaz-García J. J Fungi 2022

ORIGINAL ARTICLE

mycoses WILEY

Clonal spread of fluconazole-resistant *C. parapsilosis* in patients admitted to a referral hospital located in Burgos, Spain, during the COVID-19 pandemic

María de los Ángeles Mantecón-Vallejo[1] | Aina Mesquida[2,3] | María de Valle Ortiz[4] | Luis Buzón-Martín[5] | Sergio Ossa-Echeverri[4] | Lourdes Fisac-Cuadrado[4] | Gregoria Megías-Lobón[1] | María Pilar Ortega-Lafont[1] | Patricia Muñoz[2,3,6,7] | Pilar Escribano[2,3,8] | Jesús Guinea[2,3,6,8]

Primera detección fenotípica de CPRF (fluco>4 mg/L E-test)

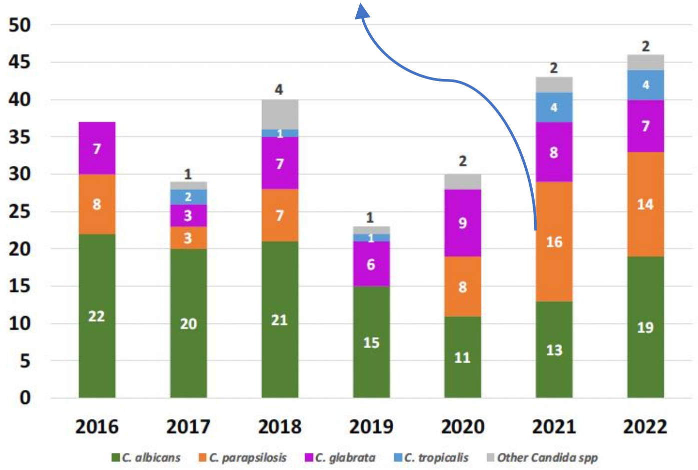


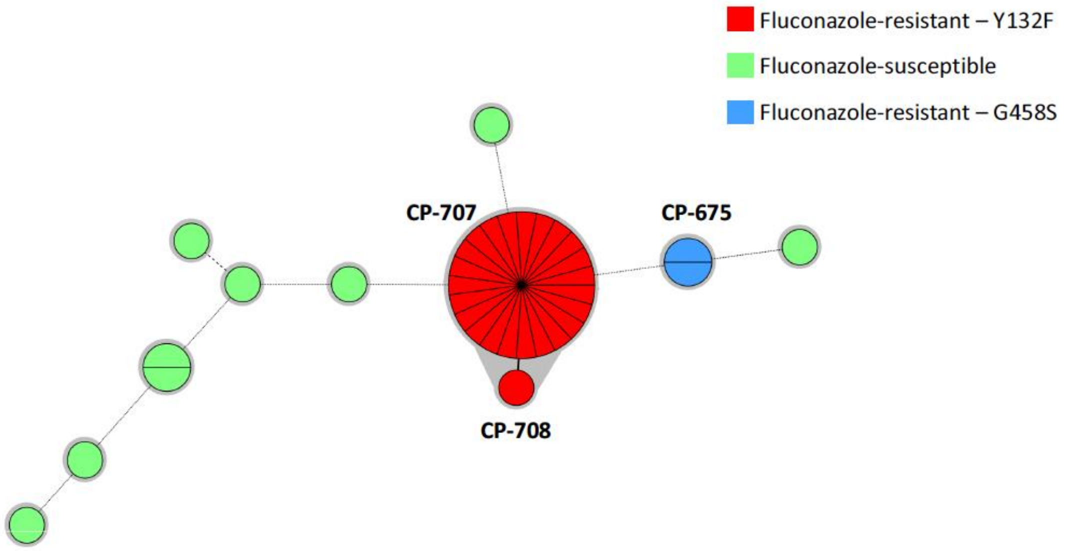
FIGURE 1 Cases of candidaemia (broken down per species) accounted at the hospital from 2016 to 2022.

Hospital terciario de referencia (777 camas)

Población de 257.003 habitantes

Todas *C. parapsilosis* desde Abril 2021 a Junio 2022

Resistencia a fluconazol 74%



Igual al genotipo de Madrid

Igual al genotipo de Madrid

Ref. Mantecón-Vallejo M. Mycoses 2024

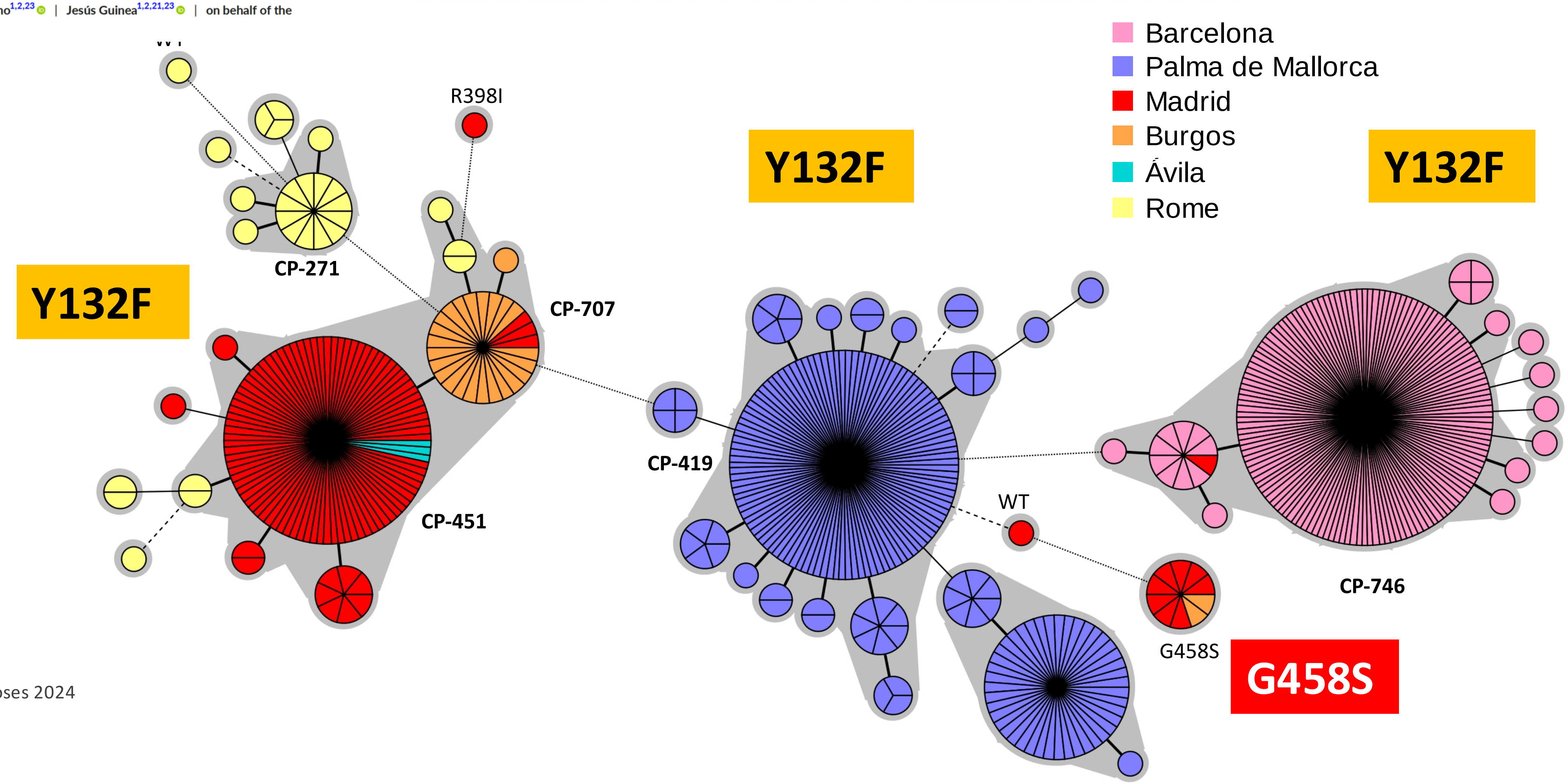
ORIGINAL ARTICLE

mycoses WILEY

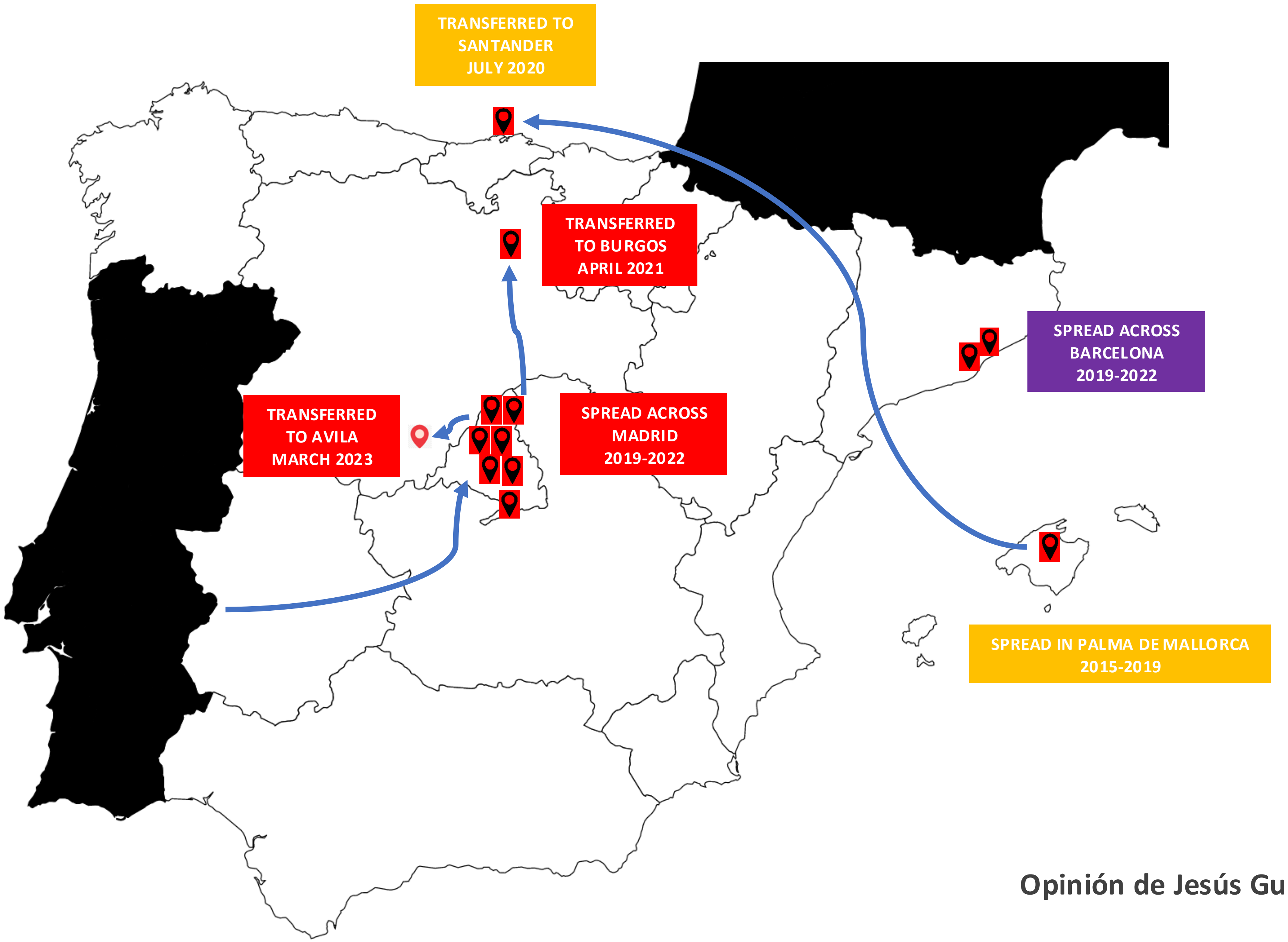
Fluconazole-resistant *Candida parapsilosis* genotypes from hospitals located in five Spanish cities and one in Italy: Description of azole-resistance profiles associated with the Y132F ERG11p substitution

Aina Mesquida[1,2] | Eva Alcoceba[3] | Eduardo Padilla[4] | Aída Ramírez[5] | Paloma Merino[6,7] | Fernando González-Romo[6,7] | Elena De Carolis[8] | Maurizio Sanguinetti[8] | María de los Ángeles Mantecón-Vallejo[9] | María Muñoz-Algarra[10] | Teresa Durán-Valle[11] | Ana Pérez-Ayala[12,13] | Elia Gómez-García-de-la-Pedrosa[14,15,16] | María del Carmen Martínez-Jiménez[17] | Miguel Ángel Sánchez-Castellano[17] | Inmaculada Quiles-Melero[18] | María Soledad Cuétara[19] | Aída Sánchez-García[20] | Patricia Muñoz[1,2,21,22] | Pilar Escribano[1,2,23] | Jesús Guinea[1,2,21,23] | on behalf of the CANDIMAD study group

A total of 53 genotypes (ranging from 1 to 19 per city; Table S1) were found; 26 were clusters involving 2 to 138 isolates per cluster. The genotypes found were different from genotypes involving fluconazole-susceptible isolates collected at sourcing hospitals or in our database, which includes about 900 genotypes, and were previously characterised (data not shown).



Ref. Mesquida A. Mycoses 2024



Opinión de Jesús Guinea

Trabajos futuros

PROYECTOS DE I+D+I EN SALUD

DATOS DE LA SOLICITUD

Para que esta solicitud sea válida ES IMPRESCINDIBLE que tenga entrada entre el martes, 13 de febrero de 2024 y el martes, 19 de marzo de 2024 inclusive, en el REGISTRO ELECTRÓNICO DEL ISCIII, con la firma ELECTRÓNICA reconocida del representante legal del centro solicitante.

DATOS DE SOLICITUD			
Nº de Expediente	PI24/00373	**Investigador Principal**	JESUS VICENTE GUINEA ORTEGA

TÍTULO DEL PROYECTO
Epidemiología de la fungemia en España: monitorización y vigilancia de cepas resistentes a antifúngicos

BÚSQUEDA ACTIVA DE Y132F

Screening de colonización

Sobre aislados obtenidos en rutina

C. parapsilosis sensu stricto

Y132F +

- **Medidas de aislamiento de paciente. ¿Es como *C. auris*?**
- **Genotipado para control de diseminación de clones**

Opinión de Jesús Guinea

Detección rápida de *C. parapsilosis*

Clinical Microbiology and Infection 30 (2024) 1447–1452

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

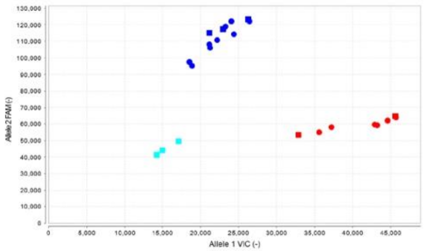
journal homepage: www.clinicalmicrobiologyandinfection.com

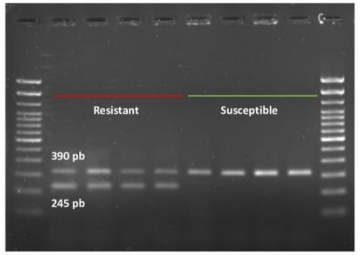
Original article

Fluconazole-resistant *Candida parapsilosis*: fast detection of the Y132F ERG11p substitution, and a proposed microsatellite genotyping scheme

Jesús Guinea [1, 2, 3, 4, *, †], Eva Alcoceba [5], Eduardo Padilla [6], Aída Ramírez [7], Elena De Carolis [8], Maurizio Sanguinetti [8], María Muñoz-Algarra [9], Teresa Durán-Valle [10], Inmaculada Quiles-Melero [11], Paloma Merino [12, 13], Fernando González-Romo [12, 13], Aída Sánchez-García [14], Elia Gómez-García-de-la-Pedrosa [15, 16, 17], Ana Pérez-Ayala [18, 19], María Ángeles Mantecón-Vallejo [20], Javier Pemán [21], María Soledad Cuétara [22], Nelly Daniela Zurita [23], Coral García-Esteban [24], María del Carmen Martínez-Jiménez [25], Miguel Ángel Sánchez Castellano [25], Elena Reigadas [1, 2, 3, 26], Patricia Muñoz [1, 2, 3, 26], Pilar Escribano [1, 2, 4, †]

Ref. Guinea J. CMI, 2024





Both formats 100% specificity and sensitivity

Turnaround time:

3 hours (conventional) and 1.5 hours (real time)

Despitaje de Y132F en aislados clínicos

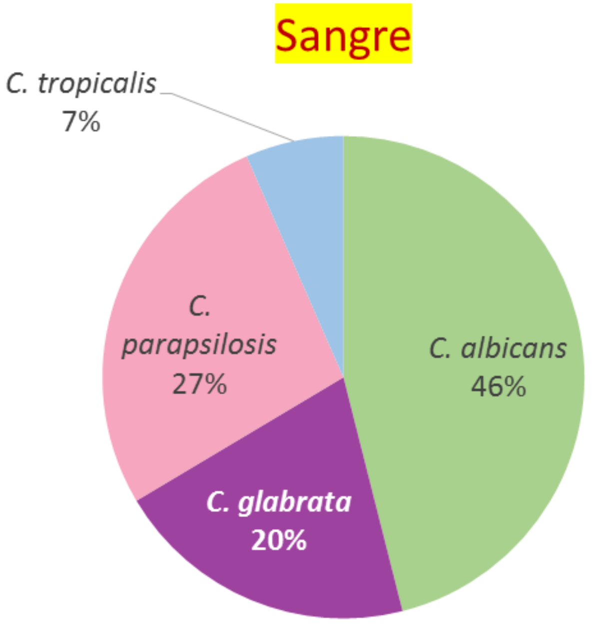
ERG11 gene sequencing and genotyping if Y132F is confirmed

AMERICAN SOCIETY FOR MICROBIOLOGY Antimicrobial Agents and Chemotherapy®

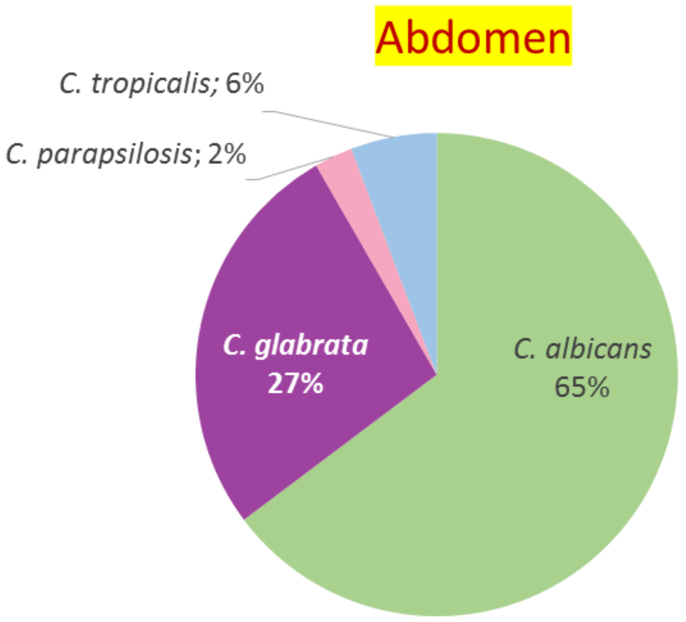
SUSCEPTIBILITY

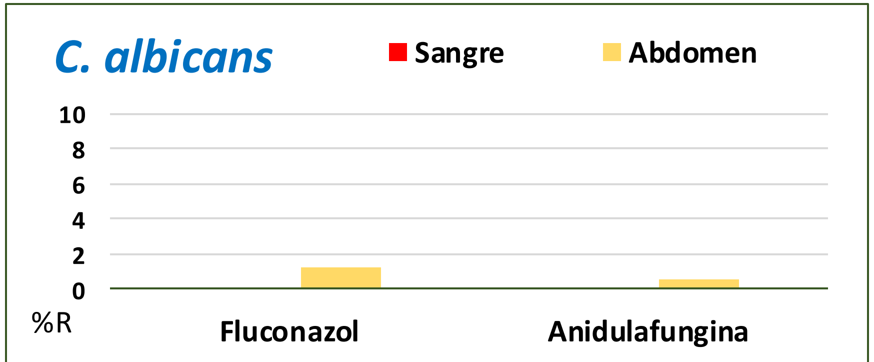
Antifungal Susceptibility Testing Identifies the Abdominal Cavity as a Source of *Candida glabrata*-Resistant Isolates

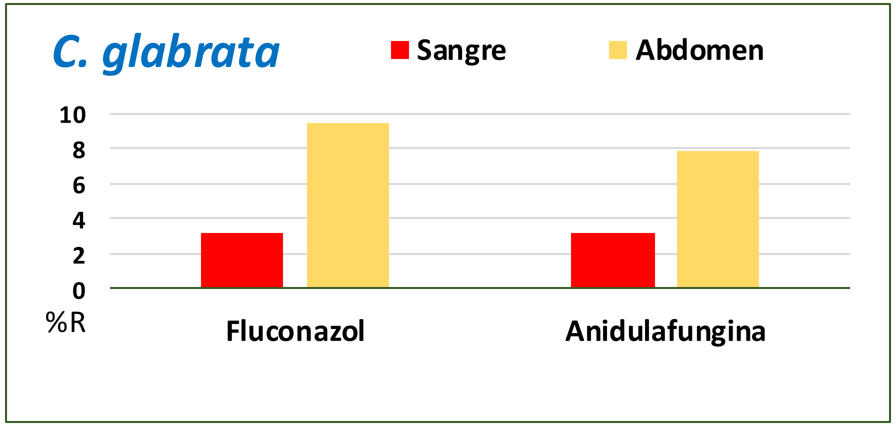
Judith Díaz-García,[a,b] Aina Mesquida,[a,b] Ana Gómez,[a,b] Marina Machado,[a,b] Pablo Martín-Rabadán,[a,b] Luis Alcalá,[a,b] Carlos Sánchez-Carrillo,[a,b,c] Elena Reigadas,[a,b] Teresa Vicente,[a,b] Patricia Muñoz,[a,b,c,d] Pilar Escribano,[a,b] Jesús Guinea[a,b,c]

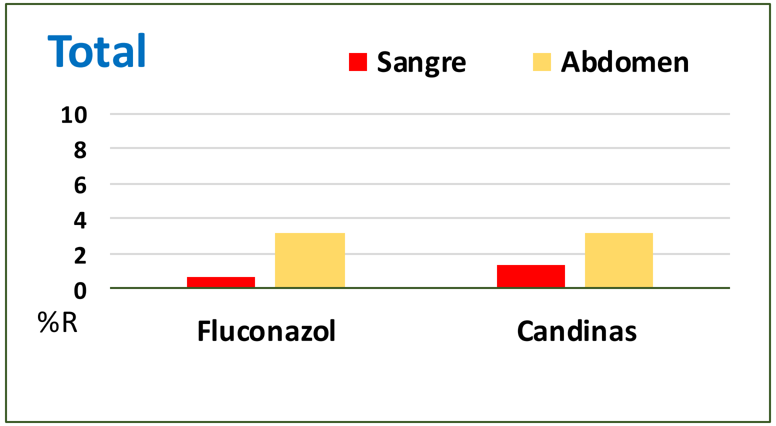


$P < 0.05$









Ref. Diaz-García J et al. AAC 2021

Clinical Microbiology and Infection 29 (2023) 1604.e1–1604.e6

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

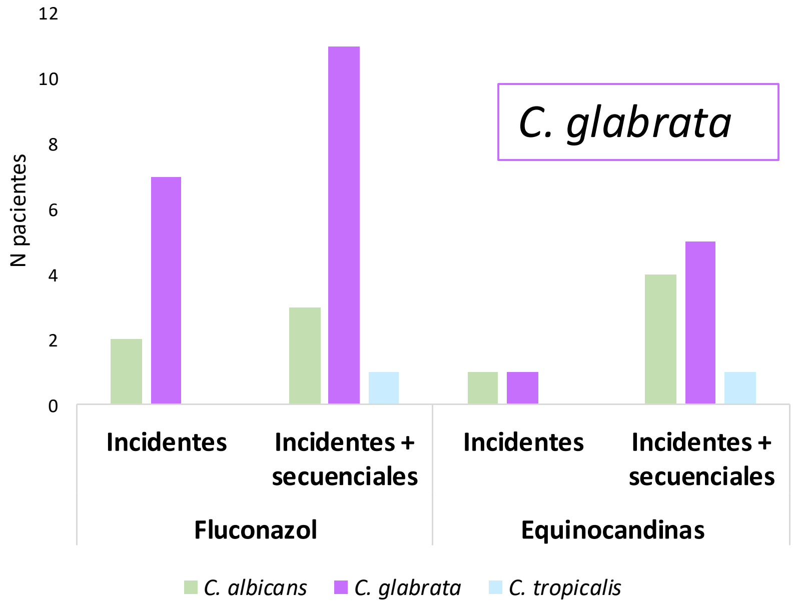
Research note

Antifungal resistance in *Candida* spp within the intra-abdominal cavity: study of resistance acquisition in patients with serial isolates

Judith Díaz-García [1,2], Marina Machado [1,2], Luis Alcalá [1,2,3], Elena Reigadas [1,2,3], Carlos Sánchez-Carrillo [1,2,3,†], Ana Pérez-Ayala [4,5], Elia Gómez-García de la Pedrosa [6,7,8], Fernando González-Romo [9,10], Paloma Merino [9,10], María Soledad Cuétara [11], Coral García-Esteban [12], Inmaculada Quiles-Melero [13], Nelly Daniela Zurita [14], María Muñoz-Algarra [15], María Teresa Durán-Valle [16], Gabriela Andrea Martínez-Quintero [16], Aída Sánchez-García [17], Patricia Muñoz [1,2,3,18], Pilar Escribano [1,2,19], Jesús Guinea [1,2,3,19,*] on behalf of the CANDIMAD study group[‡]

Patients	Isolates	Resistance rates per patient
Patients with sequential isolates (n = 112)	i) Incident + sequential	13.4% FLZ-R 8.9% ECND-R
	ii) Incident	8.0% FLZ-R 1.8% ECND-R

Resistance would have been overlooked in 61% patients if only incident isolate had been studied



Aspergillus sección *Fumigati*

	Anfo B	Azoles	Resistencia
A. fumigatus sensu stricto	S	S o R	Secundaria
N. pseudofisheri	S o R	R	Intrínseca
A. fumigatiaffinis *A. viridinutans* *A. lentulus* *N. udagawae*	R	R	Intrínseca

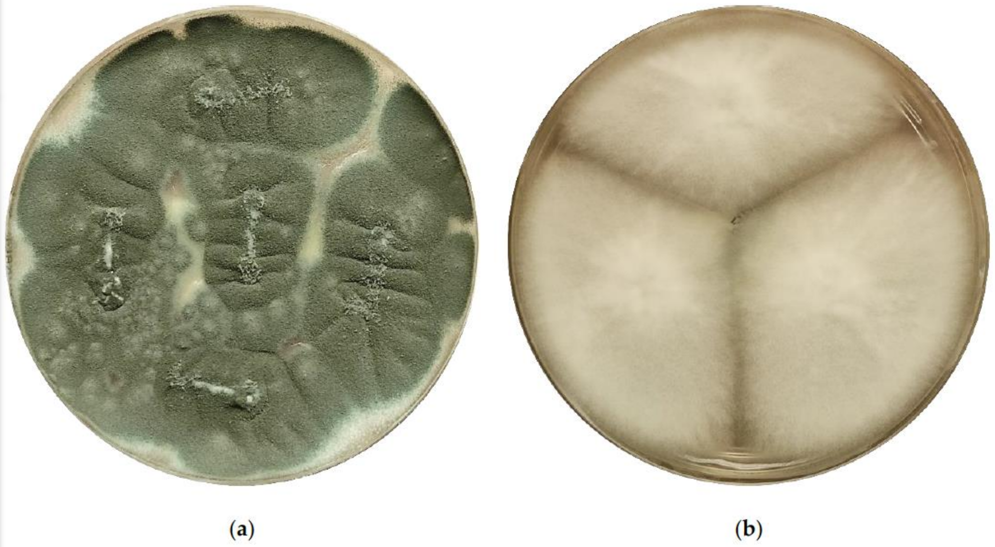


Figure 1. (a) *A. fumigatus sensu stricto* and (b) *A. lentulus* isolates grown on potato dextrose agar plates.

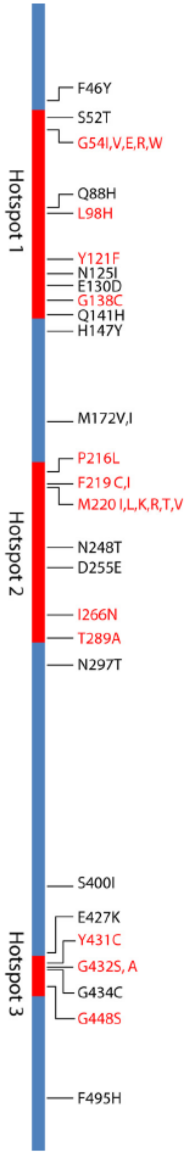
Ref: Van der Linden JWM *et al.* Med Mycol. 2011 Apr;49 Suppl 1:S82-9; Krishnan S *et al.* Mycoses 2009 May;52(3):206-22; Guinea J. J. Fungi 2020, 6, 343.

Anfo B: anfotericina B. **S**: sensible. **R**: resistente.

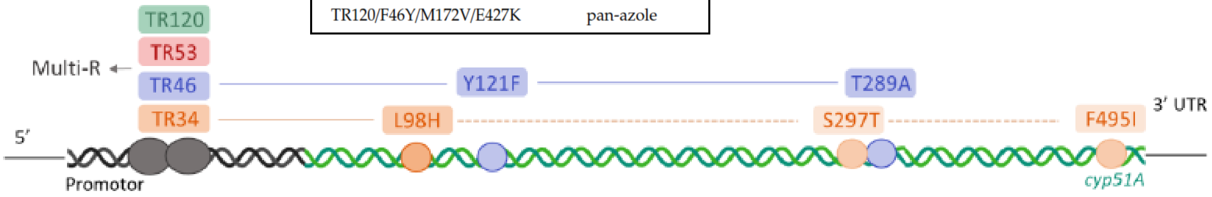
Resistencia a azoles en *Aspergillus fumigatus ss*

Diana: 14-α esterol desmetilasa (gen *Cyp51A*)

Alteraciones en gen *cyp51A*: mutaciones puntuales o sobreexpresión génica



cyp51A Polymorphism	Reported Azole Resistance
TR34/L98H	pan-azole
TR46/Y121F/T289A	voriconazole, posaconazole
TR53	itraconazole, voriconazole
TR120/F46Y/M172V/E427K	pan-azole



cyp51A Polymorphism	Resistance
N22	itraconazole
G54	itraconazole, posaconazole
Y121	voriconazole
G138	pan-azole
Q141	pan-azole
H147	pan-azole
P216	itraconazole, posaconazole
F219	itraconazole, posaconazole
M220	itraconazole, posaconazole
M236	itraconazole

cyp51A Polymorphism	Resistance
P394	itraconazole
Y431	pan-azole
G432	itraconazole, voriconazole
G434	pan-azole
T440	itraconazole
G448	itraconazole
Y491	itraconazole

Ruta clínica

Inhalación esporas **S**

Tratamiento azoles

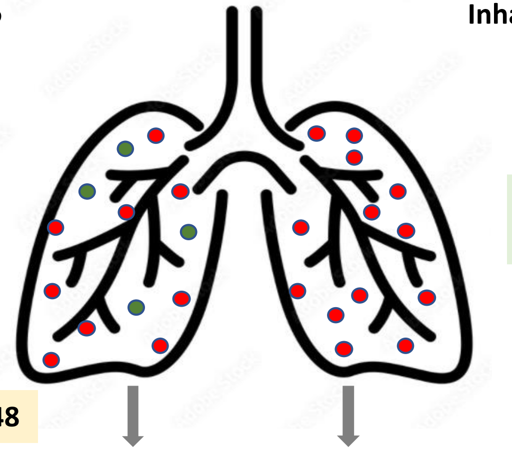
Desarrollo R

G54, P216, M220, G448

Ruta ambiental

Inhalación esporas **R**

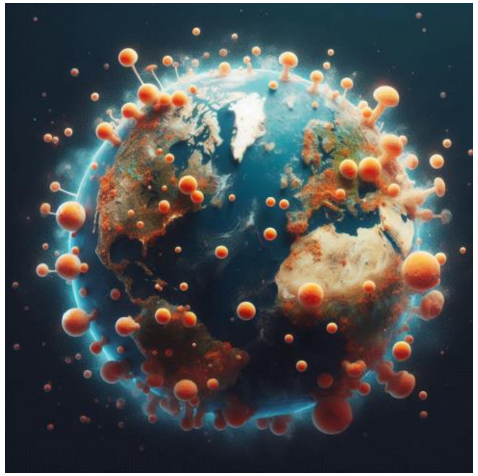
TR34, TR46, TR53, TR120



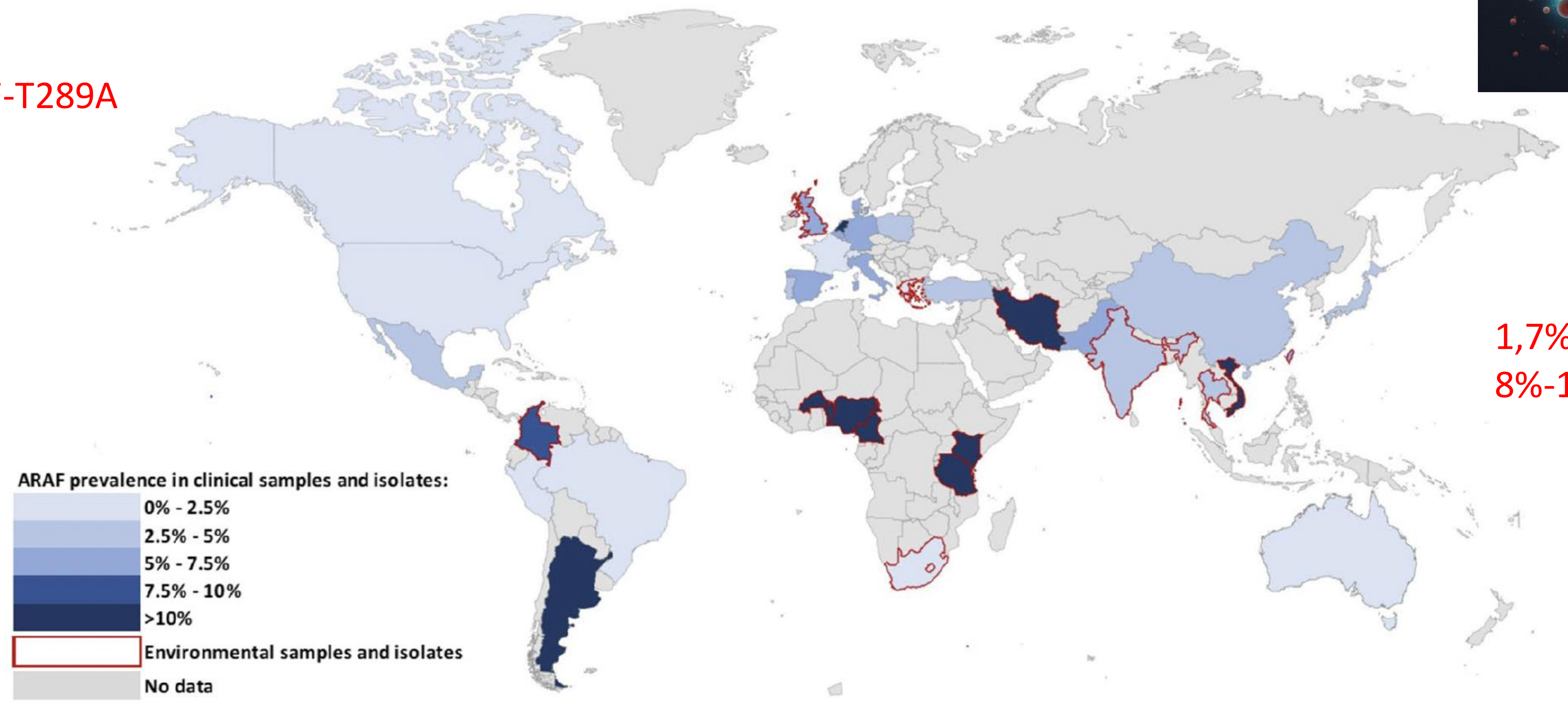
FALLO TERAPÉUTICO

Dudakova *et* al. Clin Microbiol Rev. 2017 Oct;30(4):1065-1091
van der Torre M. J. Fungi. 2020, 10;6(1):12
González-Jiménez I. Tesis Doctoral. Origen, desarrollo y nuevos mecanismos de resistencia a azoles en *Aspergillus fumigatus*. 2022
Wiederhold NP and Verweij PE. Curr Opin Infect Dis. 2020 Aug;33(4):290-297

Resistencia en el mundo



TR$_{34}$-L98H
TR$_{46}$-Y121F-T289A



HOLANDA
1,7%-6% 1997-2007
8%-15% 2013-2018

Fig. 1 Worldwide prevalence of azole resistant *Aspergillus fumigatus* (ARAF) clinical and environmental samples and isolates

Estudio *ASPE*IN (resistencia azoles *A. fumigatus* España)

- 30 hospitales invitados
- Cepas *A. fumigatus complex* (feb - mayo, 2019)
- Una colonia por cultivo y morfotipo
- Hospital Gregorio Marañón ID MALDI-TOF
- Sensibilidad antifúngica EUCAST
- Caracterización cepas resistentes *B-tubulina* y *cyp51A*

Contents lists available at ScienceDirect

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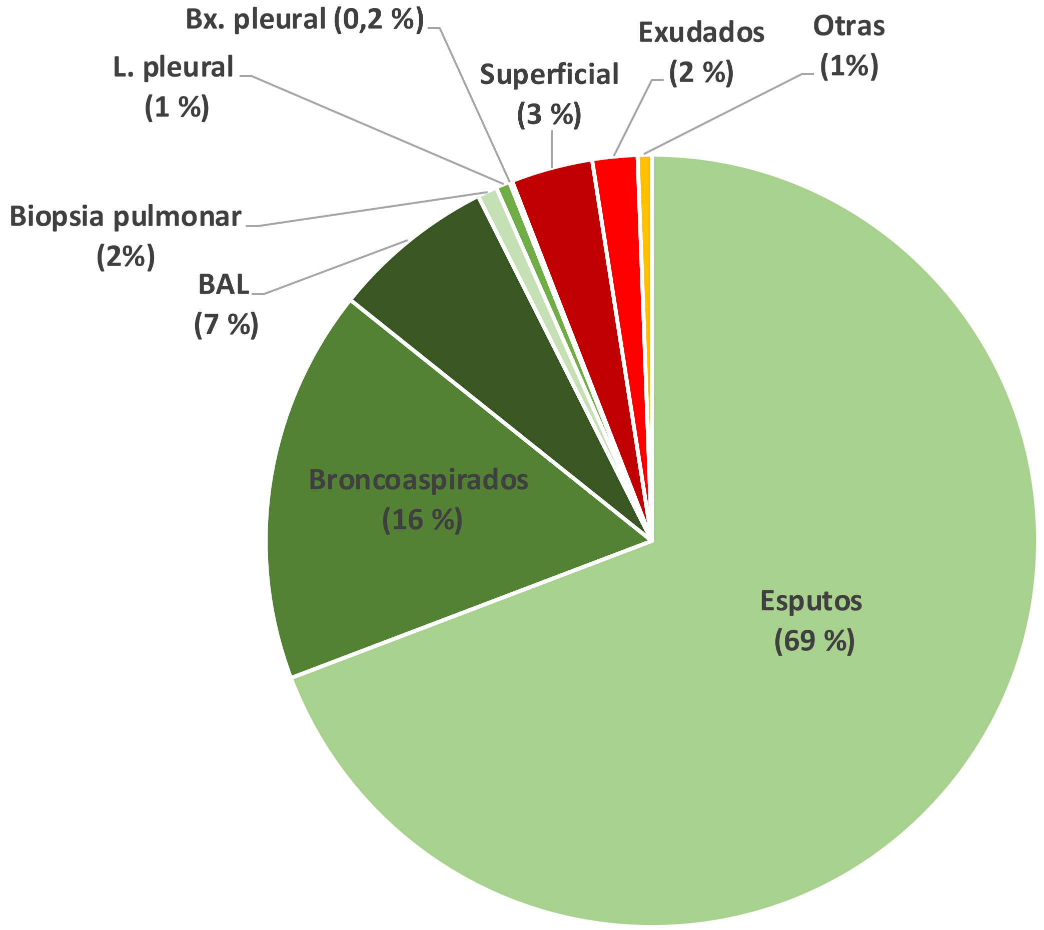
journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Azole resistance survey on clinical *Aspergillus fumigatus* isolates in Spain

Pilar Escribano [1,2], Belén Rodríguez-Sánchez [1,2,3], Judith Díaz-García [1,2], María Teresa Martín-Gómez [4], Elisa Ibáñez [5], María Rodríguez-Mayo [6], Teresa Peláez [7,8], Elia García-Gómez de la Pedrosa [9,10,11], Rocío Tejero-García [11,12,13], José María Marimón [14], Elena Reigadas [1,2], Antonio Rezusta [15,16], Cristina Labayru-Echeverría [17], Ana Pérez-Ayala [18,19], Josefina Ayats [3,20], Fernando Cobo [21,22], Carmen Pazos [23], Leyre López-Soria [24,25], Ana Alastruey-Izquierdo [11,26], Patricia Muñoz [1,2,3,27], Jesús Guinea [1,2,3,*] on behalf of the ASPEIN study group[†]

Ref: Escribano P *et al.* Clin Microbiol infect. 27 (2021) 1170.e1e1170.e7



725 pacientes

814 muestras

847 aislados *A. fumigatus sensu lato*

828 *A. fumigatus sensu stricto*

19 especies crípticas:

- ***A. lentulus*, n = 6**
- ***A. fumigatiaffinis*, n = 5**
- ***A. tsurutae*, n = 3**
- ***A. udagawae*, n = 2**
- ***A. novofumigatus*, n = 2**
- ***A. thermomutatus*, n = 1**

BX: biopsia; **L:** líquido; **BAL:** *lavado broncoalveolar*. **Ref:** Escribano P *et al.* Clin Microbiol infect. 27 (2021) 1170.e1e1170.e7

Tasas de resistencia (cepas)

Hospitales participantes

13 (1,5 %) cepas especies crípticas resistencia a AmB

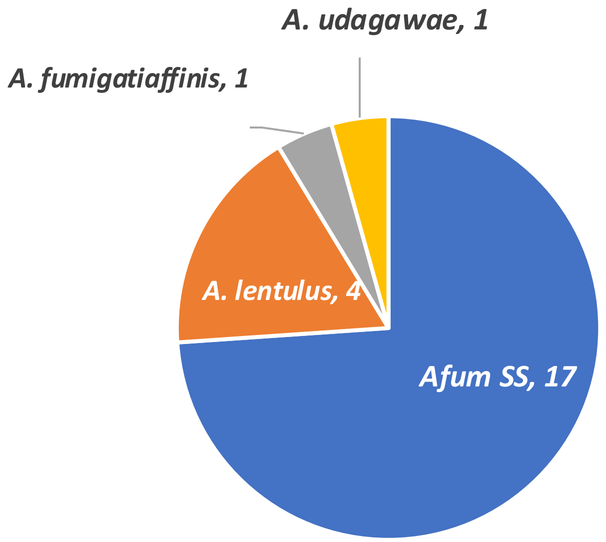
63 (7,4 %) cepas con R a azoles

R a azoles > en crípticas que en A_{fum} *SS* (95 % *vs.* 5,5 %)

Voriconazol compuesto con > tasa de R

Centro referencia micología (ISCIII)

23 aislados mismo periodo



Proporción de crípticas > que en hospitales (26 % *vs.* 1,5 %)

Porcentaje de R > que en hospitales (30 % *vs.* 5,5 %)

AmB: Anfotericina B; **R:** Resistencia; **ISCIII:** Instituto de Salud Carlos III; A_{fum}***SS***: *A fumigatus sensu stricto*.

Ref: Escribano P *et al.* Clin Microbiol infect. 27 (2021) 1170.e1e1170.e7

Estudios **ASPEIN*

ASPEIN I	ASPEIN II	ASPEIN III	ASPEIN VI
Febrero-Marzo 2019	Febrero-Marzo 2022	Febrero-Marzo 2023	Febrero-Marzo 2024
847 cepas	1975 cepas	1867 cepas	Pendiente
A. fumigatus	*Aspergillus* spp	*Aspergillus* spp	*Aspergillus* spp
30 hospitales	84 hospitales	81 hospitales	78 hospitales

Escribano E *et al.* Clin Microbiol Infect. 2021 27(8):1170.e1-1170.e7
Escribano E *et al.* TIMM. 2023 (P062)
Escribano E *et al.* ECCMID. 2024 (P2926)
Escribano E *et al.* SEIMC. 2024 (0073)
Escribano E *et al.* ECCMID. 2025 (Enviado)

*** Azole resistance in *ASPErgillus* in SpaIN**

		Anfotericina B			Azoles		
		Total	Crípticas	*Afum ss*	Total	Crípticas	*Afum ss*
ASPEIN I	**2019**	1,5%	68%	**0%**	7,4%	95%	**5,5%**
ASPEIN II	**2022**	2,2%	100%	**0%**	6,4%	100%	**4,3%**
ASPEIN III	**2023**	3%	97%	**0%**	7%	93.3%	**4,2%**

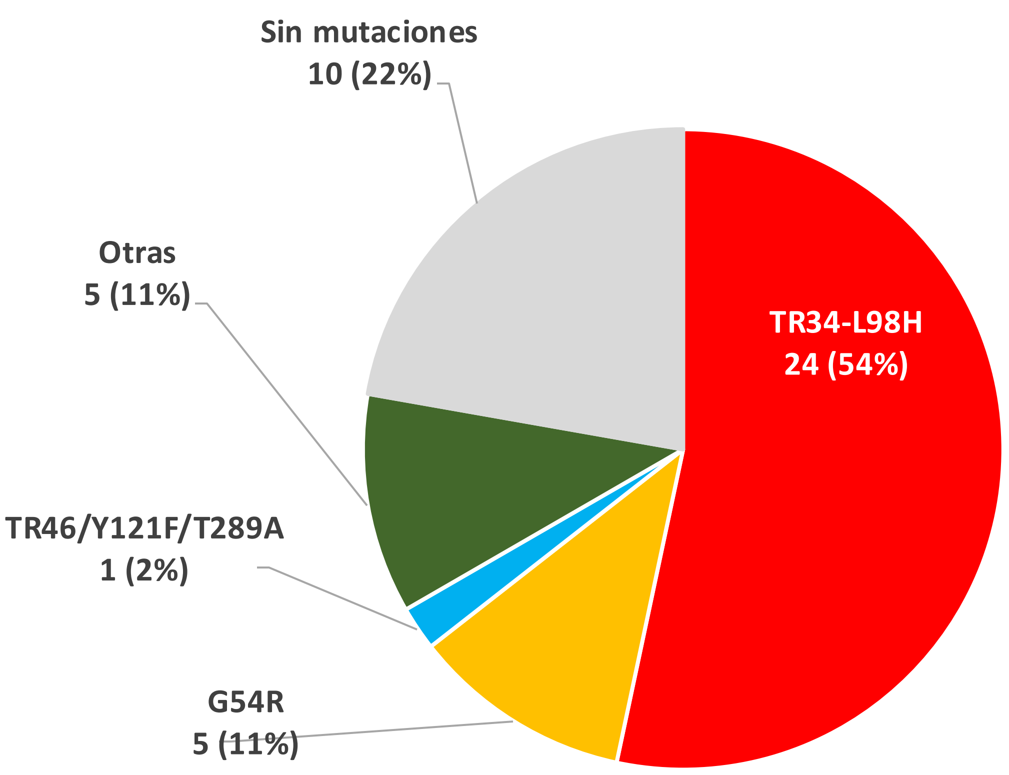
ASPEIN IV

DATOS PARA EL 2025

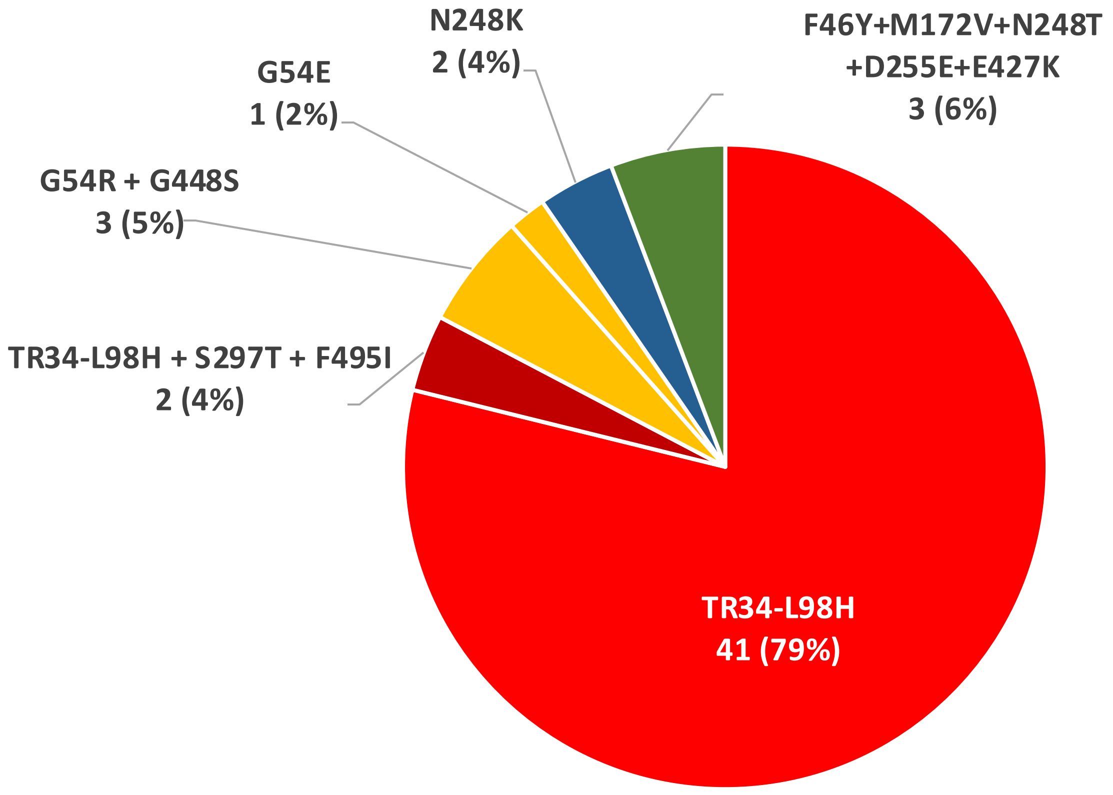
Escribano E *et al*. Clin Microbiol Infect. 2021 27(8):1170.e1-1170.e7
Escribano E *et al*. TIMM. 2023 (P062)
Escribano E *et al*. ECCMID. 2025 (Enviado)

Aspergillus fumigatus sensu stricto 2019 y 2022

ASPEIN I



ASPEIN II

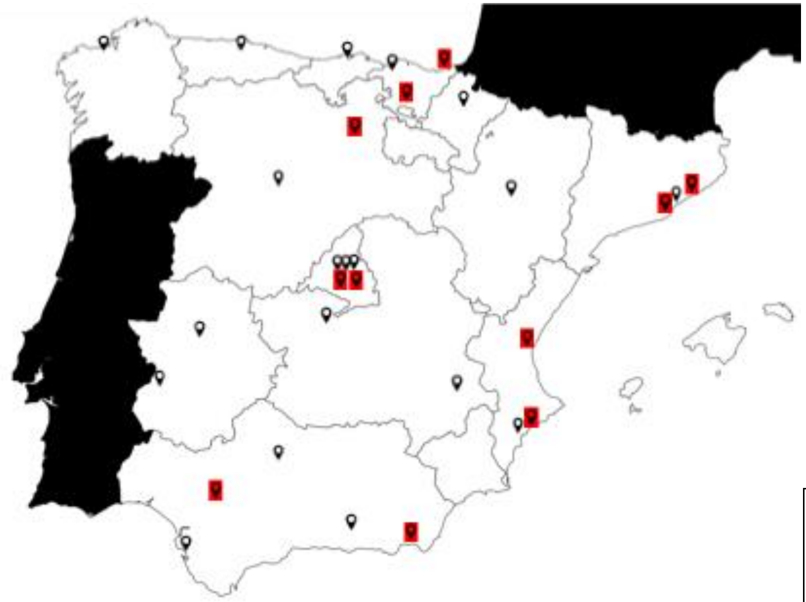


Escribano E *et al.* Clin Microbiol Infect. 2021 27(8):1170.e1-1170.e7
Escribano E *et al.* TIMM. 2023 (P062)

Aspergillus fumigatus sensu stricto 2019 y 2022

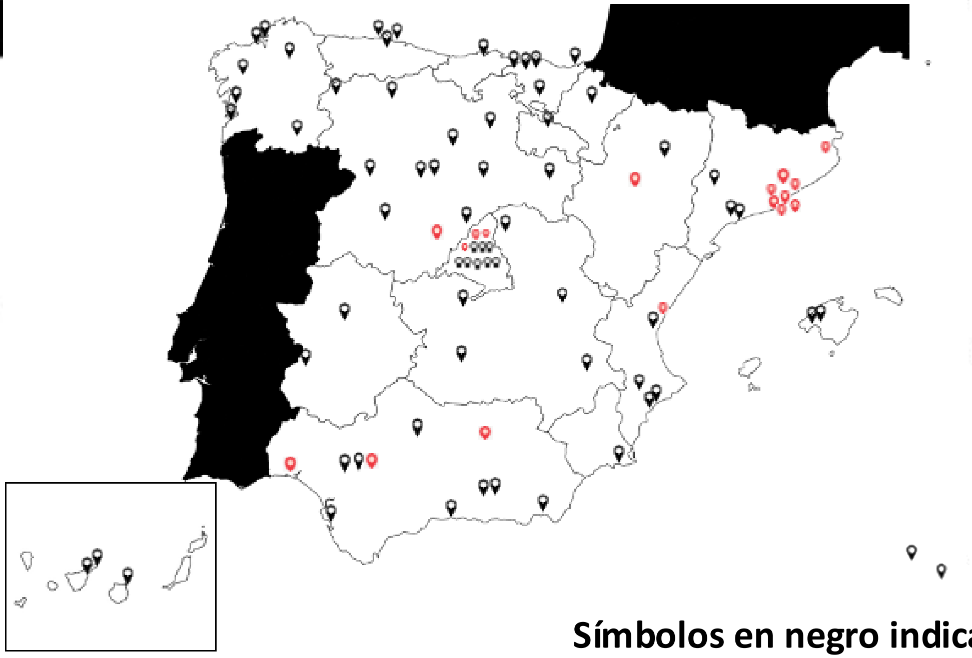
***ASPE*IN I**

10 ciudades afectadas
TR_{34}-L98H o TR_{46}



***ASPE*IN II**

12 ciudades afectadas
TR_{34}-L98H o TR_{46}



***ASPE*IN III**

11 ciudades afectadas
TR_{34}-L98H o TR_{46}

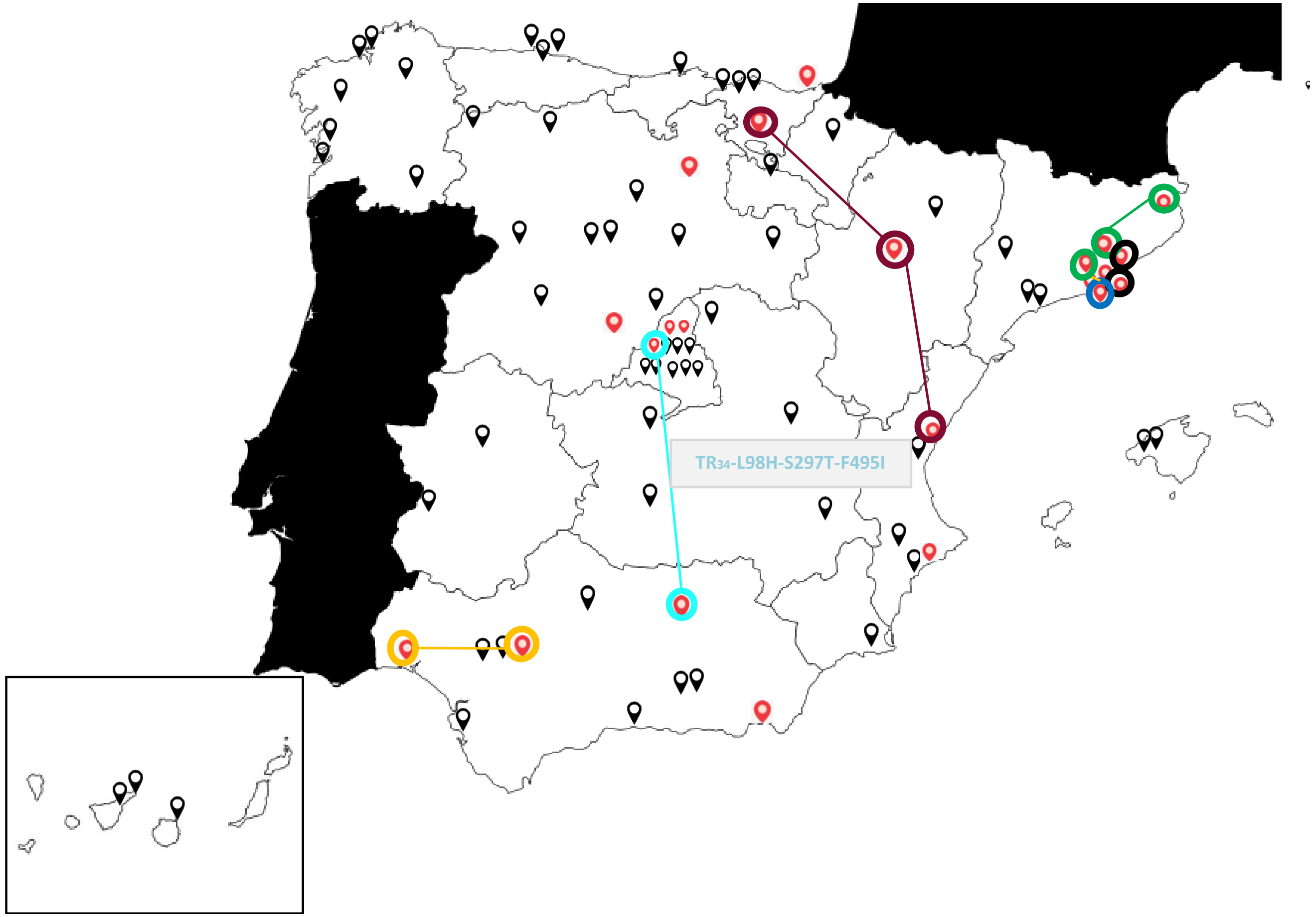
Símbolos en negro indican ciudades muestreadas
Símbolos en rojo indican ciudades con TR_{34}-L98H o TR_{46}

Escribano E *et al.* Clin Microbiol Infect. 2021 27(8):1170.e1-1170.e7
Escribano E *et al.* TIMM. 2023 (P062)
Escribano E *et al.* ECCMID. 2025 (Enviado)

Aspergillus fumigatus sensu stricto 2019 y 2022

41 genotipos únicos

48 genotipos con aislados portadores de TR_{34}-L98H±(S297T-F495I)



7 clusters

2 *clusters* en una única ciudad
Barcelona (2 pacientes en 1 hospital y 2 pacientes en otro hospital)

3 *clusters* involucrando 2 ciudades
Madrid-Jaén
Barcelona-Badalona
Sevilla-Huelva

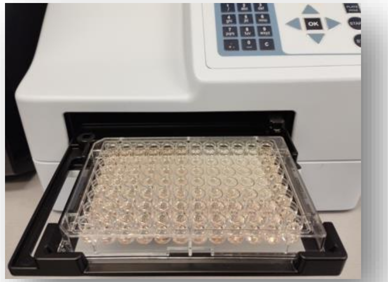
2 *clusters* involucrando a 3 ciudades
Zaragoza/Valencia/Vitoria
Badalona/Sabadell/Girona

Escribano E *et al.* ECCMID. 2023 (O1099)

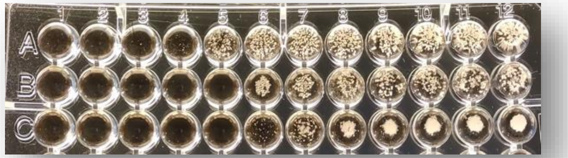
Methods for antifungal susceptibility testing

- **Broth microdilution:** reference methods

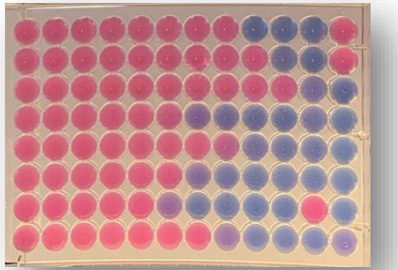
EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases



CLINICAL AND LABORATORY STANDARDS INSTITUTE ™



- **Marketed methods**
    - **Sensititre™ YeastOne™** (ThermoFisher Scientific)
    - **Etest®** (bioMérieux)
    - **VITEK®2** (bioMérieux)



Sensititre™ YeastOne™

Etest®

VITEK®2

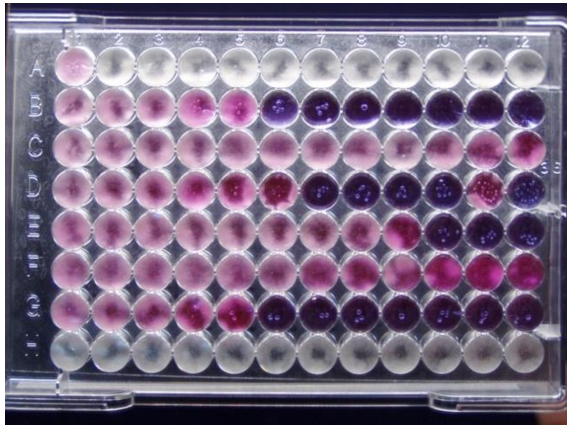
Standard vs Commercial methods: strengths and pitfalls

	Standard procedures	Commercial methods
Availability • Implemented in hospitals • Staff familiar with performance	😩	😀
Proper validation • Resistant isolates tested • Sensitivity and Specificity	😀	😩
Easy to MIC reading • Low subjectivity • Clear and well defined endpoints	😀	😩
Easy to MIC interpretation • Guidance to classify isolates • Breakpoints validated	😀	😩

Standard vs Commercial methods: strengths and pitfalls

	Standard procedures	Commercial methods
Availability • Implemented in hospitals • Staff familiar with performance	😩	😀
Proper validation • Resistant isolates tested • Sensitivity and Specificity	😀	😩
Easy to MIC reading • Low subjectivity • Clear and well defined endpoints	😀	😩
Easy to MIC interpretation • Guidance to classify isolates • Breakpoints validated	😀	😩

- **Visual reading (colour change)**
- **CLSI Breakpoints**



Sensititre YeastOne Plates

Antimicrobial	Dilution Range		
Part Number:	**YO4IVD**	**YO11**	**YO10**
Classification:	IVD	Research Use Only (RUO)	IVD
Availability:	U.S. only	U.S. only	Outside of U.S.
5-Flucytosine	0.03-64		0.06-64
Amphotericin B		0.12-8	0.12-8
Anidulafungin		0.015-8	0.015-8
Caspofungin	0.015-16	0.03-4	0.008-8
Fluconazole	0.12-128	0.5-64	0.12-256
Isavuconazole		0.008-4	
Itraconazole	0.008-16	0.015-16	0.015-16
Micafungin	0.008-16	0.008-8	0.008-8
Posaconazole		0.008-8	0.008-8
Rezafungin	0.008-8	0.008-4	
Voriconazole	0.008-8	0.008-2	0.008-8

	1	2	3	4	5	6	7	8	9	10	11	12
A	CONTROL POS	AND 0.015	AND 0.03	AND 0.06	AND 0.125	AND 0.25	AND 0.5	AND 1	AND 2	AND 4	AND 8	AB 0.12
B	MF 0.008	MF 0.015	MF 0.03	MF 0.06	MF 0.125	MF 0.25	MF 0.5	MF 1	MF 2	MF 4	MF 8	AB 0.25
C	CAS 0.008	CAS 0.015	CAS 0.03	CAS 0.06	CAS 0.125	CAS 0.25	CAS 0.5	CAS 1	CAS 2	CAS 4	CAS 8	AB 0.5
D	5FL 0.06	5FL 0.125	5FL 0.25	5FL 0.5	5FL 1	5FL 2	5FL 4	5FL 8	5FL 16	5FL 32	5FL 64	AB 1
E	PZ 0.008	PZ 0.015	PZ 0.03	PZ 0.06	PZ 0.125	PZ 0.25	PZ 0.5	PZ 1	PZ 2	PZ 4	PZ 8	AB 2
F	VOR 0.008	VOR 0.015	VOR 0.03	VOR 0.06	VOR 0.125	VOR 0.25	VOR 0.5	VOR 1	VOR 2	VOR 4	VOR 8	AB 4
G	ITR 0.015	ITR 0.03	ITR 0.06	ITR 0.125	ITR 0.25	ITR 0.5	ITR 1	ITR 2	ITR 4	ITR 8	ITR 16	AB 8
H	FZ 0.125	FZ 0.25	FZ 0.5	FZ 1	FZ 2	FZ 4	FZ 8	FZ 16	FZ 32	FZ 64	FZ 128	FZ 256

S **I** **R**

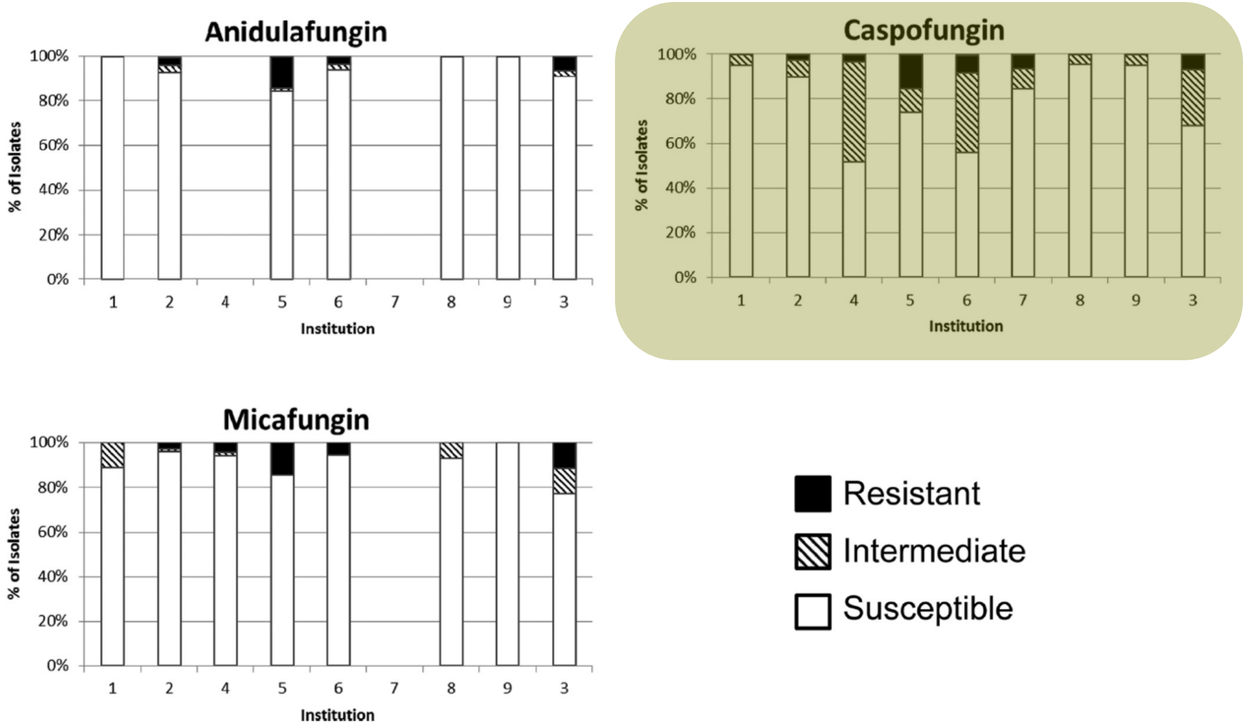
AAC
Journals.ASM.org

Real-World Experience with Echinocandin MICs against *Candida* Species in a Multicenter Study of Hospitals That Routinely Perform Susceptibility Testing of Bloodstream Isolates

Gregory A. Eschenauer,[a] M. Hong Nguyen,[b] Shmuel Shoham,[c] Jose A. Vazquez,[d*] Arthur J. Morris,[e] William A. Pasculle,[f] Christine J. Kubin,[g] Kenneth P. Klinker,[h] Peggy L. Carver,[i] Kimberly E. Hanson,[j] Sharon Chen,[k] Simon W. Lam,[l] Brian A. Potoski,[m] Lloyd G. Clarke,[a] Ryan K. Shields,[b] Cornelius J. Clancy[b,n]

- 15 USA hospitals
- 2,897 isolates
- Candins tested using SYO
- CLSI breakpoints

Modal MIC for each centre within narrow ranges (≤ 2)



C. glabrata

Careful!

Ref: Eschenauer *et al*. AAC 2014

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Multicenter Study of Epidemiological Cutoff Values and Detection of Resistance in *Candida* spp. to Anidulafungin, Caspofungin, and Micafungin Using the Sensititre YeastOne Colorimetric Method

A. Espinel-Ingroff,[a] M. Alvarez-Fernandez,[b] E. Cantón,[c] P. L. Carver,[d] S. C.-A. Chen,[e] G. Eschenauer,[d,f] D. L. Getsinger,[g] G. M. Gonzalez,[h] N. P. Govender,[i] A. Grancini,[j] K. E. Hanson,[k] S. E. Kidd,[l] K. Klinker,[m] C. J. Kubin,[n] J. V. Kus,[o] S. R. Lockhart,[p] J. Meletiadis,[q] A. J. Morris,[r] T. Pelaez,[s] G. Quindós,[t] M. Rodriguez-Iglesias,[u] F. Sánchez-Reus,[v] S. Shoham,[w] N. L. Wengenack,[x] N. Borrell Solé,[y] J. Echeverria,[z] J. Esperalba,[aa] E. Gómez-G. de la Pedrosa,[bb] I. García García,[cc] M. J. Linares,[dd] F. Marco,[ee] P. Merino,[ff] J. Pemán,[gg] L. Pérez del Molino,[hh] E. Roselló Mayans,[ii] C. Rubio Calvo,[jj] M. Ruiz Pérez de Pipaon,[kk] G. Yagüe,[ll] G. Garcia-Effron,[mm] J. Guinea,[s] D. S. Perlin,[nn] M. Sanguinetti,[oo] R. Shields,[f] J. Turnidge[pp]

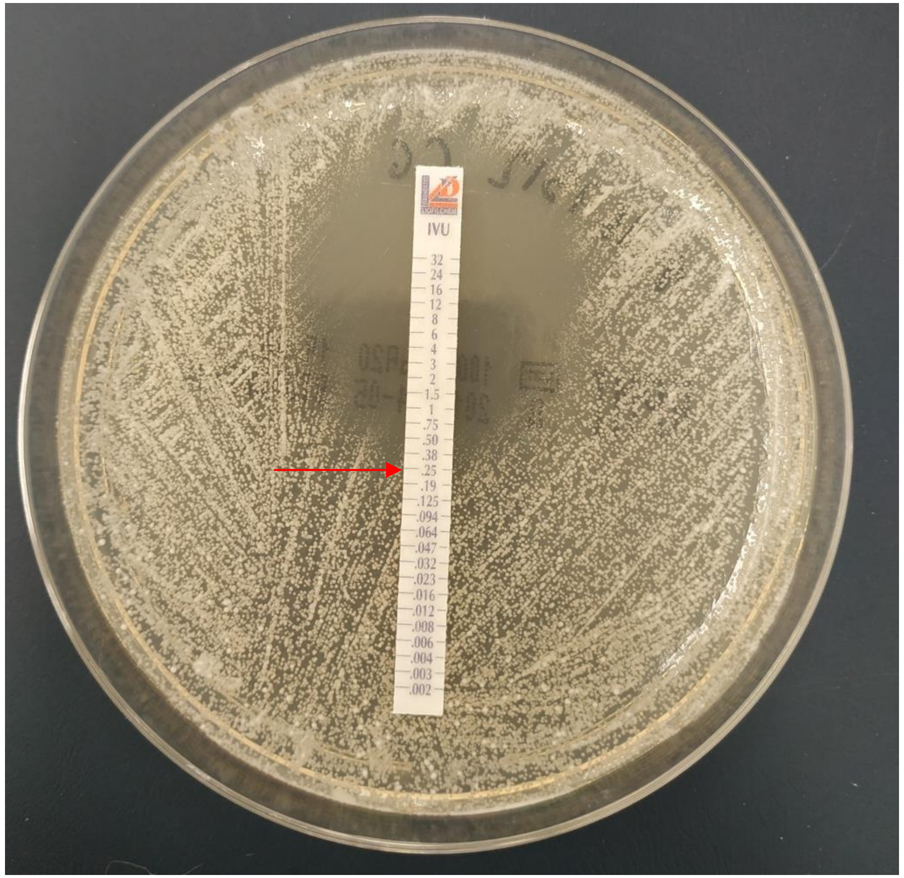
TABLE 4 Application of SYO ECVs of anidulafungin, caspofungin, and micafungin to identify *Candida* isolates harboring *fks1* and *fks2* gene mutations (non-WT strains)[a]

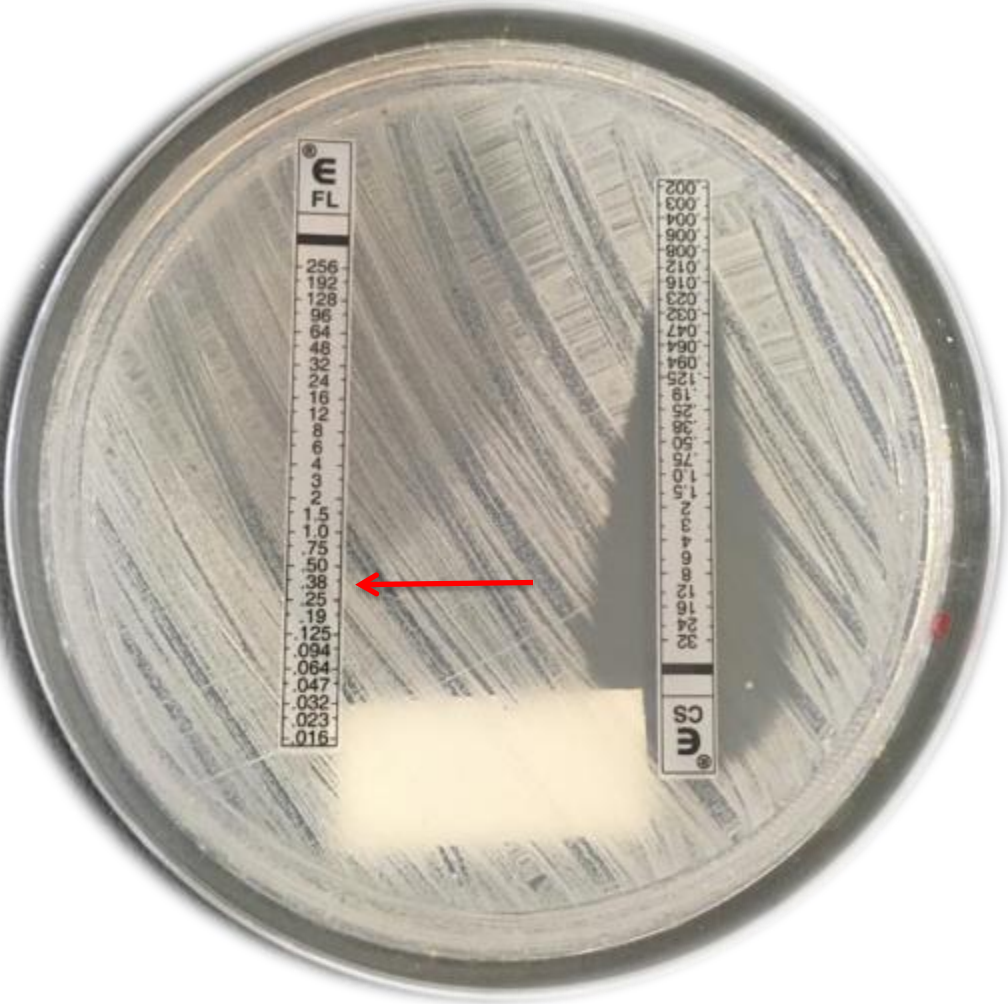
Agent	Species	No. of mutants identified/no. of mutants (%)[b]	SYO ECV (μg/ml)[c]	MIC for wild type (μg/ml)[d]
Anidulafungin	*C. albicans*	36/41 (87.8)	0.12	0.016–0.03
	C. glabrata	24/26 (92)	0.12	0.016–0.12
	C. krusei	5/5 (ND)	0.25	NA
	C. tropicalis	6/8 (87.5)	0.5	0.03–0.06
	C. dubliniensis	1/1 (ND)	0.25	NA
	Total	72/81 (88.9)		
Caspofungin	*C. albicans*	38/41 (92.7)	0.25	0.06–0.12
	C. glabrata	24/26 (92)	0.25	0.06–0.25
	C. krusei	4/5 (ND)	1	NA
	C. tropicalis	7/8 (87.5)	0.25	0.016–0.25
	C. dubliniensis	1/1 (ND)	0.25	NA
	Total	74/81 (91.4)		
Micafungin	*C. albicans*	38/41 (92.7)	0.06[e]	0.008–0.03
	C. glabrata	24/26 (92)	0.03	0.016–0.03
	C. krusei	5/5 (ND)	0.25	NA
	C. tropicalis	8/8 (100)	0.06	0.03–0.06
	C. dubliniensis	1/1 (ND)	0.12	NA
	Total	76/81 (93.8)		

Gradient diffusion plastic strips

Drug	Manufacturer
Fluconazole	Liofilchem / BioMeriéux
Itraconazole	Liofilchem / BioMeriéux
Voriconazole	Liofilchem / BioMeriéux
Posaconazole	Liofilchem / BioMeriéux
Isavuconazole	Liofilchem / BioMeriéux
Caspofungin	Liofilchem / BioMeriéux
Micafungin	Liofilchem / BioMeriéux
Anidulafungin	Liofilchem / BioMeriéux
Amphotericin B	Liofilchem / BioMeriéux

- Ellipse of growth inhibition
- Intersection with plastic strip





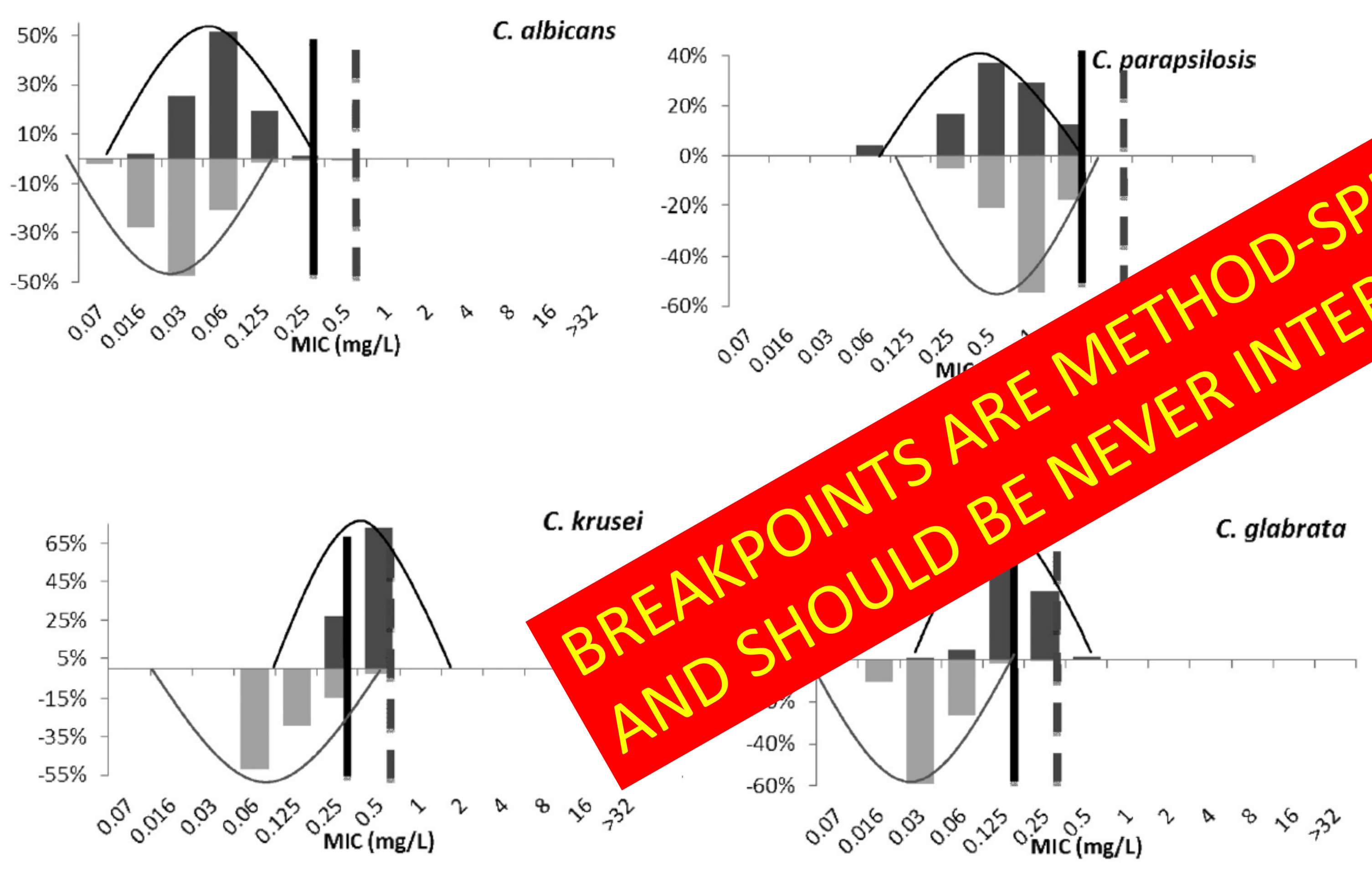
FLUCONAZOLE SUSCEPTIBLE OR RESISTANT?

Caspofungin Etest Susceptibility Testing of *Candida* Species: Risk of Misclassification of Susceptible Isolates of *C. glabrata* and *C. krusei* when Adopting the Revised CLSI Caspofungin Breakpoints

Maiken Cavling Arendrup,[a] Michael A. Pfaller,[b] and the Danish Fungaemia Study Group

Unit of Mycology, Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark,[a] and JMI Laboratories and the University of Iowa, Iowa City, Iowa, USA[b]

Anidulafungin-susceptible
Application revised BP for caspo Etest



MICs determined by Etest
MICs determined by CLSI
Solid line: CLSI breakpoint separating S and I
Broken line: CLSI breakpoint separating I and R

	Etest misclassifications	
	I	R
C. krusei	73%	
C. glabrata	31%	1.5%
Overall	13%	

Ref: Arendrup. AAC 2012

Title: Detecting echinocandin resistance in *C. glabrata* using commercial methods: are CLSI or EUCAST breakpoints suitable for categorical classification?

Figure 1a

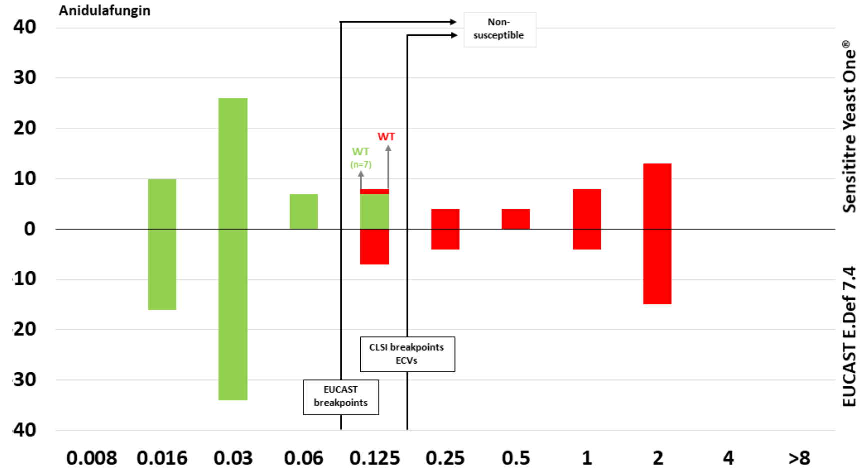


Figure 1b

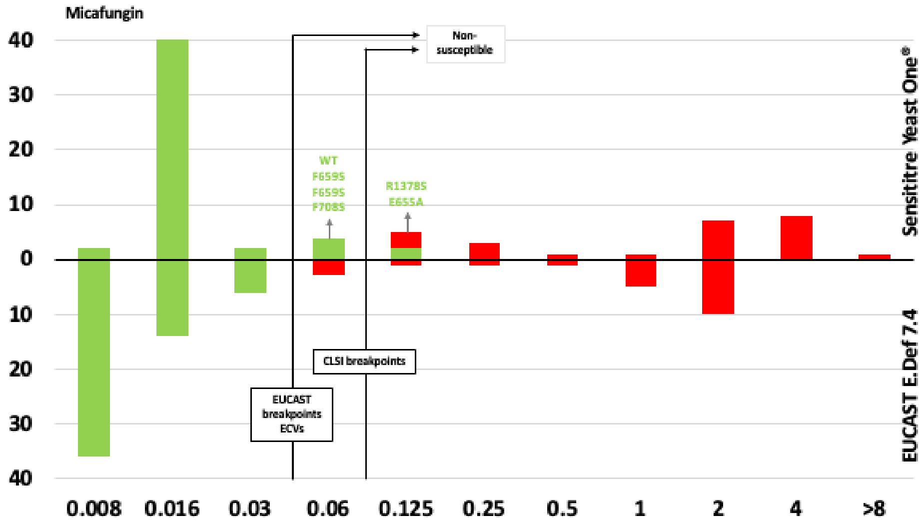


Figure 2a

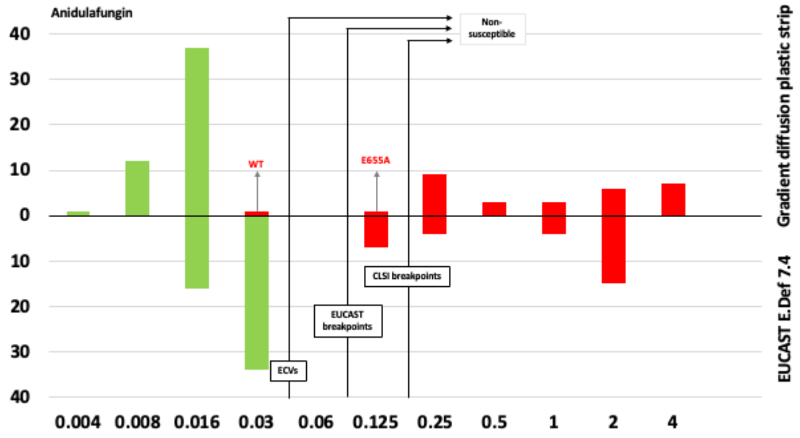
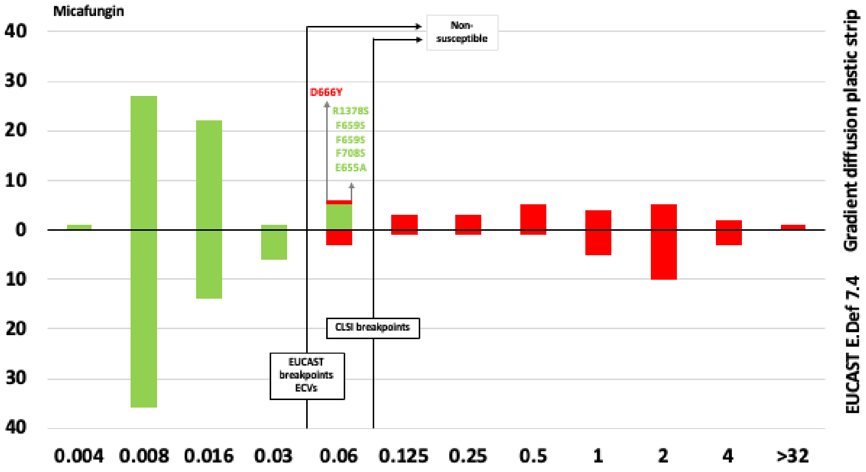
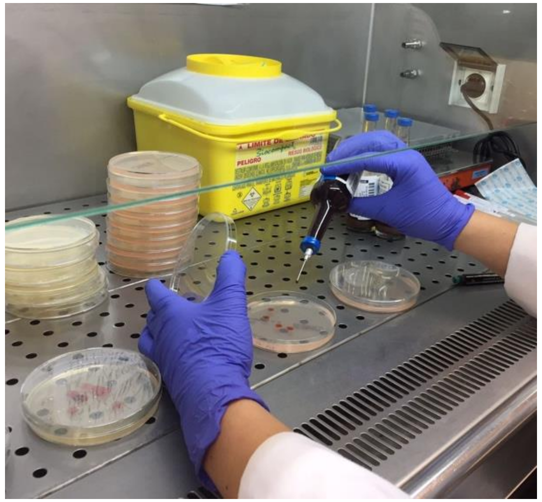


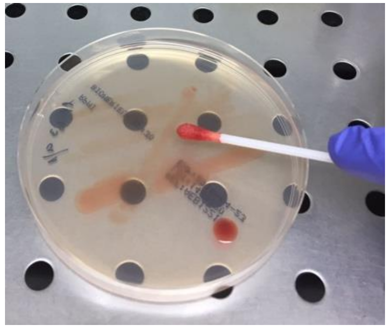
Figure 2b

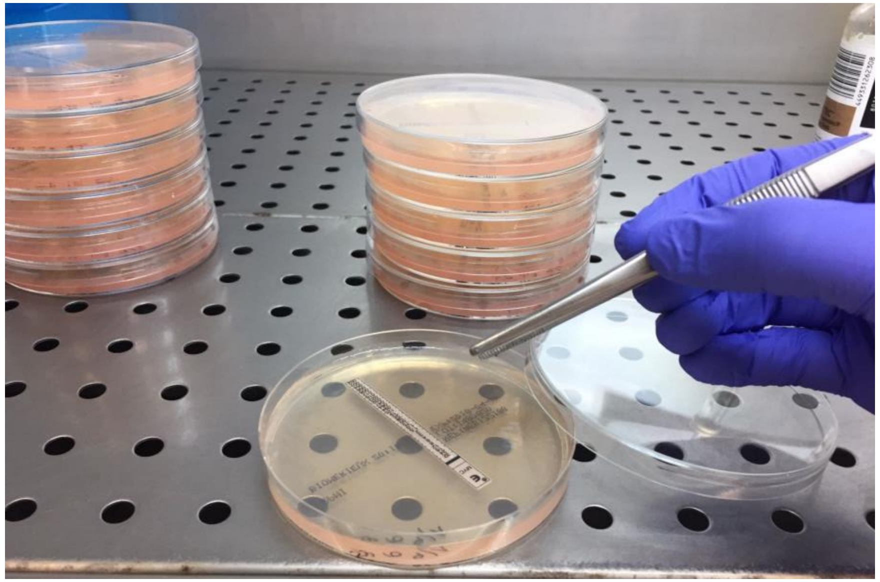


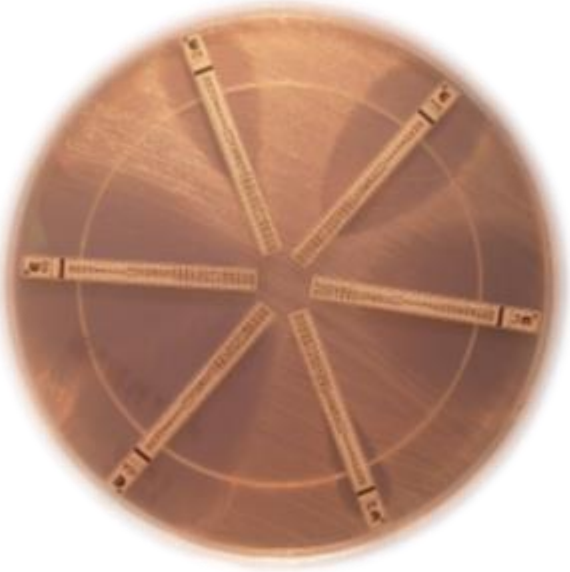
Unreported data.
Manuscript submitted

Gradient diffusion plastic strips performed directly on blood cultures









Rapid Antifungal Susceptibility Determination for Yeast Isolates by Use of Etest Performed Directly on Blood Samples from Patients with Fungemia▽

Jesús Guinea,[1,2,3]* Sandra Recio,[1] Pilar Escribano,[1] Marta Torres-Narbona,[1] Teresa Peláez,[1,2,3] Carlos Sánchez-Carrillo,[1,2] Marta Rodríguez-Créixems,[1,2,3] and Emilio Bouza[1,2,3]

JCM 2010

The Etest Performed Directly on Blood Culture Bottles Is a Reliable Tool for Detection of Fluconazole-Resistant *Candida albicans* Isolates

Pilar Escribano,[a,b] Laura Judith Marcos-Zambrano,[a,b] Ana Gómez,[a,b] Carlos Sánchez,[a,b,c] M. Carmen Martínez-Jiménez,[a,b] Emilio Bouza,[a,b,c,d] Jesús Guinea[a,b,c,d]

AAC 2017

Resistance to Echinocandins in *Candida* Can Be Detected by Performing the Etest Directly on Blood Culture Samples

María Ángeles Bordallo-Cardona,[a,b] Laura Judith Marcos-Zambrano,[a,b] Carlos Sánchez-Carrillo,[a,b] Emilio Bouza,[a,b,c,d] Patricia Muñoz,[a,b,c,d] Pilar Escribano,[a,b] Jesús Guinea[a,b,c,d]

AAC 2018

AMERICAN SOCIETY FOR MICROBIOLOGY Antimicrobial Agents and Chemotherapy®

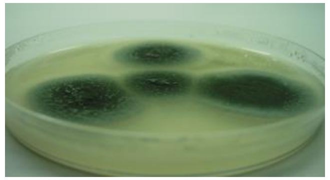
SUSCEPTIBILITY

Detection of Echinocandin-Resistant *Candida glabrata* in Blood Cultures Spiked with Different Percentages of *FKS2* Mutants

María Ángeles Bordallo-Cardona,[a,b] Carlos Sánchez-Carrillo,[a,b] Emilio Bouza,[a,b,c,d] Patricia Muñoz,[a,b,c,d] Pilar Escribano,[a,b] Jesús Guinea[a,b,c,d]

AAC 2019

Aspergillus: detección resistencia en el laboratorio



Identificación correcta

A. flavus
A. terreus
A. niger
A. nidulans

EUCAST E.Def 10.2 June 2022 — EUCAST agar screening for resistance in *Aspergillus* spp

EUCAST DEFINITIVE DOCUMENT E.Def 10.2

Screening method for the detection of azole and echinocandin resistance in *Aspergillus* using antifungal-containing agar plates, exemplified by *A. fumigatus*

J. Meletiadis[1,2], J Guinea[3,4,5], S. Arikan-Akdagli[6], N. Friberg[7], G. Kahlmeter[8], MC Arendrup[9,10,11] and the Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)*

Agares con antifúngicos

A. fumigatus

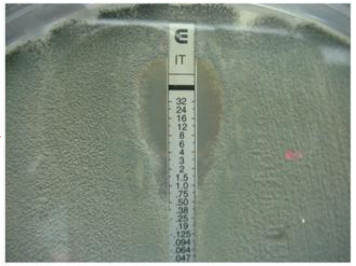
EUCAST E.DEF 9.4 March 2022 — EUCAST antifungal microdilution method for moulds

EUCAST DEFINITIVE DOCUMENT E.DEF 9.4

Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds

J Guinea*[1,2,3], J Meletiadis*[4,5], S Arikan-Akdagli[6], K Muehlethaler[7], G Kahlmeter[8], M C Arendrup[9,10,11] and the Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)**

*Both authors contributed equally



Tiras plásticas gradiente difusión

https://www.eucast.org/astoffungi/methodsinantifungalsusceptibilitytesting/ast_of_moulds último acceso 06/2024

Azole and Amphotericin B MIC Values against *Aspergillus fumigatus*: High Agreement between Spectrophotometric and Visual Readings Using the EUCAST EDef 9.3.2 Procedure

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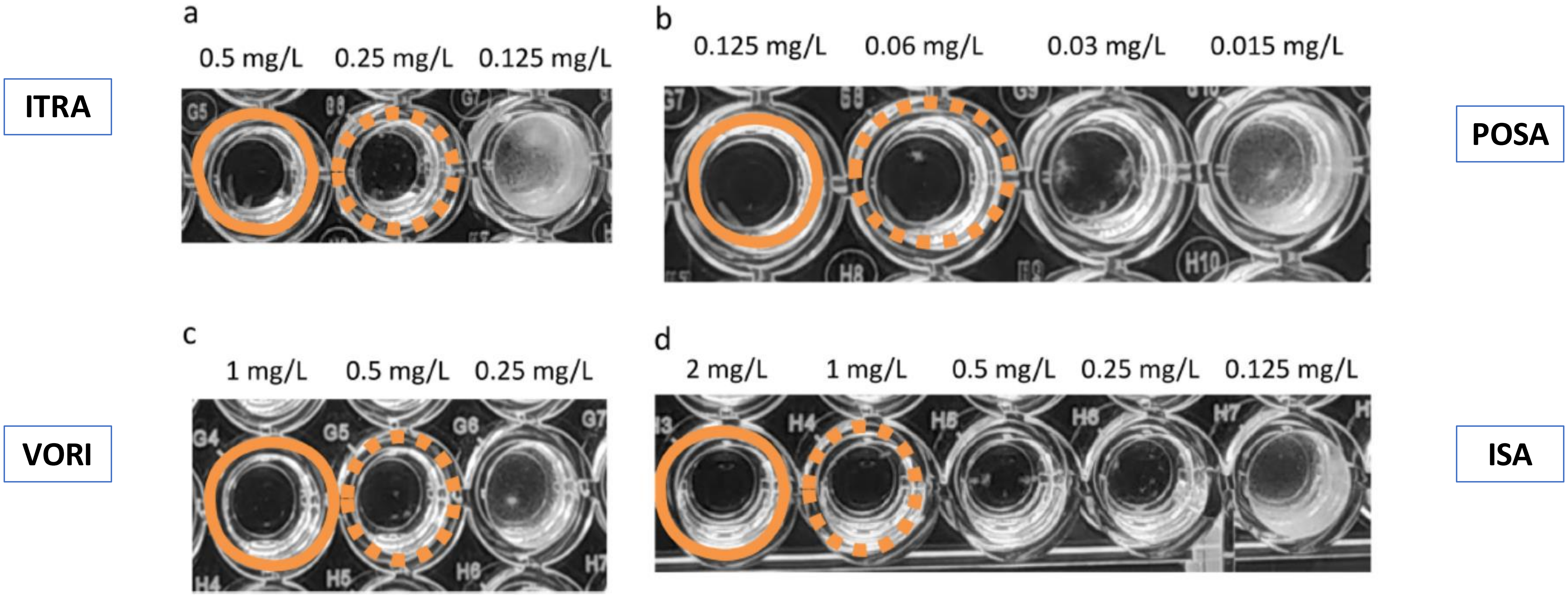
Antimicrobial Agents and Chemotherapy®

Julia Serrano-Lobo,[a,b] Ana Gómez,[a,b] Waldo Sánchez-Yebra,[c] Miguel Fajardo,[d] Belén Lorenzo,[e] Ferrán Sánchez-Reus,[f] Inmaculada Vidal,[g] Marina Fernández-Torres,[h] Isabel Sánchez-Romero,[i] Carlos Ruiz de Alegría-Puig,[j] José Luis del Pozo,[k] Patricia Muñoz,[a,b,l,m] Pilar Escribano,[a,b] Jesús Guinea,[a,b,l] on behalf of the ASPEIN Study Group



Itra: Itraconazol; **Vori:** voriconazol; **Posa:** posaconazol; **Isa:** isavuconazol.
Ref: Serrano-Lobo J *et al.* Azole and Amphotericin B MIC Values against Aspergillus fumigatus: High Agreement between Spectrophotometric and Visual Readings Using the EUCAST EDef 9.3.2 Procedure Antimicrobial Agents and Chemotherapy. 2021; January 1 e01693-20

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®
SUSCEPTIBILITY

Azole-Resistant *Aspergillus fumigatus* Clinical Isolate Screening in Azole-Containing Agar Plates (EUCAST E.Def 10.1): Low Impact of Plastic Trays Used and Poor Performance in Cryptic Species

Julia Serrano-Lobo,[a,b] Ana Gómez,[a,b] Belén Rodríguez-Sánchez,[a,b] Patricia Muñoz,[a,b,c,d] Pilar Escribano,[a,b]
Jesús Guinea[a,b,c] on behalf of the ASPEIN Study Group

	Sensibilidad	Especificidad
Itraconazol	97%	96%
Voriconazol	79%	95%
Resistencia azoles	100%	95%

Good performance on *Aspergillus fumigatus* sensu stricto
Poor performance on cryptic species

Received: 14 March 2022 | Revised: 30 June 2022 | Accepted: 1 July 2022
DOI: 10.1111/myc.13492
ORIGINAL ARTICLE
mycoses WILEY

Screening of azole resistance in *Aspergillus fumigatus* using the EUCAST E.Def 10.2 azole-containing agar method: A single study suggests that filtration of conidial suspensions prior to inoculum preparation may not be needed

Julia Serrano-Lobo[1,2] | Ana Gómez[1,2] | Elena Reigadas[1,2,3] | Patricia Muñoz[1,2,3,4] | Pilar Escribano[1,2] | Jesus Guinea[1,2,3] | on behalf of the *ASPE* IN Study Group

Sensibilidad	Especificidad
100%	98%

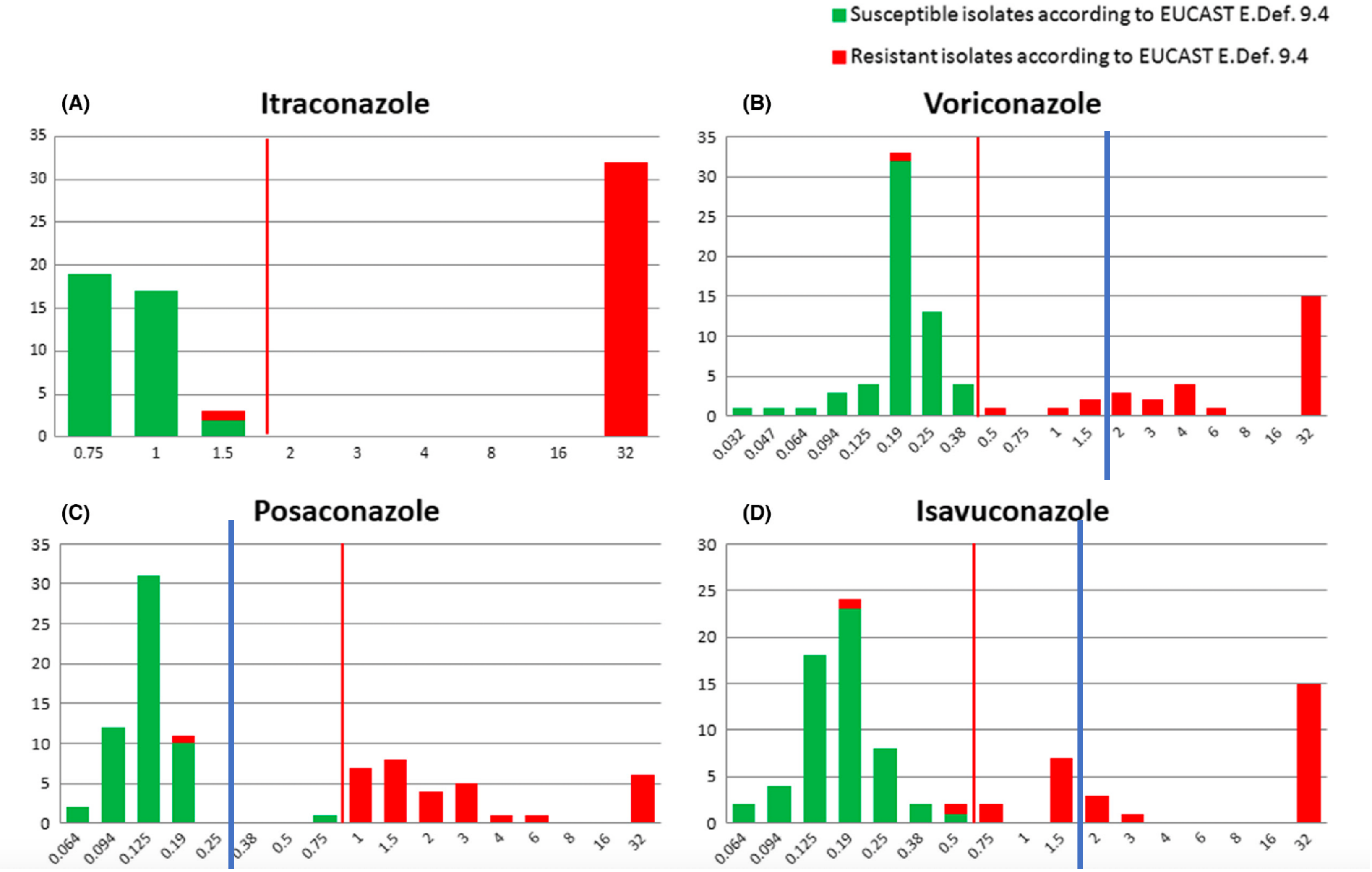
	Acuerdo filtrado *vs* sin filtrar
Itraconazol	100%
Voriconazol	100%
Posaconazol	98%

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology
Antimicrobial Chemotherapy | Full-Length Text

Azole resistance screening in *Aspergillus fumigatus sensu stricto* using the azole-containing agar method (EUCAST E.Def 10.2): conidial suspension filtration and inoculum adjustment before inoculum preparation may not be needed

Julia Serrano-Lobo,[1,2] Elena Reigadas,[1,2,3,4] Patricia Muñoz,[1,2,3,4] Pilar Escribano,[1,2,5] Jesús Guinea,[1,2,3,5] on behalf of the ASPEIN Study Group

A. fumigatus sensu stricto



Puntos de corte de EUCAST

Received: 8 September 2022 | Revised: 20 October 2022 | Accepted: 21 October 2022
DOI: 10.1111/myc.13541
ORIGINAL ARTICLE
mycoses WILEY

Gradient diffusion strips for detecting azole resistance in *Aspergillus fumigatus* sensu lato

Julia Serrano-Lobo[1,2] | Ana Gómez[1,2] | Elena Reigadas[1,2,3] | Patricia Muñoz[1,2,3,4] | Pilar Escribano[1,2] | Jesús Guinea[1,2,3] | on behalf of the ASPEIN Study Group

	Sensibilidad	Especificidad
Itra	97%	100%
Vori	97%	100%
Posa	97%	100%
Isa	93,3%	100%

Ref: Serrano-Lobo J, Gómez A, Reigadas E, et al. Gradient diffusion strips for detecting azole resistance in *Aspergillus fumigatus* sensu lato. Mycoses. 2023;66(3):196-201

Article type: Original Paper

Title: Effective Detection of Azole Resistance in *Aspergillus fumigatus sensu stricto* Using a Gradient Diffusion Plastic Strip: A Comparison of Filtered adjusted vs. Unfiltered unadjusted Inocula

We conclude that the gradient diffusion plastic strip is a method for screening for azole resistance in *A. fumigatus sensu stricto* isolates when MICs obtained are interpreted as follows: itraconazole >1 mg/L, voriconazole >0.5 mg/L, and posaconazole >0.25 mg/L.

Skipping filtration and adjustment of conidial suspensions did not negatively influence the performance of the method.

Unreported data.
Manuscript submitted

Detección molecular de resistencia a azoles *A. fumigatus*

PATHONOSTICS

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

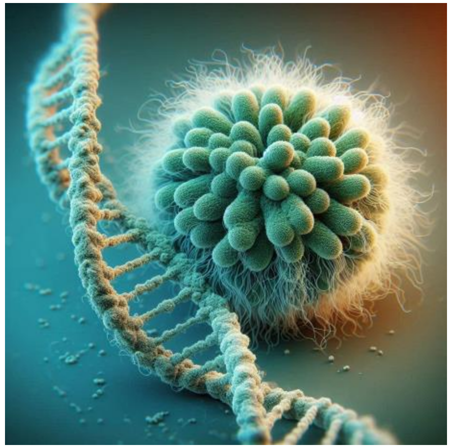
Species multiplex
- *Aspergillus fumigatus*
- *Aspergillus terreus*
- *Aspergillus* species
- Internal Amplification Control (IAC)

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

Detección molecular de resistencia a azoles *A. fumigatus*



MOLECULAR

- PCR
- Mutaciones gen *cyp51A* + promotor

  MycoGENIE® *Aspergillus fumigatus* (Adamtech)

  Fungiplex® *Aspergillus* Azole-R IVD (Bruker Daltonik GmbH)

  AsperGenius® (PathoNostics B.V.)

No se detectan todas las mutaciones
Exclusivo *A. fumigatus* SS

Paciente hematológico
Sensibilidad 97%
Especificidad 100%

Clinical Infectious Diseases
MAJOR ARTICLE
IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Clinical Impact of Polymerase Chain Reaction–Based *Aspergillus* and Azole Resistance Detection in Invasive Aspergillosis: A Prospective Multicenter Study

Sammy Huygens,[1,a] Albert Dunbar,[1,a] Jochem B. Buil,[2] Corné H. W. Klaassen,[3] Paul E. Verweij,[2] Karin van Dijk,[4] Nick de Jonge,[5] Jeroen J. W. M. Janssen,[5] Walter J. F. M. van der Velden,[6] Bart J. Biemond,[7] Aldert Bart,[8] Anke H. W. Bruns,[9] Pieter-Jan A. Haas,[10] Astrid M. P. Demandt,[11] Guy Oudhuis,[12] Peter von dem Borne,[13] Martha T. van der Beek,[14] Saskia K. Klein,[15,16] Peggy Godschalk,[17] Lambert F. R. Span,[16] Douwe F. Postma,[18] Greetje A. Kampinga,[19] Johan Maertens,[20,21] Katrien Lagrou,[21,22] Toine Mercier,[20,21] Ine Moors,[23] Jerina Boelens,[24] Dominik Selleslag,[25] Marijke Reynders,[26] Willemien Zandijk,[3] Jeanette K. Doorduijn,[27] Jan J. Cornelissen,[27] Alexander F. A. D. Schauwvlieghe,[25] and Bart J. A. Rijnders[1]

Infection
https://doi.org/10.1007/s15010-024-02236-7
BRIEF REPORT
Check for updates

Report of three azole-resistant *Aspergillus fumigatus* cases with TR34/L98H mutation in hematological patients in Barcelona, Spain

Patricia Monzo-Gallo[1,6] · Ana Alastruey-Izquierdo[2,5] · Mariana Chumbita[1,6] · Tommaso Francesco Aiello[1,6] · Antonio Gallardo-Pizarro[1,6] · Olivier Peyrony[3] · Christian Teijon-Lumbreras[1] · Laura Alcazar-Fuoli[2,5] · Mateu Espasa[4] · Alex Soriano[1,5,6] · Francesc Marco[4] · Carolina Garcia-Vidal[1,5,6]

Bosetti and Nofytos. Curr Fungal Infect Rep. 2023, 18:1-10.

Para llevar a casa

- Realizar estudios de sensibilidad si se van a tomar decisiones
- Decidir tipos de muestras
- Métodos estandarizados, difícil en rutina
- Métodos comerciales, interpretar correctamente
- Cepas resistentes, requieren una caracterización posterior

!Muchas gracias a tod@s¡

Microbiología (Laboratorio)

Elena Reigadas

Luis Alcalá

Teresa Vicente

Pablo Martín-Rabadán

Julia Serrano

Microbiología (enfermedades infecciosas)

Maricela Valerio

Marina Machado

María Palomo

Ana Álvarez-Uría

Belén Padilla

Patricia Muñoz

Grupo estudio *ASPE*IN

***ASPE*IN study group**: Complejo Hospitalario Torrecárdenas (Waldo Sánchez-Yebra Romera, Juan Antonio Sánchez-Gómez), Hospital Universitario Puerta del Mar (Inmaculada Guerrero Lozano), Hospital Universitario Reina Sofía (Rocío Tejero-García), Hospital Virgen de las Nieves (Fernando Cobo Martínez), Hospital Universitario Clínico San Cecilio (Laura Viñuela), Hospital Universitario Juan Ramón Jiménez (Francisco Franco-Álvarez de Luna), Complejo Hospitalario de Jaén (Ana Lara Oya, Isabel Casanovas Moreno-Torres), Hospital Regional Universitario de Málaga (María Pilar Bermúdez Ruiz), Hospital Universitario Virgen Macarena (Ana Isabel Suárez Barrenechea), Hospital Universitario Valme (Carmen Castro Méndez), Hospital Universitario Virgen del Rocío (Maite Ruiz Pérez de Pipaón), Hospital Universitario San Jorge (Héctor Condado Condado, Rocío Cebollada Sánchez), Hospital Universitario Miguel Servet (Concepción López Gómez), Hospital Universitario San Agustín (Emilio David Valverde Romero; Henar Villar Pérez), Hospital Universitario de Cabueñes (Marta Arias Temprano, Alba Guiu Martínez), Hospital Universitario Central Asturias (Teresa Peláez, Cristian Castello-Abietar), Hospital Universitario Son Llátzer (Carmen Collado Giner), Hospital Universitario Son Espases (Eva Alcoceba Cruixent, Carla Iglesias Escobar), Hospital de Galdakao (Ana Patricia Martínez Lafuente, Ana Gual de Torrella), Hospital Universitario Cruces (Leyre López-Soria, Olga Bello Jiménez), Hospital Universitario de Basurto (Miren Josebe Unzaga Barañano, Iris Sharon Pérez Ramos), Hospital Universitario Donostia (Miren Nekane Azkue Albizu), Hospital Universitario de Álava (Marina Fernández-Torres), Hospital Universitario de Gran Canaria Doctor Negrín (Raúl Gilarranz Luengo), Hospital Universitario Insular de Gran Canaria (Isabel de Miguel Martínez), Hospital Universitario Nuestra Señora de Candelaria (Ángeles Sampere Martínez), Hospital Universitario de Canarias (Silvia Rosa Campos Gutiérrez), Hospital Universitario Marqués de Valdecilla-IDIVAL (María Pía Roiz Mesones), Hospital General Universitario de Albacete (Julia Lozano Serra), Hospital General Universitario de Ciudad Real (Cristina Colmenarejo Serrano), Hospital Virgen de la Luz (Quilez Malo Casero), Hospital General Universitario de Guadalajara (María Elena Rodríguez Zurita), Hospital Universitario de Toledo (Rosa Jiménez Barrena), Hospital Santos Reyes (Marta Fernández Esgueva), Hospital Nuestra Señora de Sonsoles (Oscar Fraile Santos, Beatriz Pascual Sánchez), Hospital Universitario de Burgos (Cristina Labayru Echeverría), Hospital El Bierzo (Lisbeth Gonçalves De Freitas, Laura Milian Gay), Complejo Asistencial Universitario de León (Raquel Calleja Fernández, Alicia Barreales Fonseca), Complejo Asistencial Universitario de Palencia (Almudena Silvia Tinajas Puertas, Irene López-Ramos, Moisés García Bravo), Complejo Asistencial Universitario de Salamanca (Juan Luis Muñoz Bellido), Complejo Asistencial de Segovia (Noelia Arenal Andrés, Cristina Veintimilla Yánez, Susana Hernando Real, M. 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