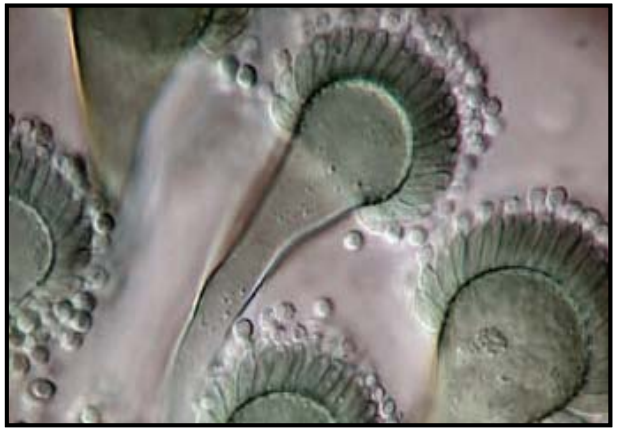
Détermination de la sensibilité aux antifongiques:

Pourquoi ? Quand ? Comment ?



Florent Morio

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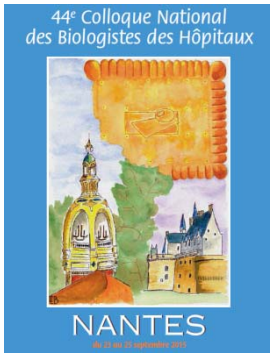
Florent.morio@chu-nantes.fr

- 44ème Colloque National des Biologistes des Hôpitaux, Nantes, 23-25 Septembre 2015-

ACNBH

ODPC N° 1495



44ème Colloque National
des Biologistes des Hôpitaux
Nantes, 23-25 septembre 2015

ODPC habilité à dispenser des programmes de DPC

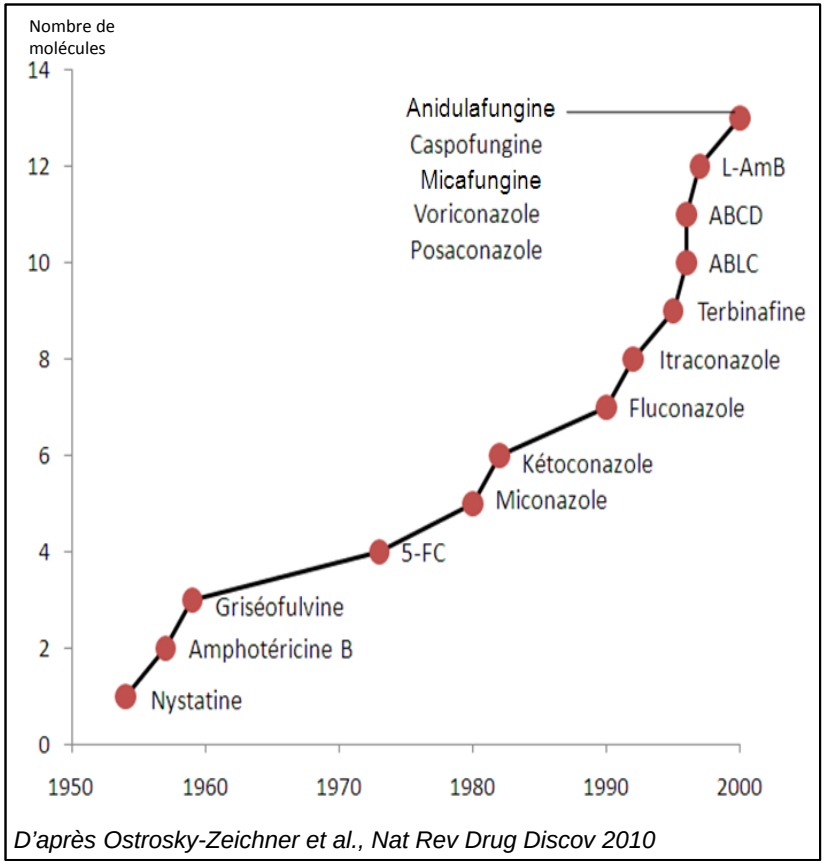
DECLARATION D'INTERET
DANS LE CADRE DE MISSIONS DE FORMATION REALISEES POUR L'ACNBH

Dr **Florent MORIO**
Exerçant au **CHU de NANTES**
déclare sur l'honneur

avoir un/des intérêt(s) avec les entreprises pharmaceutiques, du diagnostic ou d'édition de logiciels **en relation avec le DMDIV et/ou le sujet présenté** :

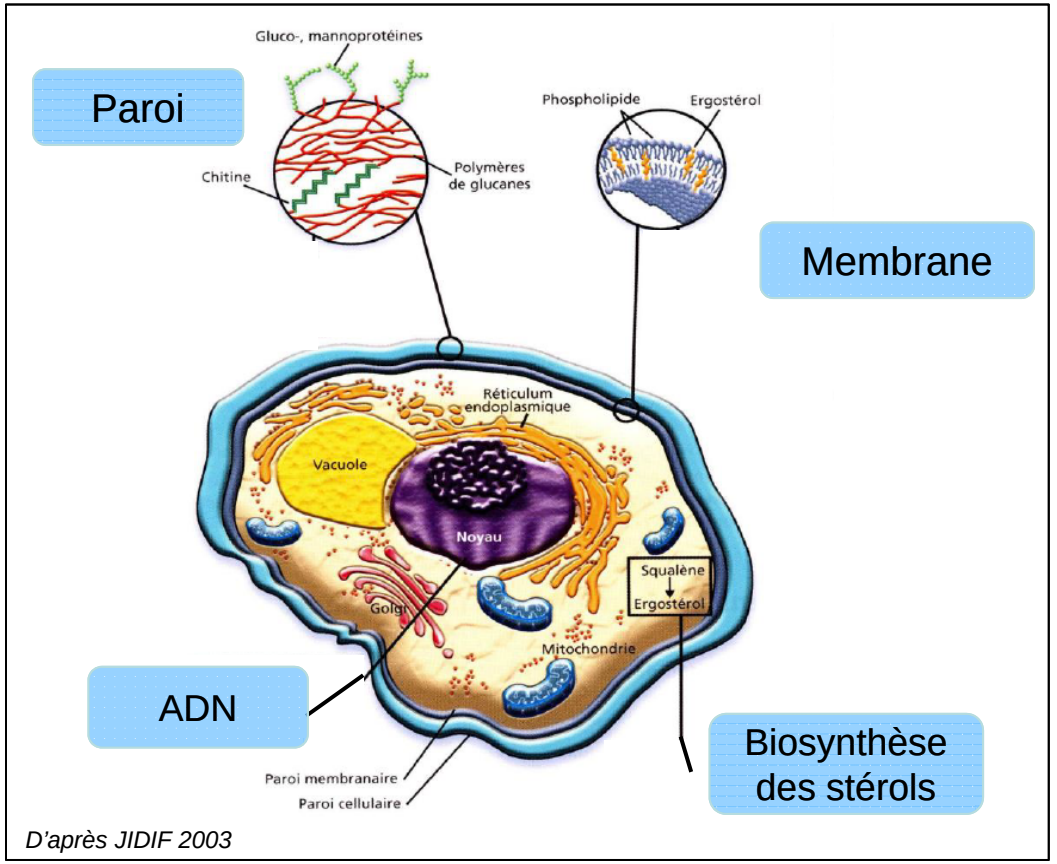
- Astellas Pharma
- Gilead Sciences
- Merck
- Pfizer

Antifongiques disponibles et modes d'action



D'après Ostrosky-Zeichner et al., Nat Rev Drug Discov 2010

Explosion du développement des antifongiques systémiques des années 50 aux années 2000



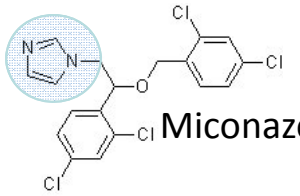
D'après JIDIF 2003

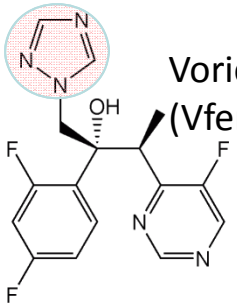
Cibles des antifongiques

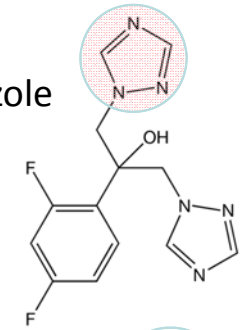
Nombre croissant de molécules mais nombre de cibles reste limité…

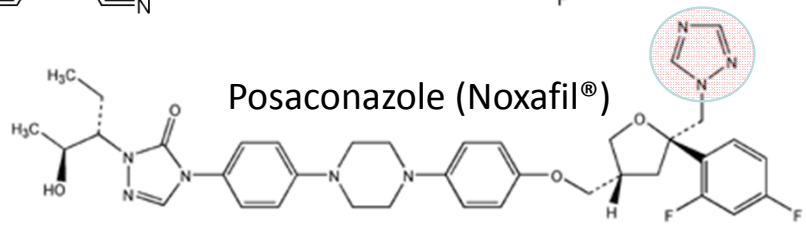
Antifongiques : quatre classes disponibles

Azolés

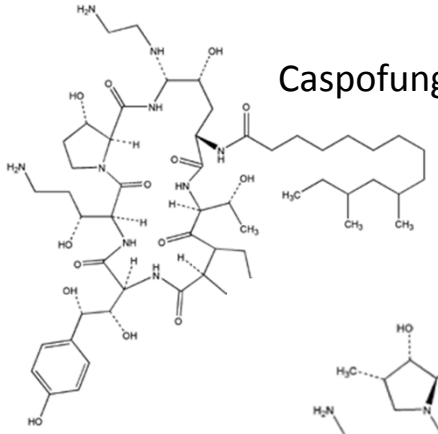

Miconazole

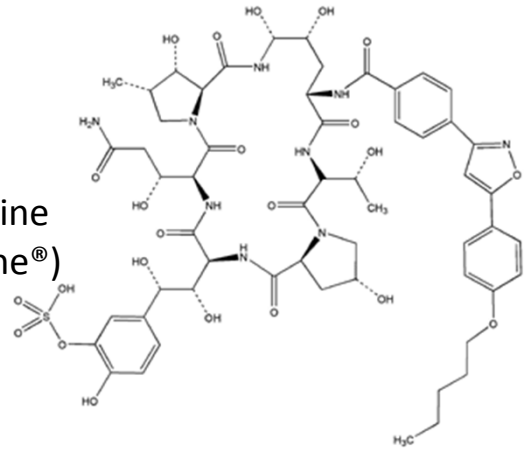

Voriconazole (Vfend®)


Fluconazole

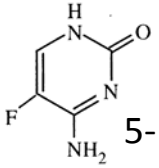

Posaconazole (Noxafil®)

Echinocandines


Caspofungine (Cancidas®)

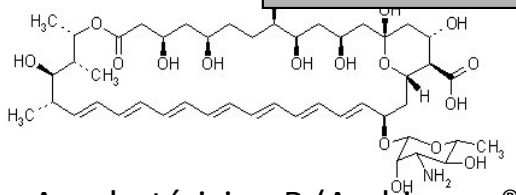

Micafungine (Mycamine®)

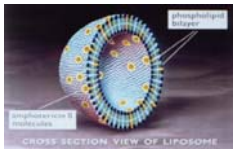
Pyrimidines

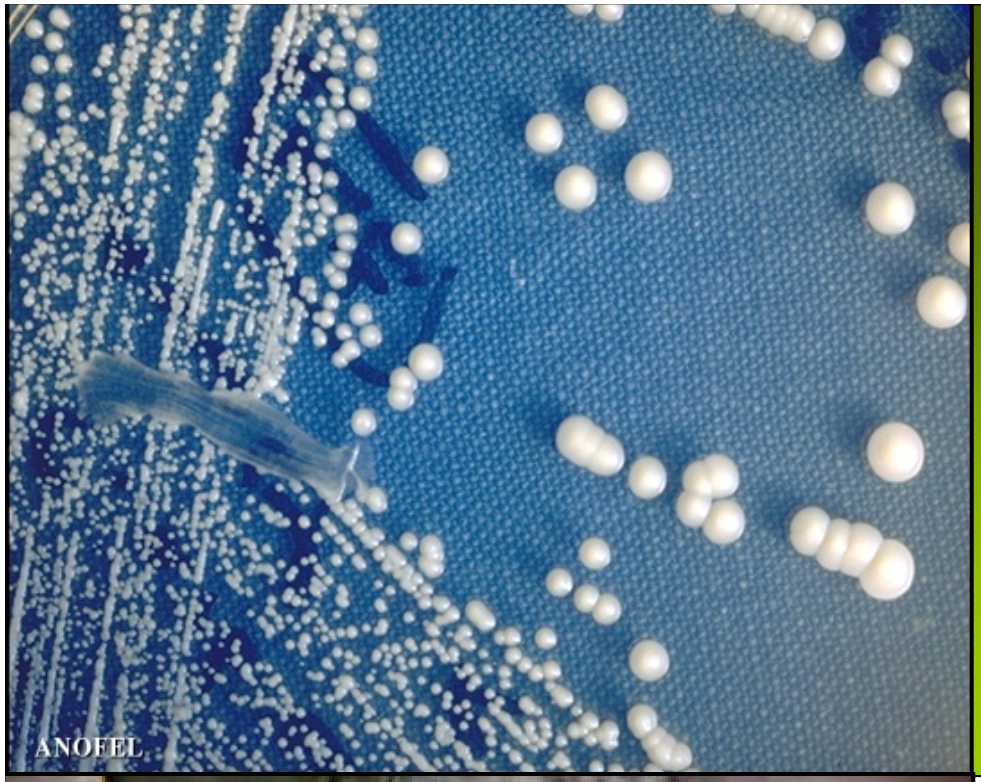

5-Fluorocytosine

Indications en curatif, empirique et prophylaxie des mycoses

Polyènes


Amphotéricine B (Ambisome®)





Pourquoi ?

Pourquoi réaliser un antifongigramme ?

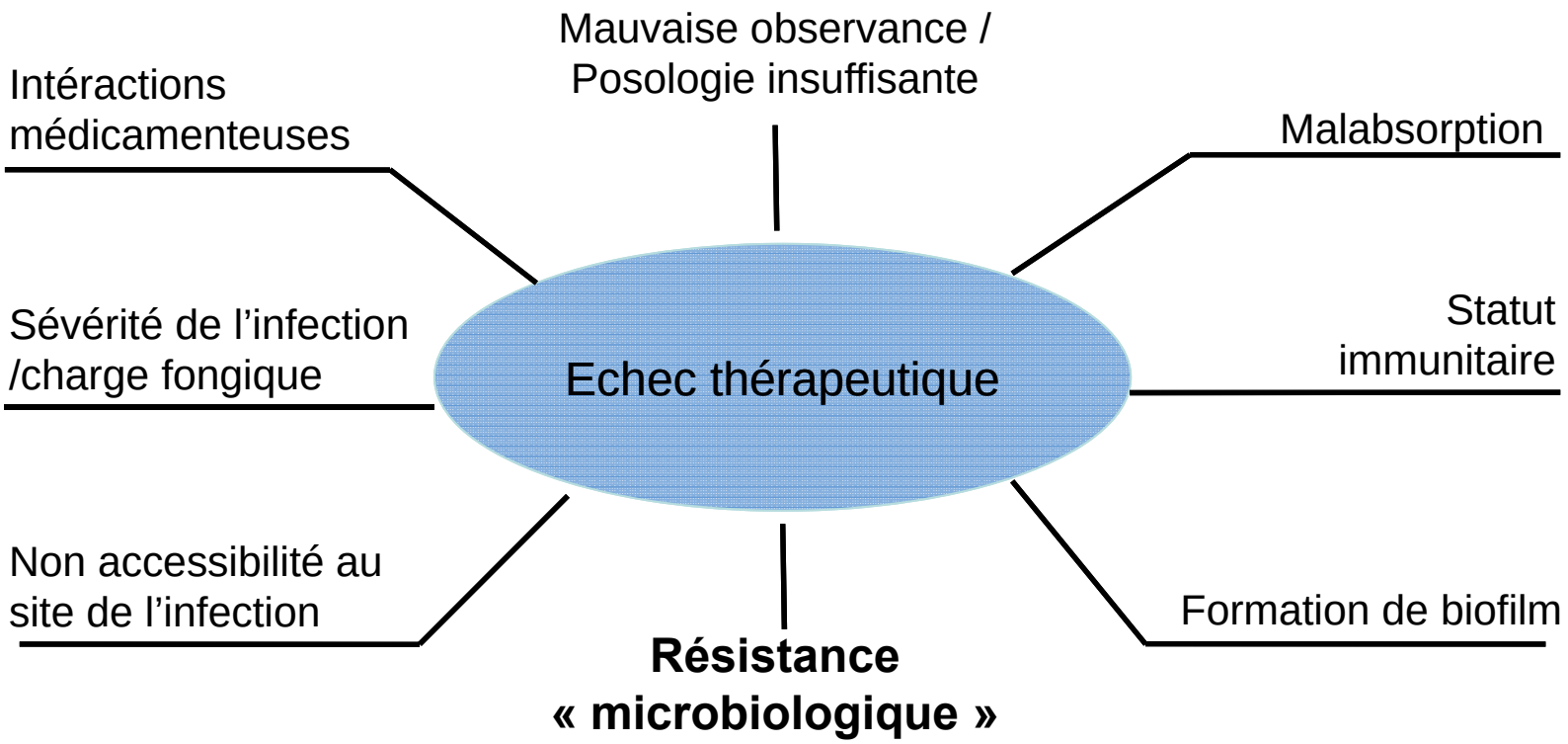
- **<u>Objectif</u>: prédire l'efficacité du traitement antifongique**
- **Permet d'apporter la preuve d'une résistance acquise !**
- <u>Règle des « 90 /60 »[1]</u> :
  - ✓ *Une infection due à une souche sensible, répond au traitement dans 90% des cas*
  - ✓ *Une infection due à une souche résistante, répond dans 60% des cas*

L'antifongigramme aide au choix de l'antifongique et à la surveillance du traitement mais ne peut prédire à lui seul la réponse thérapeutique

[1]Rex and Pfaller, Clin Infect Dis 2002

La résistance n'est qu'une des causes d'échec thérapeutique..

Les causes d'échec thérapeutique sont <u>multiples</u>



Adapté de White *et al*., Clin Microbiol Rev 1998

Résistance des champignons aux antifongiques

Résistance « intrinsèque »

Résistance qui se développe en l'absence d'exposition à l'antifongique.
Elle concerne toutes les souches de la même espèce et en constitue un trait génétique.

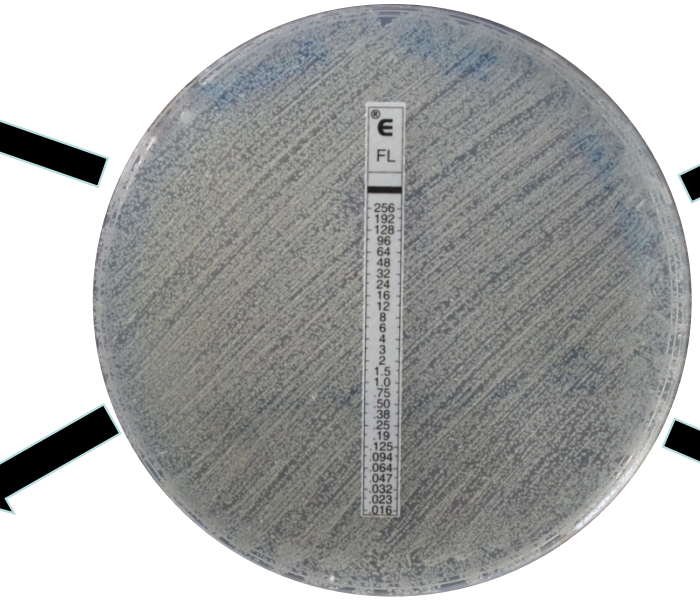
VS

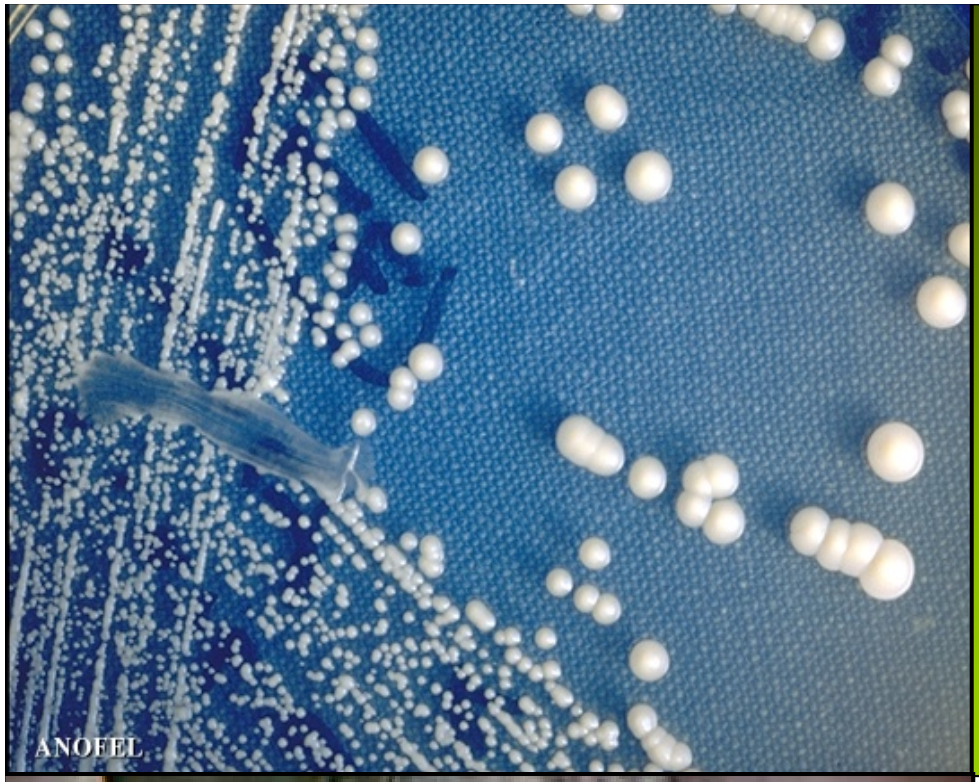
Résistance acquise

Résistance secondaire à une exposition à un antifongique (souche initialement sensible).
Elle ne touche que certaines souches d'une même espèce.

Problématique de la résistance aux antifongiques

Problématique d'actualité & axe de recherche en plein essor





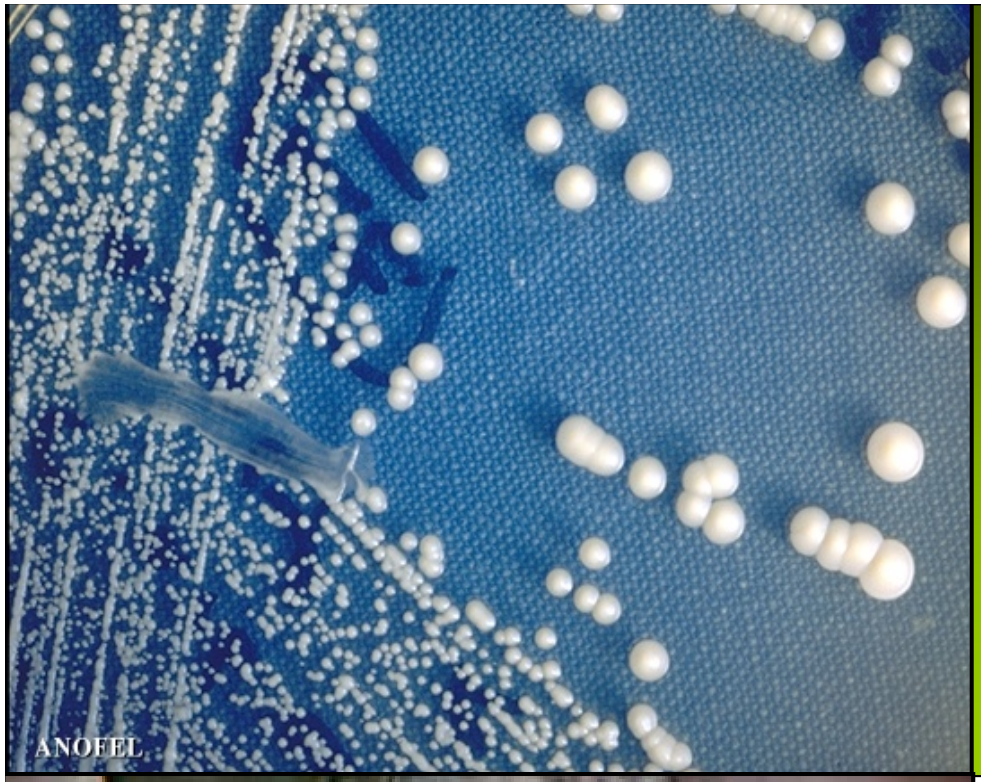
Quand ?

Indications de l'antifongigramme

- **Il ne doit pas être systématique**
- Recommandée dans plusieurs situations cliniques
  - ✓ Isolement d'une souche normalement d'un site stérile
  - ✓ Infection invasive
  - ✓ Infection par une espèce rare
  - ✓ Suspicion d'échec thérapeutique
- A visée épidémiologique

Pas de réel consensus sur les indications mais bon sens !!

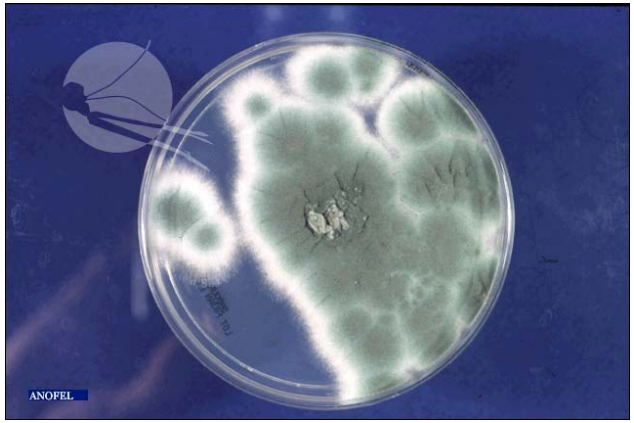
Cuenca-Estrella *et al*., Clin Microbiol Infect 2012; Pfaller *et al*., J Clin Microbiol 2012



Comment ?

Détermination de la CMI des champignons aux antifongiques

- **Non réalisable pour tous les antifongiques** (surtout ATFs systémiques)
- Méthodes de référence
  - CLSI : M27-A3, M38-A2
  - EUCAST: E.DEF 7.2, E.DEF 9.2
- Méthodes commerciales
  - Etest® (bioMérieux)
  - Sensititre YeastOne® (Trek Diagnostic)
  - Vitek2® AST Card (bioMérieux)
  - Neo-Sensitabs® (Rosco Diagnostica)

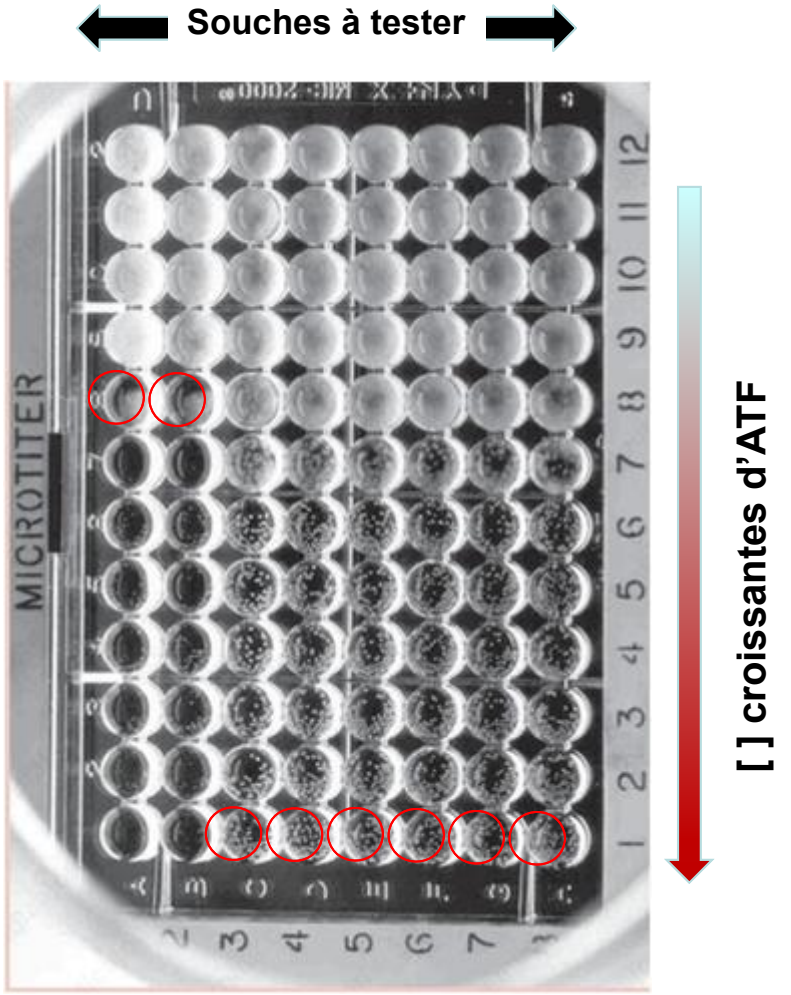


	Candida spp.	*Aspergillus* spp.
Etest®	oui	oui
SYO®	oui	?
Vitek2®	oui	non
Sensitabs®	oui	oui

En pratique: Tester 1 colonie ? laquelle ? plusieurs ? Combien ?

Méthodes de références

- <u>Deux standards disponibles:</u>
  - ✓ CLSI (USA), EUCAST (Europe)
- Principe de microdilution (liquide ++)
  - ✓ *Plaque avec quantités croissantes d'ATF*
  - ✓ *Inoculum standardisé*
  - ✓ *Incubation puis interprétation*
  - ✓ *Règles de lecture codifiées*
- <u>Avantages</u> :
  - ✓ *Standardisation (fiabilité, robustesse)*
  - ✓ *Comparaison interlaboratoires*
- <u>Inconvénients:</u>
  - ✓ *Long et fastidieux*
  - ✓ *Acquisition des poudres d'ATFs*



*Clinical Laboratory Institute,**EUropean Committee on Antimicrobial Susceptibility Testing

CLSI *vs* EUCAST : différences méthodologiques….

Détermination des CMIs des levures

	CLSI M27-A3	EUCAST (E.DEF 7.2)
Inoculum	0.5-2.5x10^3 CFU/mL	0.5-2.5x10^5 CFU/mL
Milieu	RPMI 1640 G0.2%	RPMI 1640 G2%
T° incubation	35°C	35°C
Incubation	24-48h	18-24h
Lecture	visuelle	spectrophotométrique

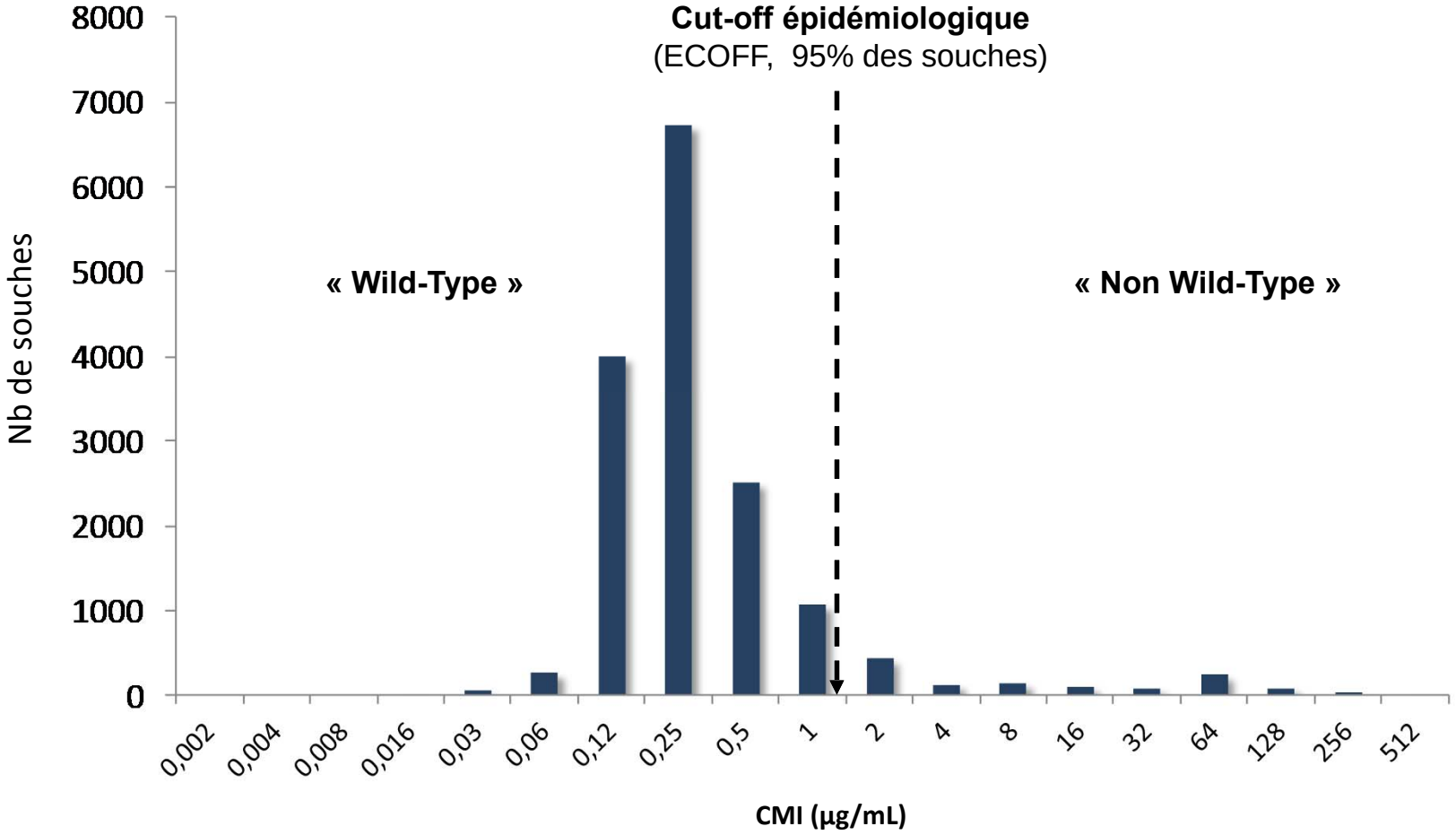
Standardisation permet d'établir le profil de sensibilité de chaque espèce et pour chaque antifongique

Détermination des seuils épidémiologiques (ECV/ECOFF) et seuils d'interprétation clinique : S, I/SDD, R

Principe de la détermination des seuils épidémiologiques (ECOFFs)

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

Fluconazole et *Candida albicans* (n=15991)

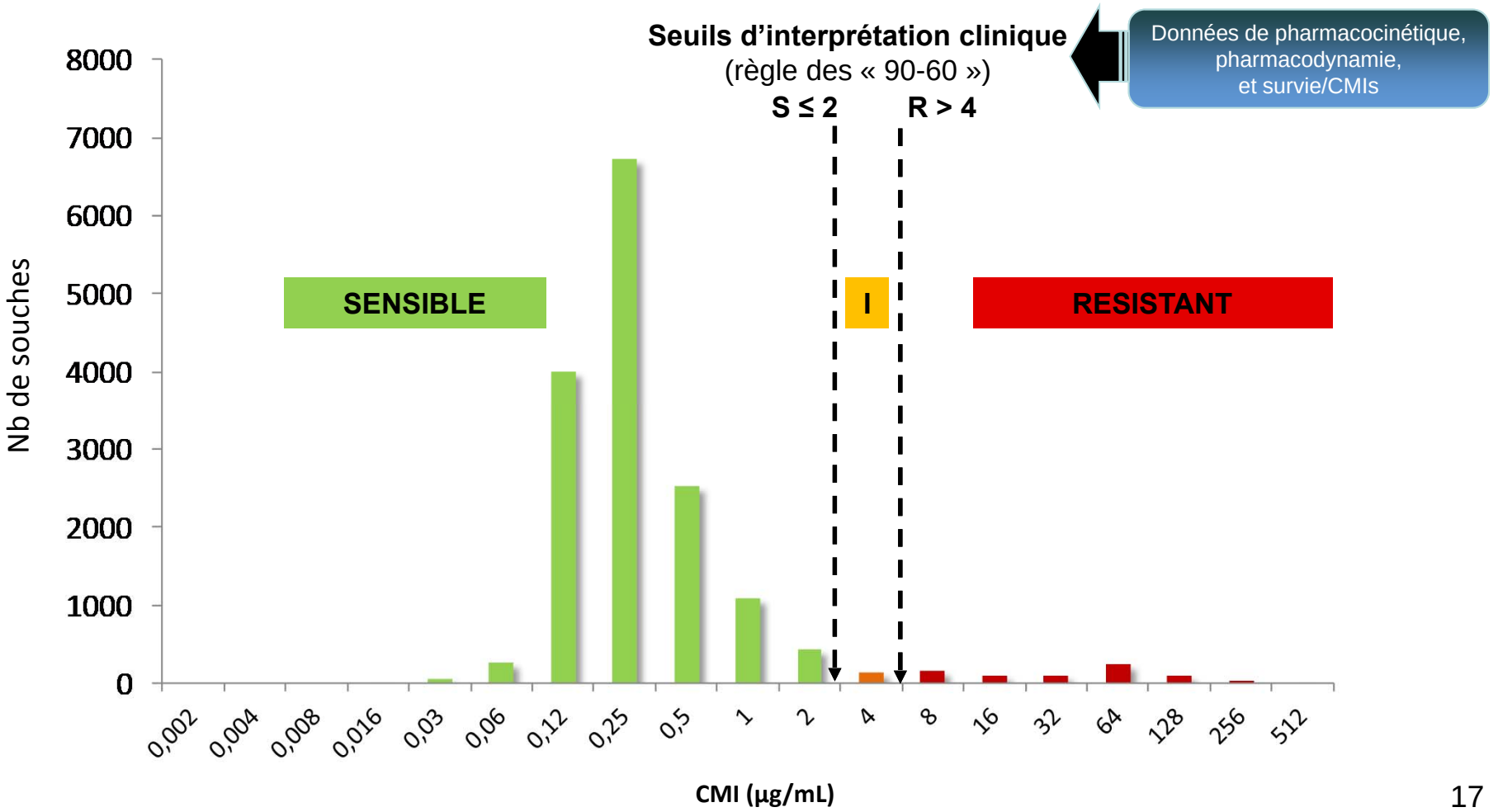


*http://www.eucast.org/ast_of_fungi

Principe de la détermination des seuils d'interprétation clinique

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

Fluconazole et *Candida albicans* (n=15991)



*http://www.eucast.org/ast_of_fungi

Seuils établis par le CLSI et l'EUCAST pour l'interprétation des CMIs (mg/L)

Antifongique(s)	Espèce(s)	Concentrations Minimales Inhibitrices (mg/L)			
		CLSI M27-A3		EUCAST	
		S	R	S	R
Fluconazole	*C. albicans*	≤2	≥8	≤2	>4
	C. glabrata	-	≥64	≤0.002	>32
	C. tropicalis	≤2	≥8	≤2	>4
	C. krusei[1]	-	-	-	-
	C. parapsilosis	≤2	≥8	≤2	>4
Voriconazole	*C. albicans*	≤0.125	≥1	≤0.12	>0.12
	C. glabrata[2]	-	-	-	-
	C. tropicalis	≤0.125	≥1	≤0.12	>0.12
	C. krusei	≤0.5	≥2	-	-
	C. parapsilosis	≤0.125	≥1	≤0.12	>0.12
Micafungine	*C. albicans*	≤0.25	≥1	≤0.016	>0.016
	C. glabrata	≤0.06	≥0.25	≤0.03	>0.03
	C. tropicalis	≤0.25	≥1	-	-
	C. krusei	≤0.25	≥1	-	-
	C. parapsilosis	≤2	≥8	≤0.002	>2
Amphotéricine B	*C. albicans*	-	-	≤1	>1
	C. glabrata	-	-	≤1	>1
	C. tropicalis	-	-	≤1	>1
	C. krusei	-	-	≤1	>1
	C. parapsilosis	-	-	≤1	>1

[1]La détermination de la CMI n'est pas recommandée pour cette espèce (non sensible à cet antifongique) ;
[2]Les données disponibles pour cette espèce ne permettent pas d'établir de relation formelle entre la sensibilité *in vitro* et le succès thérapeutique ;

Morio F *et al*., Actualités en Microbiologie Clinique 2014

Seuils établis par le CLSI et l'EUCAST pour l'interprétation des CMIs (mg/L)

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	C. krusei[1]	-	-	-	-
	C. parapsilosis	≤2	≥8	≤2	>4
Voriconazole	*C. albicans*	≤0.125	≥1	≤0.12	>0.12
	C. glabrata[2]	-	-	-	-
					.12
					.12
Micafungine					.016
	C. glabrata	≤0.06	≥0.25	≤0.03	>0.03
	C. tropicalis	≤0.25	≥1	-	-
	C. krusei	≤0.25	≥1	-	-
	C. parapsilosis	≤2	≥8	≤0.002	>2
Amphotéricine B	*C. albicans*	-	-	≤1	>1
	C. glabrata	-	-	≤1	>1
	C. tropicalis	-	-	≤1	>1
	C. krusei	-	-	≤1	>1
	C. parapsilosis	-	-	≤1	>1

Importants efforts d'harmonisation ces dernières années entre CLSI et EUCAST

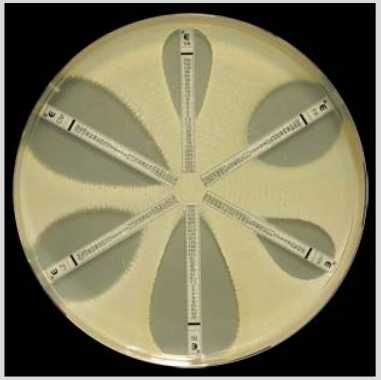
[1]La détermination de la CMI n'est pas recommandée pour cette espèce (non sensible à cet antifongique) ;
[2]Les données disponibles pour cette espèce ne permettent pas d'établir de relation formelle entre la sensibilité *in vitro* et le succès thérapeutique ;

Morio F *et al*., Actualités en Microbiologie Clinique 2014

Méthodes commercialisées pour déterminer les CMIs en mycologie

Diffusion en milieu gélosé

Bandelettes Etest®
(bioMérieux)



Neo-Sensitabs®
(Rosco Diagnostica)

Microdilution en milieu liquide

Vitek2 AST Card®
(bioMérieux)

SensititreYeastOne®
(TREK Diagnostic Systems)

Bonne concordance avec les techniques de référence du CLSI et de l'EUCAST
(surtout évaluée pour les levures)

Matar *et al*., 2003; Alexander *et al*., 2007; Cuenca-Estrella *et al*., 2010; Pfaller *et al*., 2012; Ranque *et al*., 2012; Pfaller *et al*., 2013

Méthodes commerciales pour déterminer les CMIs

Antifongiques étudiées selon la méthode utilisée

	Azolés								Polyènes		Echinocandines			Autres		
	FLC	VRC	ITC	POS	KET	MIC	ECO	CLO	AMB	NYS	CAS	MIC	ANI	5FC	TER	CICLO
Etest®																
Neo-Sensitabs®																
Sensititre YeastOne®																
Vitek2 AST®																

Acronymes: *FLU: fluconazole; VRC: voriconazole; ITC: itraconazole; POS: posaconazole; KET: ketoconazole; MIC: miconazole: ECO: econazole; CLO: clotrimazole; AMB: amphotericine B; NYS: nystatine; CAS: caspofungine; MIC: micafungine; ANI: anidulafungine; 5FC: 5-fluorocytosine; TER: terbinafine; CICLO: Ciclopiroxolamine*

Disponible

Non disponible

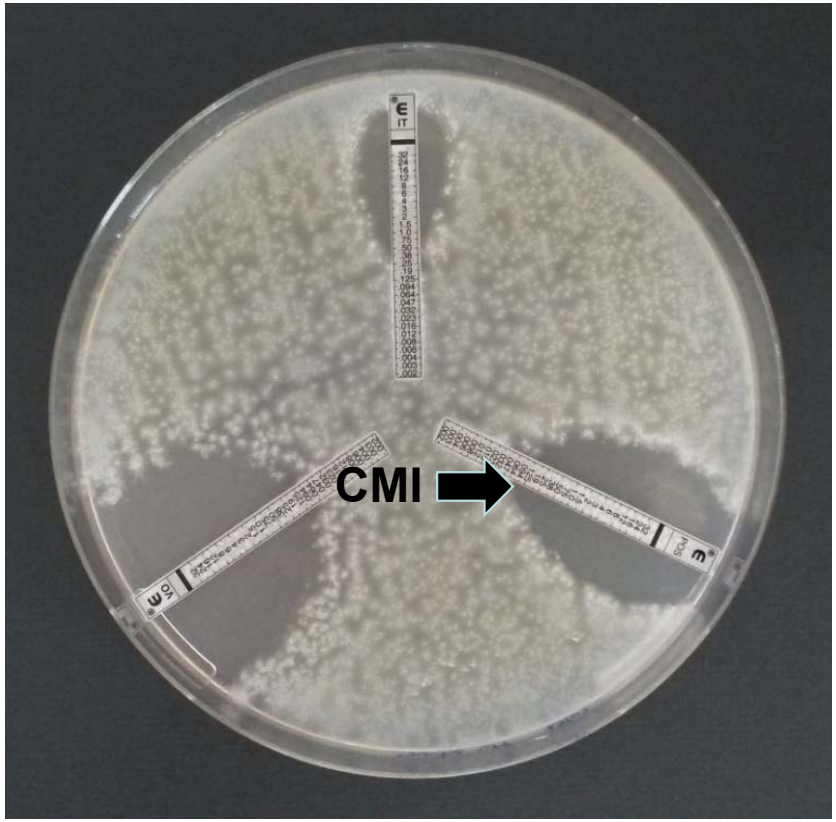
Le panel d'antifongiques disponibles dépend de la méthode utilisée !

Méthode Etest® (bioMérieux)

- **Méthode <u>commerciale</u> la plus utilisée** dans les laboratoires de mycologie
- Principe de diffusion en milieu gélosé
  - ✓ *Milieu gélosé RPMI (ref AEB 122180)*
  - ✓ *Inoculum standardisé*
  - ✓ *Dépôt de la bandelette imprégnée de l'ATF*
  - ✓ *Incubation puis interprétation*
  - ✓ *Règles de lecture codifiée (**<u>dépendante de l'ATF et du type de champignon</u>**)*
- <u>Avantages</u>

  - ✓ *Bonne concordance avec méthodes de référence*
  - ✓ *Simple et rapide*
- <u>Inconvénients</u>

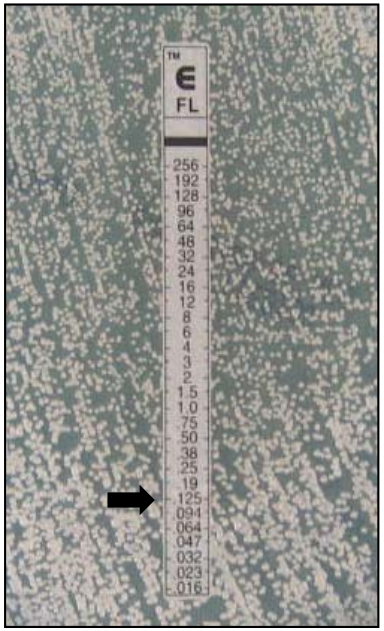
  - ✓ *Non reconnue comme méthode de référence !!*
  - ✓ *Seuils d'interprétation pas toujours disponibles*
  - ✓ *Expertise ++*



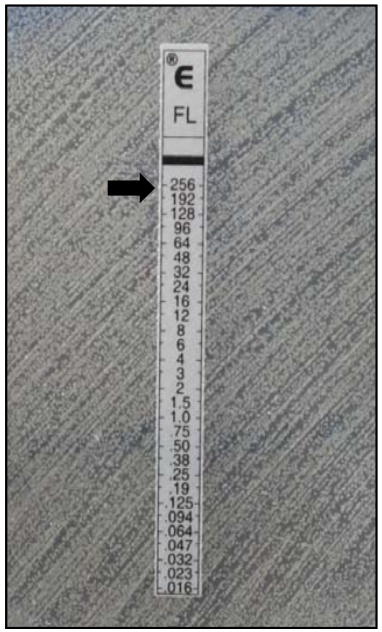
Méthode Etest® : principes et difficultés

***Candida* spp. *: e*xemples de lectures des Concentrations Minimales Inhibitrices

Azolés (lecture à 80% d'inhibition)

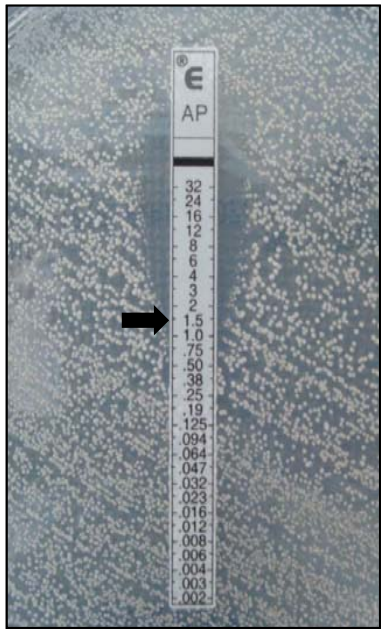


C. albicans: Le **phénomène de traîne** complique la lecture de la CMI **CMI=0.125 µg/mL**. Souche sensible.



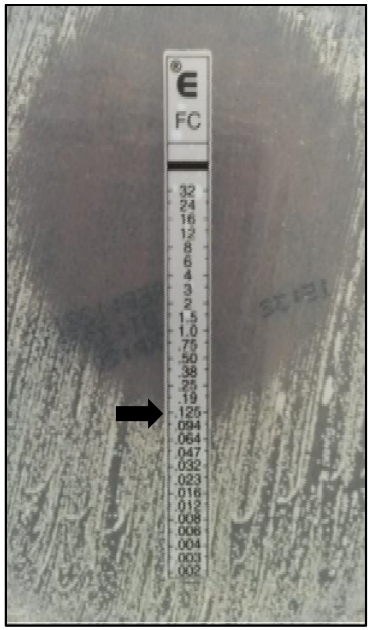
C. albicans: Souche résistante (**CMI>256 µg/mL**)

Amphotéricine B (100%)



C. albicans: CMI élevée liée à la présence d'une mutation du gène *ERG3*. **CMI=1.5 µg/mL**.

Flucytosine (90%)



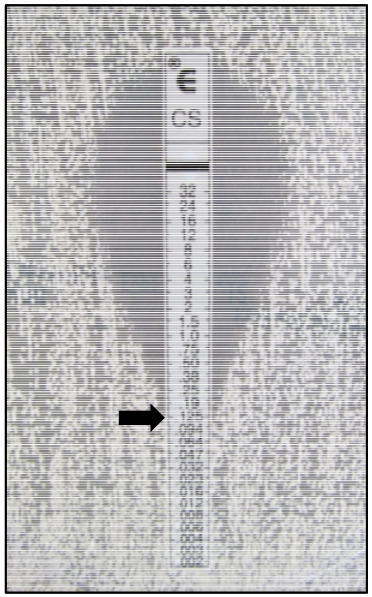
C. albicans: Souche sensible (**CMI=0.125 µg/mL**)

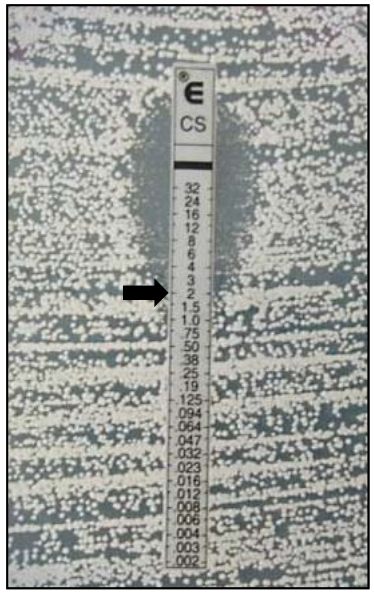
Morio F *et al*., Actualités en Microbiologie Clinique 2014

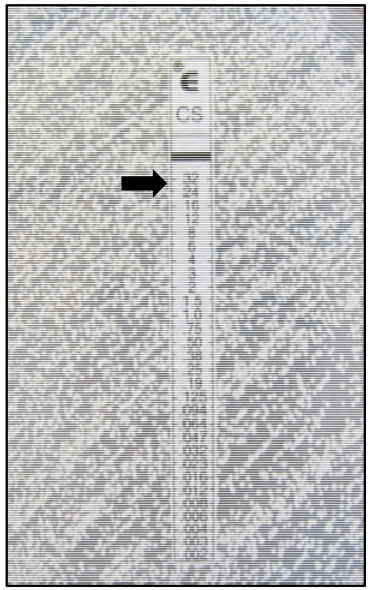
Méthode Etest® : principes et difficultés

Candida spp. *: e*xemples de lectures des Concentrations Minimales Inhibitrices

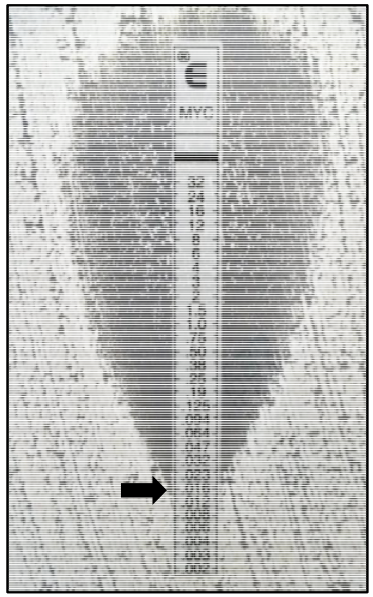
Echinocandines (lecture à 80% d'inhibition)







C. albicans: Isolats séquentiels et isogéniques obtenus chez un patient traité par caspofungine pendant plusieurs semaines. Noter l'augmentation progressive de la CMI au décours de la prise en charge thérapeutique (isolat initial à gauche).



C. tropicalis: « Eagle effect » ou phénomène de **croissance paradoxale**. CMI=0.023µg/mL)

Modalités de lecture diffèrent pour les champignons filamenteux !

Morio F *et al*., Actualités en Microbiologie Clinique 2014

Méthode Etest® : principes et difficultés

Attention à l'extrapolation des breakpoints aux techniques commerciales !!

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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]

The purpose of this study was to evaluate the performance of caspofungin Etest and the recently revised CLSI breakpoints. A total of 497 blood isolates, of which 496 were wild-type isolates, were included. A total of 65/496 susceptible isolates (13.1%) were misclassified as intermediate (I) or resistant (R). Such misclassifications were most commonly observed for *Candida krusei* (73.1%) and *Candida glabrata* (33.1%). The revised breakpoints cannot be safely adopted for these two species.

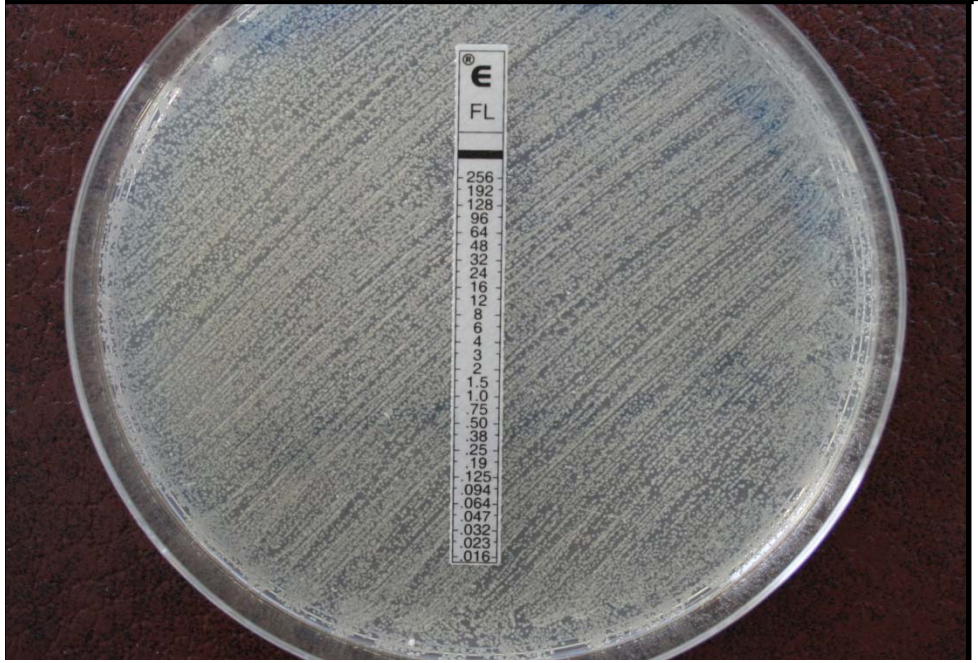
L'application des breakpoints du CLSI à la méthode Etest® conduit à surestimer la résistance de *C. glabrata* et *C. krusei* à la caspofungine

Arendup *et al.*, Antimicrob Agents Chemother 2008

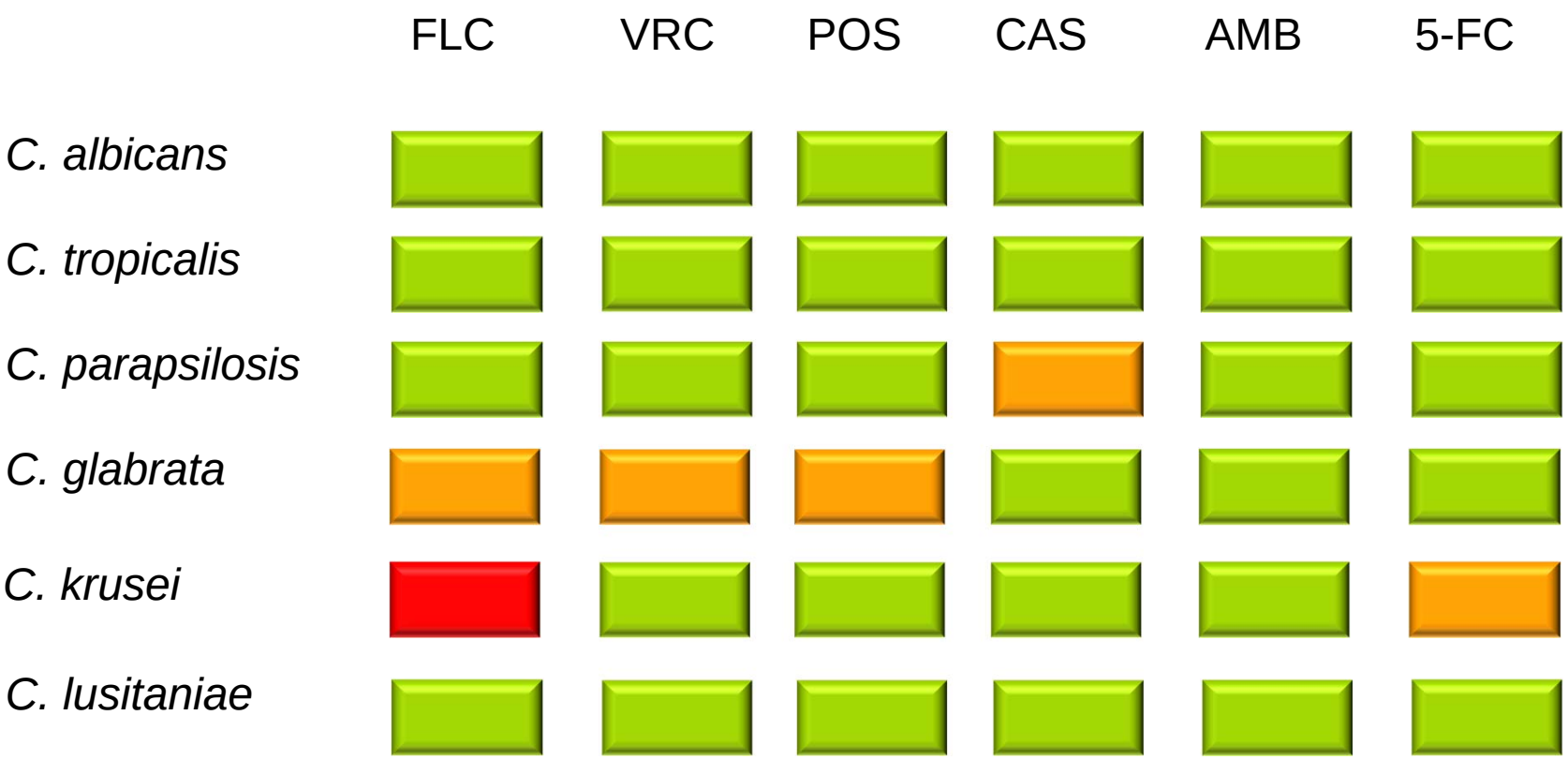
Antifongigramme

&

Candida spp.



Profil de sensibilité des principales espèces de *Candida* spp.



Source : Eric Dannaoui

Qu'en est-il des autres espèces ?

Espèces rares présentent parfois une sensibilité réduite aux ATFs !

JMM Case Reports (2014) DOI 10.1099/jmmcr.0.003707

Case Report

A rare case of breakthrough fungal pericarditis due to fluconazole-resistant *Candida auris* in a patient with chronic liver disease

Vikas Khillan,[1] Neha Rathore,[1] Shallu Kathuria[2] and Anuradha Chowdhary[2]

Correspondence
Anuradha Chowdhary
dranuradha@hotmail.com

[1]Department of Microbiology, Institute of Liver and Biliary Sciences,
[2]Department of Medical Mycology, Vallabhbhai Patel Chest Institute, Un

JOURNAL OF CLINICAL MICROBIOLOGY, June 2007, p. 2025–2027 Vol. 45, No. 6
0095-1137/07/$08.00+0 doi:10.1128/JCM.00222-07
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Outbreak of Fungemia among Neonates Caused by *Candida haemulonii* Resistant to Amphotericin B, Itraconazole, and Fluconazole

U. Khan,[1]* Noura A. Al-Sweih,[1,2] Suhail Ahmad,[1] Nawal Al-Kazemi,[2]
Seema Khan,[2] Leena Joseph,[1] and Rachel Chandy[1]

biology, Faculty of Medicine, Kuwait University, P. O. Box 24923, Safat,[1] and Department of Microbiology, Maternity Hospital, Ministry of Health,[2] Kuwait

CASE REPORT

JCM Journals.ASM.org

Echinocandin Resistance in Two *Candida haemulonii* Isolates from Pediatric Patients

Marisol Dominguez Muro,[a,b] Fábio de Araújo Motta,[b] Marion Burger,[b,c] Analy Sa Líbera Maria Dalla-Costa[a,b]

Hospital de Clínicas, Universidade Federal do Paraná Curitiba, Paraná, Brazil[a]; Hospital, Faculdades, an Brazil[b]; Secretaria Municipal da Saúde de Curitiba, Paraná, Brazil[c]; and Laboratório Especial de Micolo

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2006, p. 3578–3582 Vol. 44, No. 10
0095-1137/06/$08.00+0 doi:10.1128/JCM.00863-06
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Candida rugosa, an Emerging Fungal Pathogen with Resistance to Azoles: Geographic and Temporal Trends from the ARTEMIS DISK Antifungal Surveillance Program

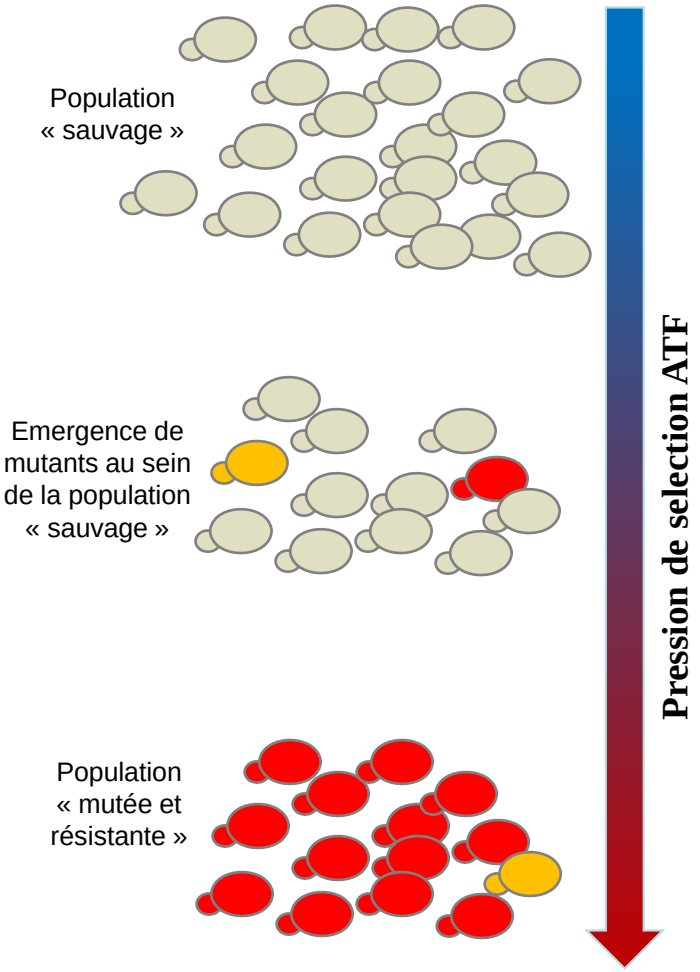
M. A. Pfaller,[1]* D. J. Diekema,[1] A. L. Colombo,[2] C. Kibbler,[3] K. P. Ng,[4] D. L. Gibbs,[5]
V. A. Newell,[5] and the Global Antifungal Surveillance Group

University of Iowa College of Medicine, Iowa City, Iowa[1]; Escola Paulista de Medicina, Sao Paulo, Brazil[2]; Royal Free Hospital, London, United Kingdom[3]; University Malaya, Kuala Lumpur, Malaysia[4]; and Giles Scientific, Inc., Santa Barbara, California[5]

Intérêt de tester la CMIs en cas d'isolement d'une espèce rare

Candida spp. & résistance acquise

- Ampleur encore inférieure à celui de la résistance aux antibiotiques
- Résultat d'un **processus de sélection** +/- rapide
- **Non transférable** (≠ bactéries)
- Repose sur **plusieurs mécanismes** pfs associés chez une même souche clinique
- En pratique:
  - ✓ *Résistance à l'AMB est exceptionnelle*
  - ✓ *Résistance aux azolés globalement stable*
  - ✓ *Emergence de la résistance aux échinocandines*



Candida spp. & résistance acquise aux azolés

- **Connue depuis les années 90** en particulier chez les patients infectés par le VIH et présentant une candidose oropharyngée
- **Evénement relativement rare** et consécutif à une exposition aux **azolés au long cours**
- **Plus rapide chez *C. glabrata***
- **Progressif et cumulatif**

NB: Décrit chez patients atteints de mucoviscidose, traités par azolés au long cours pour une colonisation aspergillaire/ABPA (=dommage collatéral)

Sanglard *et al*., Lancet Infect Dis 2002
Coste *et al*., Eukaryot Cell 2007
MacCallum *et al*., Antimicrob Agents Chemother 2010
Siikala *et al*., J Antimicrob Chemother 2010

CLSI 2010-2011	% FLC-R
C. albicans (1405)	0.4 %
C. glabrata (571)	8.8%
C. parapsilosis (565)	2.1%

Résistance est souvent croisée entre les antifongiques azolés

Candida spp. & résistance acquise aux échinocandines

Une conséquence de l'utilisation accrue des échinocandines dans nos hôpitaux ?

Emergence of echinocandin-resistant Candida spp. in a hospital setting: A consequence of 10 years of increasing use of antifungal therapy?

ARTICLE *in* EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY 33(9) · APRIL 2014

Impact Factor: 2.67 · DOI: 10.1007/s10096-014-2096-9 · Source: PubMed

Fekkar *et al.*, Eur J Clin Microbiol Infect Dis 2014

Candida spp. & résistance acquise aux échinocandines

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2011, p. 3254–3260 Vol. 55, No. 7
0066-4804/11/$12.00 doi:10.1128/AAC.01750-10
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Caspofungin Dose Escalation for Invasive Candidiasis Due to Resistant *Candida albicans*

Nathan P. Wiederhold,[1,2,3]* Laura K. Najvar,[1,3] Rosie A. Bocanegra,[1,3] William R. Kirkpatrick,[1,3] and Thomas F. Patterson[1,3]

The University of Texas Health Science Center at San Antonio, San Antonio, Texas[1]; The University of Texas at Austin College of Pharmacy, Austin, Texas[2]; and South Texas Veterans Health Care System, San Antonio, Texas[3]

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s.ASM.org

CASE REPORT

Breakthrough Invasive *Candida glabrata* in Patients on Micafungin: a Novel *FKS* Gene Conversion Correlated with Sequential Elevation of MIC

ta Yonetani,[c] Hiroshi Makino,[c] Takayasu Watanabe,[a] Naoki Tsujimoto,[a]
shitsugu Miyazaki,[b] Hajime Takizawa,[a] Hajime Goto[a]

ry Medicine, Mitaka, Tokyo, Japan[a]; Department of Chemotherapy and Mycoses, National Institute of Medicine, Department of Laboratory Medicine, Mitaka, Tokyo, Japan[c]

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Stepwise Development of a Homozygous S80P Substitution in Fks1p, Conferring Echinocandin Resistance in *Candida tropicalis*

Rasmus Hare Jensen,[a] Helle Krogh Johansen,[b] Maiken Cavling Arendru

Mycology Unit, Statens Serum Institut, Copenhagen, Denmark[a]; Department of Clinical Mi Denmark[b]

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2007, p. 1876–1878 Vol. 51, No. 5
0066-4804/07/$08.00+0 doi:10.1128/AAC.00067-07
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Acquired Echinocandin Resistance in a *Candida krusei* Isolate Due to Modification of Glucan Synthase

Jennifer Nielsen Kahn,[1] Guillermo Garcia-Effron,[2] Ming-Jo Hsu,[1] Steven Park,[2] Kieren A. Marr,[3] and David S. Perlin[2]*

Merck Research Laboratories, Rahway,[1] and Public Health Research Institute, New Jersey Medical School—UMDNJ, Newark,[2] New Jersey, and Fred Hutchinson Cancer Research Center, Seattle, Washington[3]

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Rapid Emergence of Echinocandin Resistance during *Candida kefyr* Fungemia Treatment with Caspofungin

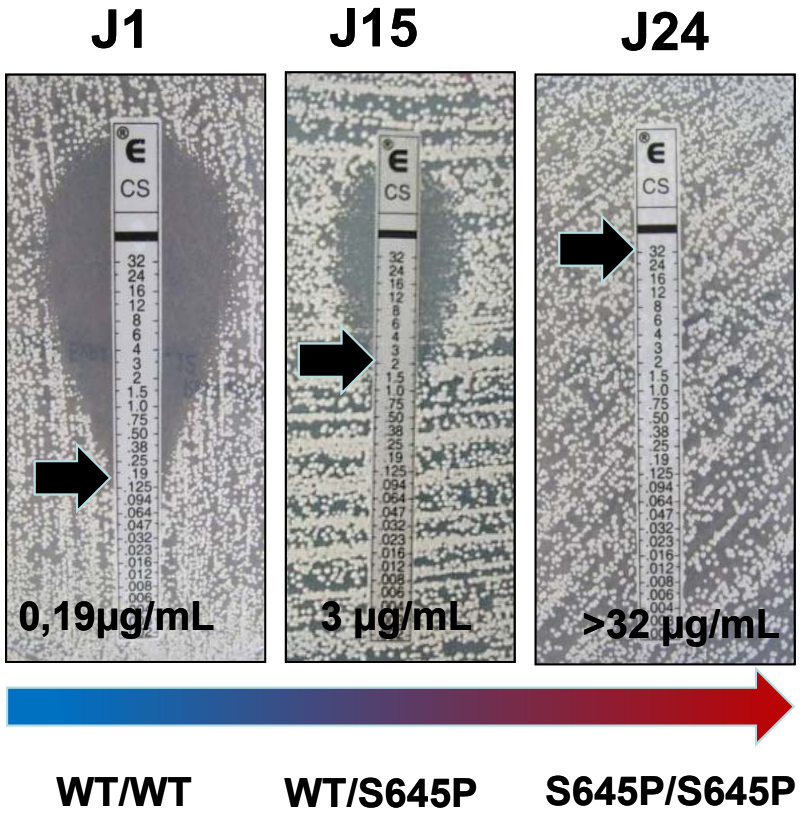
A. Fekkar,[a,b] I. Meyer,[a] J. Y. Brossas,[b] E. Dannaoui,[c] M. Palous,[a] M. Uzunov,[d] S. Nguyen,[d] V. Leblond,[d] D. Mazier,[a,b,e] A. Datry[a,b,e]

Service de Parasitologie Mycologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France[a]; INSERM, UMR S945, Paris, France[b]; Service de Microbiologie, Unité de Parasitologie-Mycologie, Hôpital Européen Georges Pompidou, Université Paris-Descartes, Faculté de Médecine, Paris, France[c]; Service d'Hématologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France[d]; Université Pierre et Marie Curie-Paris 6, UMR S945, Paris, France[e]

Candida spp. & résistance acquise aux échinocandines

- **Résistance naturelle est exceptionnelle**
- Résistance acquise de plus en plus décrite dans la littérature
- Secondaire à une **exposition** (**parfois courte)** aux échinocandines
- Associée à un **échec clinique**
- Décrites chez les plusieurs espèces (*C. glabrata* ++)
- Résistance souvent croisée
- Principal mécanisme
  - ➡ mutation(s) sur le gène *FKS1/2*

Souches séquentielles de *C. albicans*, isolées chez un patient d'hématologie traité par caspofungine (Cancidas®)



Alexander *et al*., Clin Infect Dis 2013; Dannaoui *et al*., Emerg Infect Dis 2012; Fekkar *et al*., Eur J Clin Microbiol Infect Dis 2014

Vers une multi-résistance de *Candida glabrata* aux antifongiques ?

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Frequency of Decreased Susceptibility and Resistance to Echinocandins among Fluconazole-Resistant Bloodstream Isolates of *Candida glabrata*

M. A. Pfaller,[a] M. Castanheira,[a] S. R. Lockhart,[b] A. M. Ahlquist,[b] S. A. Messer,[a] and R. N. Jones[a,c]

JMI Laboratories, North Liberty, Iowa, USA[a]; Mycotic Disease Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[b]; and Tufts University School of Medicine, Boston, Massachusetts, USA[c]

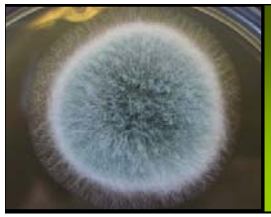
The echinocandin class of antifungal agents is considered to be the first-line treatment of bloodstream infections (BSI) due to *Candida glabrata*. Recent reports of BSI due to strains of *C. glabrata* resistant to both fluconazole and the echinocandins are of concern and prompted us to review the experience of two large surveillance programs, the SENTRY Antimicrobial Surveillance Program for the years 2006 through 2010 and the Centers for Disease Control and Prevention population-based surveillance conducted in 2008 to 2010. The *in vitro* susceptibilities of 1,669 BSI isolates of *C. glabrata* to fluconazole, voriconazole, anidulafungin, caspofungin, and micafungin were determined by CLSI broth microdilution methods. Fluconazole MICs of ≥64 μg/ml were considered resistant. Strains for which anidulafungin and caspofungin MICs were ≥0.5 μg/ml and for which micafungin MICs were ≥0.25 μg/ml were considered resistant. A total of 162 isolates (9.7%) were resistant to fluconazole, of which 98.8% were nonsusceptible to voriconazole (MIC > 0.5 μg/ml) and 9.3%, 9.3%, and 8.0% were resistant to anidulafungin, caspofungin, and micafungin, respectively. There were 18 fluconazole-resistant isolates that were resistant to one or more of the echinocandins (11.1% of all fluconazole-resistant isolates), all of which contained an acquired mutation in *fks1* or *fks2*. By comparison, there were no echinocandin-resistant strains detected among 110 fluconazole-resistant isolates of *C. glabrata* tested in 2001 to 2004. These data document the broad emergence of coresistance over time to both azoles and echinocandins in clinical isolates of *C. glabrata*.

Antifongigramme

&

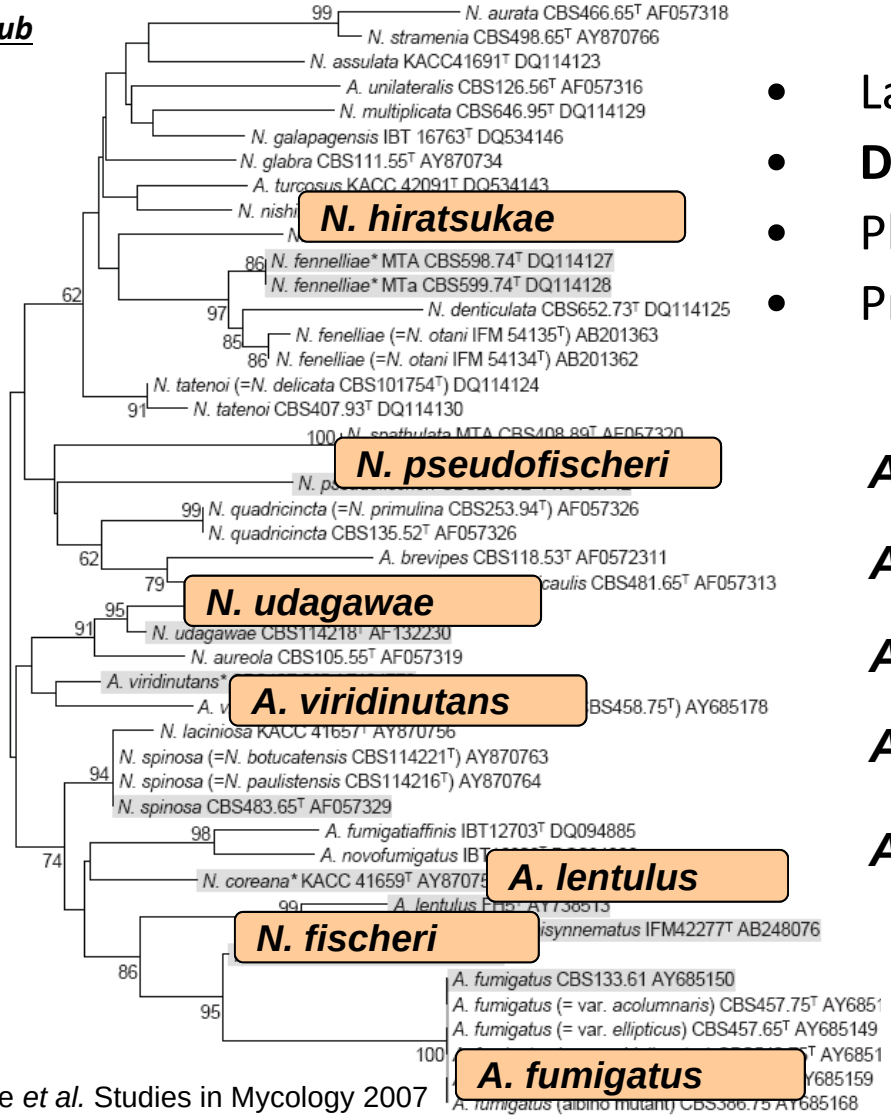
Aspergillus spp.

Aspergillus section *Fumigati*
Aspergillus section *Nidulantes*
Plus de 180 espèces distinctes d'*Aspergillus* (Pitt *et al*., 2000)
-
Nouvelles espèces régulièrement décrites
-
Certaines espèces morphologiquement proches conduisant à définir des complexes d'espèces ou « section »
-
Profil de sensibilité parfois variable au sein d'une même « section »
Aspergillus section *Terrei*
Aspergillus section *Flavi*

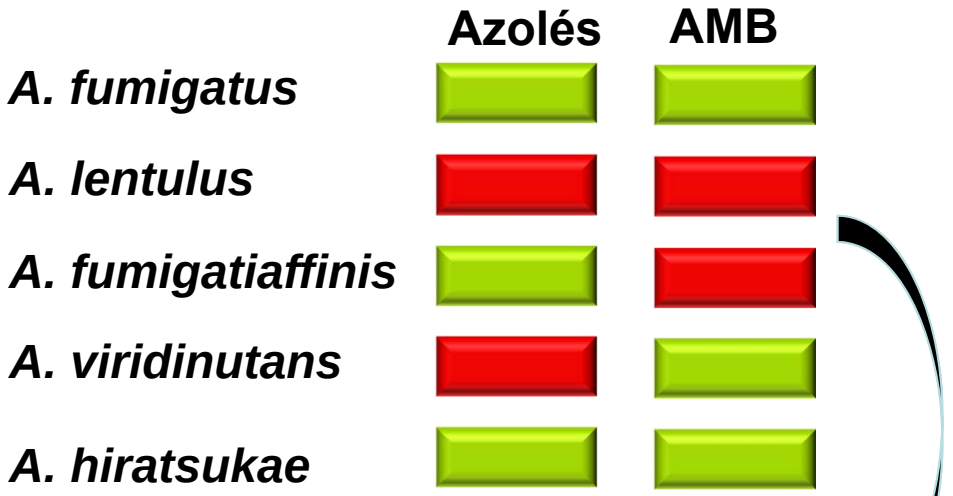


Aspergillus section *Fumigati*: une diversité méconnue !!

B-tub

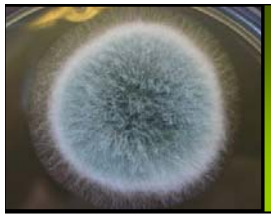


- Large complexe d'espèces (**≈33 espèces**)
- **Difficilement différenciables** en microscopie
- Plusieurs espèces **<u>impliquées en clinique</u>**
- Profil de **sensibilité variable aux ATFs**



Importance de tester les CMIs en cas d'IFIs ou chez patient à risque

Balajee *et al.* Studies in Mycology 2007
Balajee *et al.* Eukaryot Cell 2006
Alcazar-Fuoli *et al.* Antimicrob Agents Chemother 2008



État des lieux de la résistance aux azolés d'*A. fumigatus*

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

Itraconazole Resistance in *Aspergillus fumigatus*

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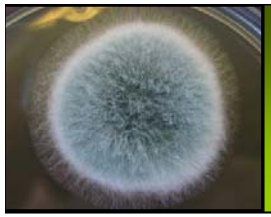
Department of Infectious Diseases and Tropical Medicine (Monsall Unit), North Manchester General Hospital, Crumpsall, Manchester M8 6RB,[1] Section of Infectious Diseases, Department of Medicine, University of Manchester,[2] and Department of Microbiology,[4] Hope Hospital, Salford M6 8HD, Department of Molecular Biology and Biotechnology, University of Sheffield,[3] and Neonatal Screening Laboratory, Sheffield Children's Hospital,[5] Sheffield S10 2UH, and Mycology Reference Laboratory, Public Health Laboratory, Kingsdown, Bristol BS2 8EL,[7] United Kingdom, and Division of Infectious Diseases, Department of Medicine, Santa Clara Valley Medical Center, San Jose, California 95128[6]

Received 8 July 1996/Returned for modification 19 November 1996/Accepted 3 March 1997

Invasive aspergillosis is an increasingly frequent opportunistic infection in immunocompromised patients. Only two agents, amphotericin B and itraconazole, are licensed for therapy. Itraconazole acts through inhibition of a P-450 enzyme undertaking sterol 14α demethylation. In vitro resistance in *Aspergillus fumigatus* to itraconazole correlated with in vivo outcome has not been previously described. For three isolates (AF72, AF90, and AF91) of *A. fumigatus* from two patients with invasive aspergillosis itraconazole MICs were elevated. A neutropenic murine model was used to establish the validity of the MICs. The isolates were typed by random amplification of polymorphic DNA. Analysis of sterols, inhibition of cell-free sterol biosynthesis from [^{14}C]mevalonate, quantitation of P-450 content, and [^{3}H]itraconazole concentration in mycelial pellets were used to determine the mechanisms of resistance. The MICs for the three resistant isolates were >16 μg/ml. In vitro resistance was confirmed in vivo for all three isolates. Molecular typing showed the isolates from the two patients to be genetically distinct. Compared to the susceptible isolate from patient 1, AF72 had a reduced ergosterol content, greater quantities of sterol intermediates, a similar susceptibility to itraconazole in cell-free ergosterol biosynthesis, and a reduced intracellular [^{3}H]itraconazole concentration. In contrast, AF91 and AF92 had slightly higher ergosterol and lower intermediate sterol concentrations, fivefold increased resistance in cell-free systems to the effect of itraconazole on sterol 14α demethylation, and intracellular [^{3}H]itraconazole concentrations found in susceptible isolates. Resistance to itraconazole in *A. fumigatus* is detectable in vitro and is present in wild-type isolates, and at least two mechanisms of resistance are responsible.

Décrite pour la première fois en 1997…



État des lieux de la résistance aux azolés d'*A. fumigatus*

- **Processus de sélection** sous l'effet de la pression antifongique
- **Exposition souvent longue** (plusieurs mois)
- Principal mécanisme=**<u>altération de la cible</u>** (*CYP51A*)
- Nombreuses mutations décrites
  - *Glycine 54 (G54R, G54K, G54V..)*
  - *Méthionine 220 (M220T, M220K, M220I…)*
  - *Glycine 448 (G448S)…*
- Impact variable sur la sensibilité aux azolés (résistance croisée possible)

Résistance longtemps considérée comme <u>uniquement</u> secondaire à une exposition au long cours aux azolés….

Genotype	Amino acid substitutions	Typical phenotype	References
Hot spot mutations commonly found in azole resistant isolates[a]			
G54	E, K, R, V, W	Itraconazole and posaconazole resistant, voriconazole susceptible	[2,17]
L98	H	Pan-azole resistant	[2,9,30]
M220	K, I, T, V	Itraconazole and posaconazole resistant, voriconazole variable	[2,17]
Mutations found less frequently in resistant isolates[b]			
N22	D	Itraconazole resistant, other azole susceptibilities not reported	[49]
S52	T	Pan-azole resistant	[45]
G138	C, R	Pan-azole resistant	[2]
Q141	H	Pan-azole resistant	[45]
H147	Y	Pan-azole resistant	[2]
P216	L	Itraconazole and posaconazole resistant, voriconazole susceptible	[2]
M236	K, T, V	Itraconazole resistant	[50]
S297	T	Itraconazole and posaconazole resistant, voriconazole susceptible	[9,30]
P394	L	Itraconazole resistant, other azole susceptibilities not reported	[49]
Y431	C	Pan-azole resistant	[2]
G434	C	Pan-azole resistant	[2]
T440	A	Itraconazole resistant, other azole susceptibilities not reported	[49]
G448	S	Pan-azole resistant	[2]
Y491	H	Itraconazole resistant, other azole susceptibilities not reported	[49]
F495	I	Itraconazole and posaconazole resistant, voriconazole susceptible	[9,30]

Bueid *et al*., J Antimicrob Chemother 2010; Howard *et al*., Med Mycol 2011; Dannaoui *et al*., J Antimicrob Chemother 2001

Journal of Antimicrobial Chemotherapy (2001) **47**, 333–340

Acquired itraconazole resistance in *Aspergillus fum*

Eric Dannaoui*, Elisabeth Borel, Marie-France Monier, Marie-Antoinette Piens, Florence Persat

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JOURNAL OF CLINICAL MICROBIOLOGY, June 2011, p. 2243–2251 Vol. 49, No. 6
0095-1137/11/$12.00 doi:10.1128/JCM.00213-11
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Aspergillus Species and Other Molds in Respiratory Samples from Patients with Cystic Fibrosis: a Laboratory-Based Study with Focus on *Aspergillus fumigatus* Azole Resistance▽

Klaus Leth Mortensen,[1]* Rasmus Hare Jensen,[1] Helle Krogh Johansen,[2] Marianne Skov,[3] herbarrow,[4] up[1]

Institut, Copenhagen, Denmark[1]; *pital Rigshospitalet, Copenhagen,*

OPEN ACCESS Freely available online PLoS MEDICINE

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

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

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RESEARCH

Clinical Implications of Azo Resistance in *Aspergillus fumig* the Netherlands, 2007–2009

Jan W.M. van der Linden, Eveline Snelders, Greetje A. Yvette I. Rebato Oosenkamp, Ed J. Kuijper, Fre

Journal of Antimicrobial Chemotherapy

resistance to azoles in *Aspergillus fumigatus* eated for haematological malignancies

[1,2], Cécile Farrugia[1,2], Françoise Foulet[1], Françoise Botterel[1,2],

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MAJOR ARTICLE

High-frequency Triazole Resistance Found In Nonculturable *Aspergillus fumigatus* from Lungs of Patients with Chronic Fungal Disease

David W. Denning,[1,2,3] Steven Park,[4] Cornelia Lass-Florl,[5] Marcin G. Fraczek,[2,3] Marie Kirwan,[1,2] Robin Gore,[2] Jaclyn Smith,[2] Ahmed Bueid,[2] Caroline B. Moore,[3] Paul Bowyer,[2] and David S. Perlin[2,4]*

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APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2009, p. 4053–4057
0099-2240/09/$08.00+0 doi:10.1128/AEM.00231-09
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Possible Environmental Origin of Re to Medical T

Eveline Snelders,[1,2] Robert A. G. Huis in ' Gert H. J. Kema,[3] Willem J. G. Melchers,[1,2]* and Paul E. Verweij[1,2]*

Department of Medical Microbiology[1] *and Nijmegen Institute for Infection, Inflammation, and Immunity,*[2] *Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands, and Plant Research International, Wageningen, The N*

tus Resistance Mechanism Conferring nce to Azole Antifungals Involves nbination of *cyp51A* Alterations▽

a-Effron,[1] L. Alcázar-Fuoli,[1] W. J. G. Melchers,[2] P. E. Verweij,[2] uenca-Estrella,[1] and J. L. Rodríguez-Tudela[1]

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4465–4468
0066-4804/11/$12.00 doi:10.1128/AAC.00185-11
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Azole Resistance in *Aspergillus fumigatu* ARTEMIS Global Surveillance Stu Due to the TR/L98H Muta the *cyp51A* Gene▽

Shawn R. Lockhart,[1]* João P. Frade,[1] Kizee A. Etienn Daniel J. Diekema,[2,3] and S. Arunmozh

Mycotic Diseases Branch, Division of Foodborne, Waterborne and Environmenta Prevention, Atlanta, Georgia 30333,[1] *and Department of Pathology*[2] *and University of Iowa Carver College of Medicine, Iowa C*

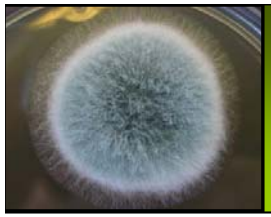
J Antimicrob Chemother
doi:10.1093/jac/dkr443

Journal of Antimicrobial Chemotherapy

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

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

[1]Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India; [2]Department of Pulmonary Medicine, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India; [3]Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; [4]Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands



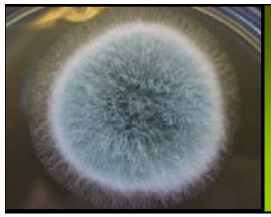
Résistance d' *A. fumigatus* : un problème émergent ?

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

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

David W Denning[†1,2] & David S Perlin[3]

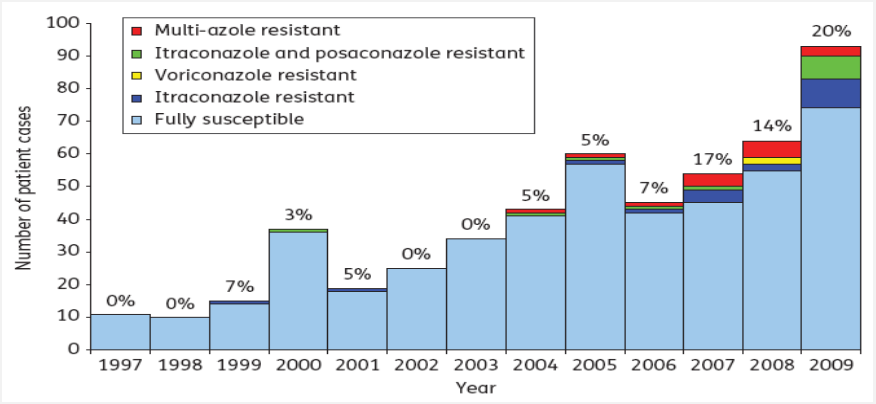
Dening & Perlin, Future Microbiology, 2011



Résistance d' *A. fumigatus* : un problème émergent ?

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

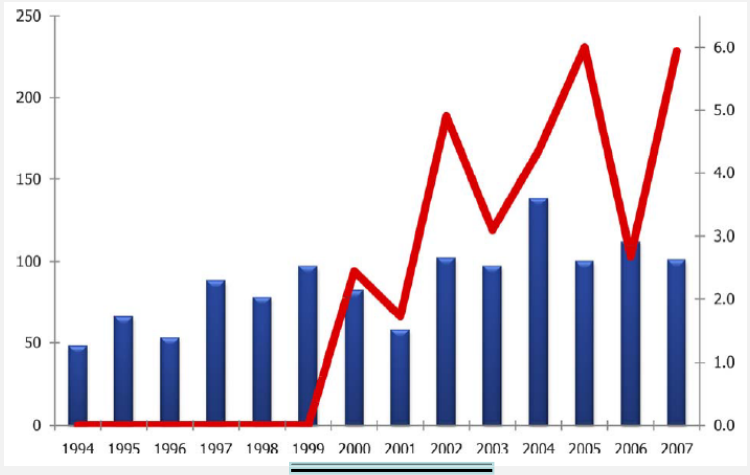
Manchester (UK)



Prévalence accrue de souches azole-R *CYP51A* mutées et non mutées (WT) !!

Bueid *et al.,* J Antimicrob Chemother 2010

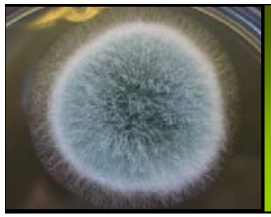
Nijmegen (Pays-Bas)



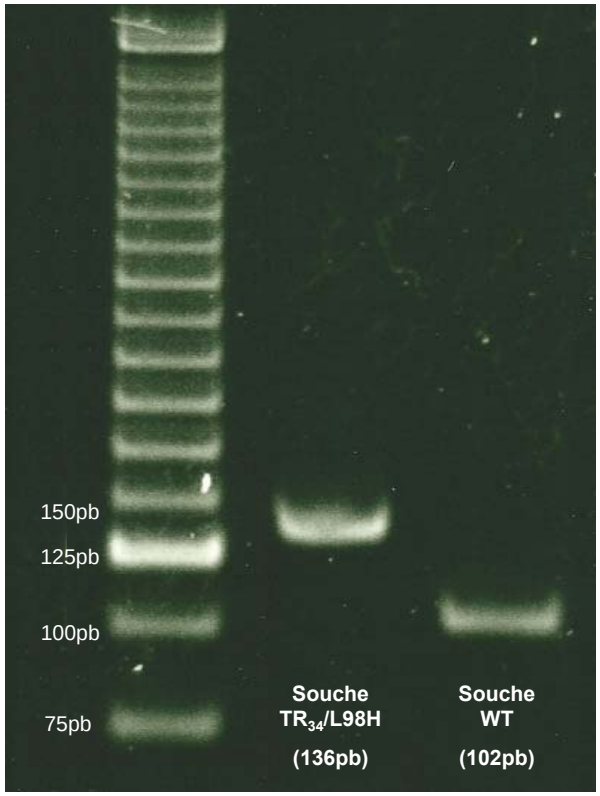
Prévalence accrue et émergence d'un nouveau mécanisme (TR_{34}/L98H)

Snelders *et al.*, PLos Med 2008

Présence de souches résistantes chez des patients n'ayant jamais reçu d'azolés (« naïf »)



État des lieux de la résistance aux azolés d'*A. fumigatus*

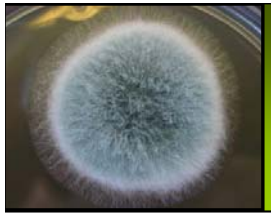


- **TR_{34}/L98H: Mécanisme original**
- Combinaison de **deux mécanismes distincts**
  - ✓ Duplication en tandem de 34 pb dans le promoteur du gène *CYP51A* (-288 à -322)
  - ✓ Substitution L98H
- **Effet synergique**
  - ➢ Diminution d'affinité de l'enzyme/cible
  - ➢ Surexpression du gène *CYP51A* (4 à 8 fois)

Résistance croisée aux azolés *in vitro* et *in vivo* (mortalité 88%)

<u>Remarque</u> :
Duplications dans région promotrice du gène *CYP51* en réponse aux pesticides azolés, décrites chez plusieurs champignons phytopathogènes (ex: *P. digitatum*)…

Snelders *et al*., Plos Med 2008; Verweij *et al*., Lancet Infect Dis 2009; Snelders *et al*., PLos One 2012; Van Der Linden *et al*., Emerg Infect Dis 2011



TR_{34}/L98H: une origine environnementale ?

Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use?

Paul E Verweij, Eveline Snelders, Gert H J Kema, Emilia Mellado, Willem J G Melchers

Invasive aspergillosis due to multi-azole-resistant *Aspergillus fumigatus* has emerged in the Netherlands since 1999, with 6·0–12·8% of patients harbouring resistant isolates. The presence of a single resistance mechanism (denoted by TR/L98H), which consists of a substitution at codon 98 of *cyp51A* and a 34-bp tandem repeat in the gene-promoter region, was found in over 90% of clinical *A fumigatus* isolates. This is consistent with a route of resistance development through exposure to azole compounds in the environment. Indeed, TR/L98H *A fumigatus* isolates were cultured from soil and compost, were shown to be cross-resistant to azole fungicides, and genetically related to clinical resistant isolates. Azoles are abundantly used in the environment and the presence of *A fumigatus* resistant to medical triazoles is a major challenge because of the possibility of worldwide spread of resistant isolates. Reports of TR/L98H in other European countries indicate that resistance might already be spreading.

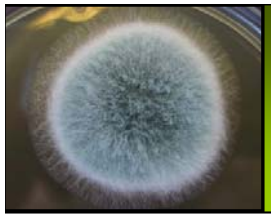
Lancet Infect Dis 2009; 9: 789–95

Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands (P E Verweij MD, E Snelders MSc, W J G Melchers PhD); Wageningen University, Plant Science Group, Plant Research International BV, Wageningen, Netherlands (G H J Kema PhD);

Arguments…

1. Mécanisme rencontré chez champignons phytopathogènes
2. Souches TR_{34}/L98H isolées de patients « naïf »
3. Souches TR_{34}/L98H présente dans l'environnement
4. Confère résistance croisée fongicides/antifongiques
5. Forte homogénéité génétique des souches TR_{34}/L98H

Références :
Camps *et al*., J Clin Microbiol 2012;50:2674:80
Snelders *et al*., PLos One 2012;7:e31801
Verweij *et al*., Lancet Infect Dis 2009;9:789:95
Snelders *et al*., Appl Environ Microbiol 2009;75:4053-7
Snelders *et al*., Plos Med 2008;5:e219



TR_{34}/L98H: une origine environnementale ?

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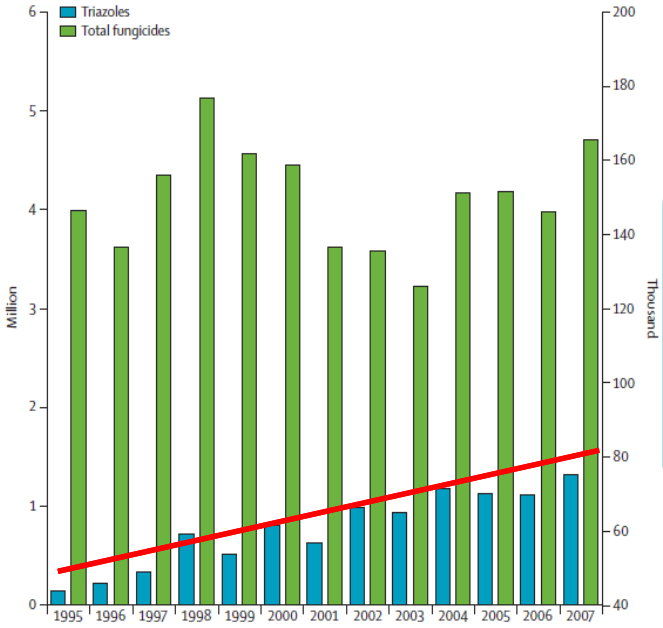
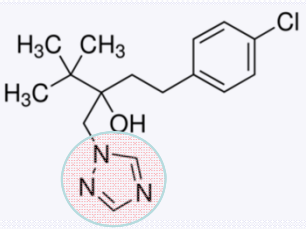


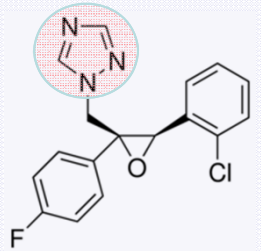
Date d'autorisation de mise sur le marché de ces composés (1990-1996) précède l'apparition des 1ere souches TR_{34}/L98H (1998)

Cinq fongicides suspectés

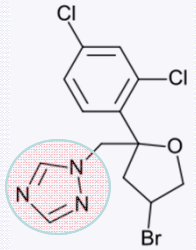
Tebuconazole



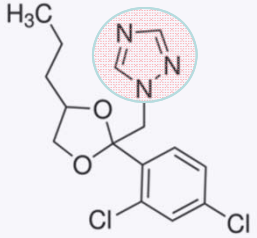
Epoxiconazole



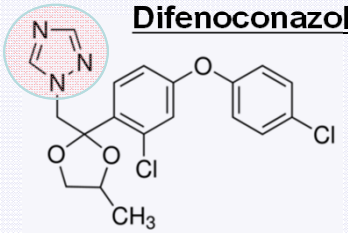
Bromuconazole



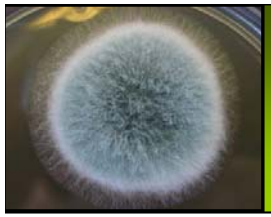
Propiconazole



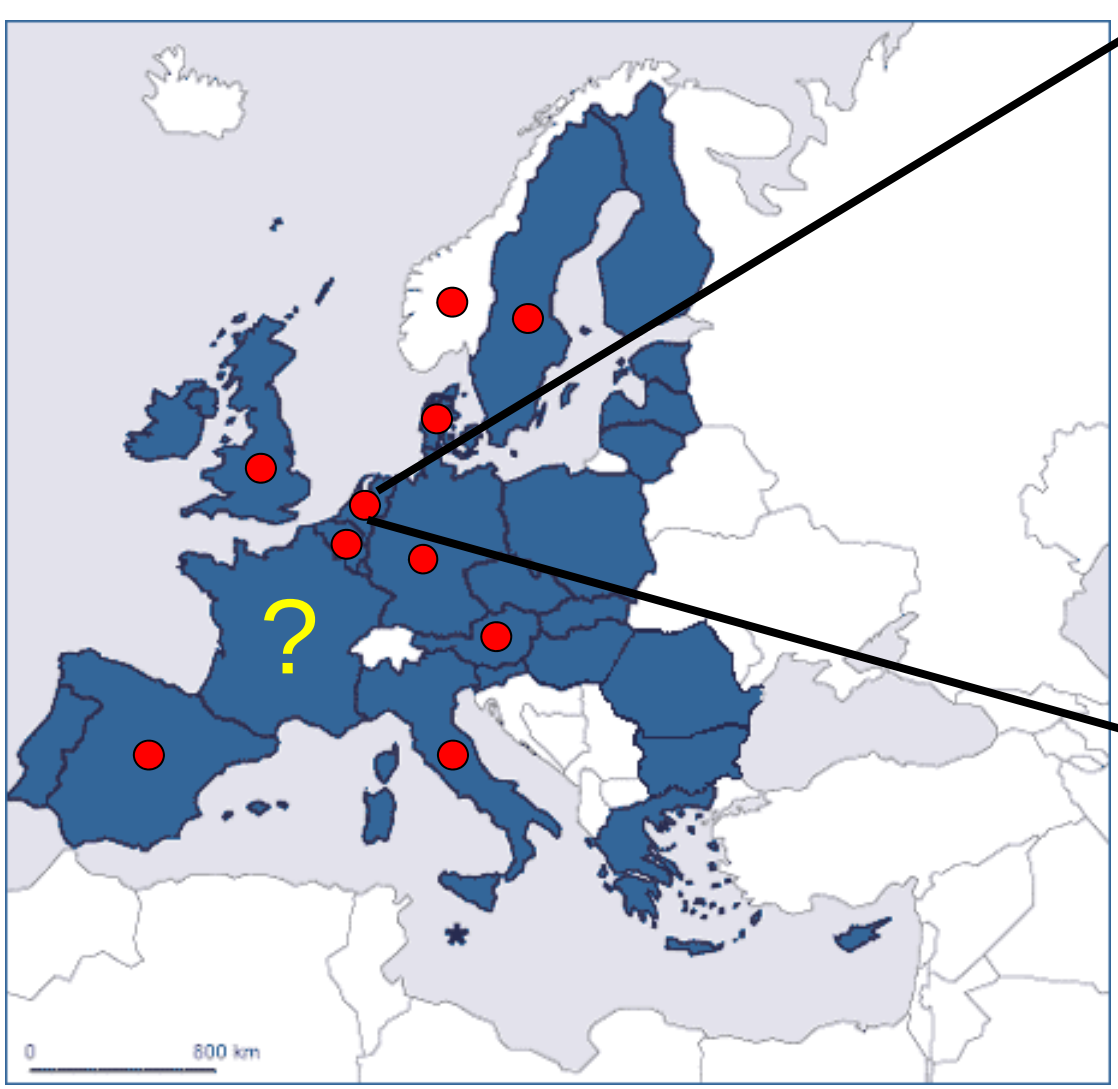
Difenoconazole

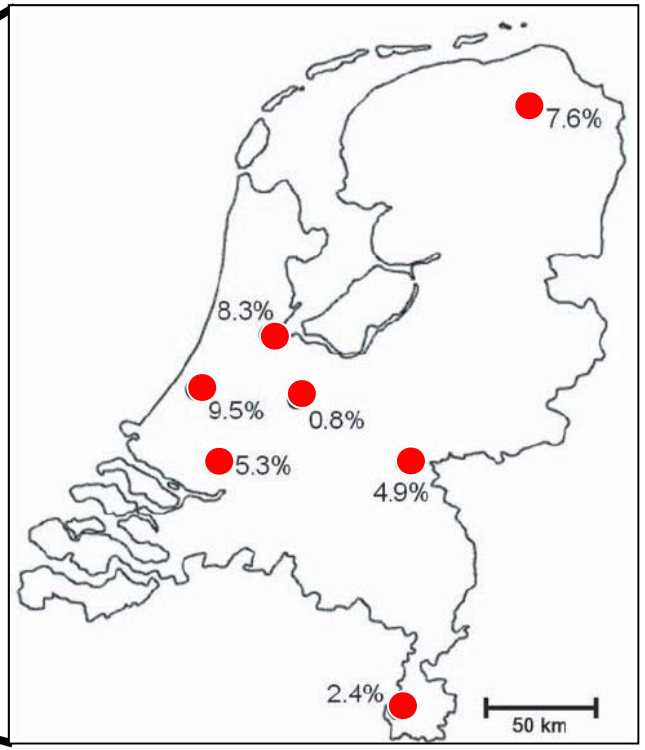


Snelders *et al*., Plos One 2012;7:e31801



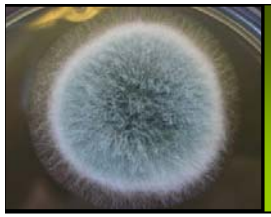
TR_{34}/L98H : dispersion en Europe





TR_{34}/L98H largement distribué en Europe…

Van Der Linden *et al*. 51st ICAAC 2011
Van Der Linden *et al*. Emerg Infect Dis 2011
Lockhart *et al*. J Clin Microbiol 2011
Chowdhary *et al*. J Antimicrob Chemother 2011



TR_{34}/L98H: Données CHU de Nantes

IICiMed

High prevalence of triazole resistance in *Aspergillus fumigatus*, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis

Journal of Antimicrobial Chemotherapy

Florent Morio[1,2]†, Guillaume G. Aubin[2]†, Isabelle Danner-Boucher[3,4], Alain Haloun[3,4], Emilie Sacchetto[2], Dea Garcia-Hermoso[5,6], Stéphane Bretagne[5,6], Michel Miegeville[1,2] and Patrice Le Pape[1,2]*

Objectives: Triazole resistance in *Aspergillus fumigatus* due to a single azole resistance mechanism (TR/L98H) is increasingly reported in European countries. Data from patients with cystic fibrosis (CF) are limited. Our study aimed to investigate the prevalence and molecular mechanisms of azole resistance in *A. fumigatus* in a cohort of patients with CF.

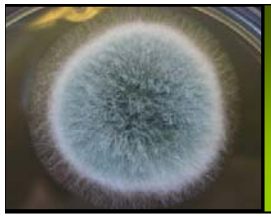
Methods: Eighty-five *A. fumigatus* isolates from 50 CF patients, collected between January 2010 and April 2011, were retrospectively analysed for azole resistance using agar plates containing 4 mg/L itraconazole. MICs of itraconazole, voriconazole and posaconazole were determined according to EUCAST methodology for each isolate able to grow on this medium. Species identification was performed by sequencing of the β-tubulin gene. Sequencing analysis of the *cyp51A* gene and its promoter region was conducted.

Results: Nine isolates (four patients, 8% prevalence) were able to grow on itraconazole-containing agar plates. Itraconazole resistance was confirmed by EUCAST methodology (MICs >2 mg/L). All isolates had mutations in the *cyp51A* gene at residues previously involved in azole resistance: L98H ($n=5$), M220T ($n=4$) and G54R ($n=1$). One patient had three genetically distinct azole-resistant isolates identified during the study. The isolates with L98H that were recovered from three patients (6% prevalence) also had the 34 bp tandem repeat in the promoter region of *cyp51A* (TR/L98H) and displayed multiazole resistance.

Conclusions: We report an 8% prevalence of itraconazole resistance in CF patients in our centre, mostly driven by TR/L98H (6%). Our data confirm that TR/L98H occurs in France and can be highly prevalent in CF patients.

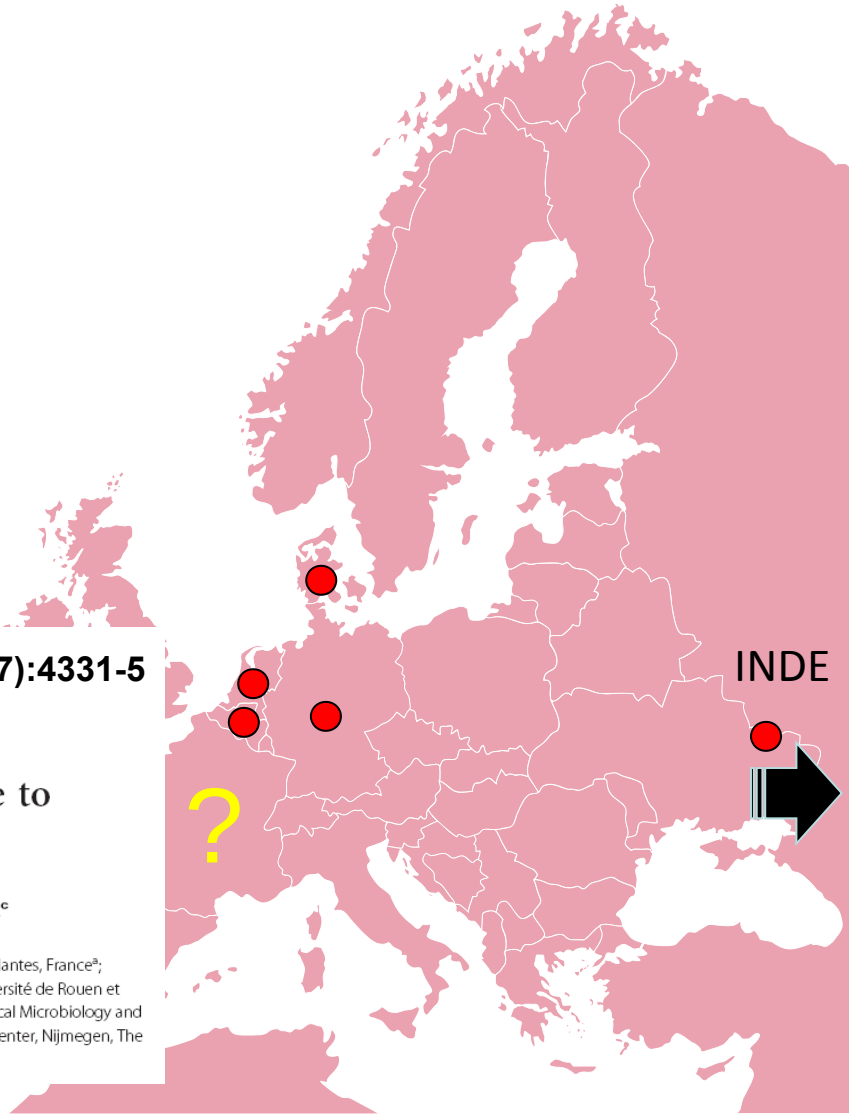
4 patients porteurs de souches résistantes aux azolés dont TR34/98H (8%)

Morio *et al.* J Antimicrob Chemother 2012



TR_{46}/Y121F/T289A: nouvelle mutation liée à l'environnement

- Première souche TR_{46}/Y121F/T289A décrite au Pays-Bas
- Combinaison de **deux mécanismes distincts**
  - ✓ *Duplication en tandem de 46 pb dans promoteur*
  - ✓ *Substitution Y121F et T289A*
- **Résistance de haut niveau au Voriconazole**
- Isolats retrouvés dans l'environnement et chez les patients…..



AAC Journals.ASM.org

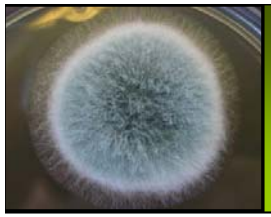
Antimicrob Agents Chemother 2015Jul;59(7):4331-5

First Description of Azole-Resistant *Aspergillus fumigatus* Due to TR_{46}/Y121F/T289A Mutation in France

Rose-Anne Lavergne,[a,b] Florent Morio,[a,b] Loïc Favennec,[c] Stéphane Dominique,[d] Jacques F. Meis,[e,f] Gilles Gargala,[c] Paul E. Verweij,[f] Patrice Le Pape[a,b]

Département de Parasitologie et Mycologie Médicale, Université de Nantes, Nantes Atlantique Universités, EA1155-IICiMed, Faculté de Pharmacie, Nantes, France[a]; Laboratoire de Parasitologie-Mycologie, Institut de Biologie, CHU de Nantes, Nantes, France[b]; EA3800, Laboratoire de Parasitologie-Mycologie, Université de Rouen et CHU de Rouen, Rouen, France[c]; Department of Pneumology, Cystic Fibrosis Center, Rouen University Hospital, Rouen, France[d]; Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands[e]; Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands[f]

Vermeuleun *et al.*, Eurosurv 2012; Van Der Linden *et al.*, Clin Infect Dis 2013; Chowdhary *et al.*, J Antimicrob Chemother 2014



Résistance d'origine environnementale : TR_{46}/Y121F/T289A

MAJOR ARTICLE

Aspergillosis due to Voriconazole Highly Resistant *Aspergillus fumigatus* and Recovery of Genetically Related Resistant Isolates From Domiciles

Jan W. M. van der Linden,[1,2,a] Simone M. T. Camps,[1,2,a] Greetje A. Kampinga,[3] Jan P. A. Arends,[3] Yvette J. Debets-Ossenkopp,[4] Pieter J. A. Haas,[5] Bart J. A. Rijnders,[6] Ed J. Kuijper,[7] Frank H. van Tiel,[8] János Varga,[9] Anna Karawajczyk,[10] J. Zoll,[1,2] Willem J. G. Melchers,[1,2] and Paul E. Verweij[1,2]

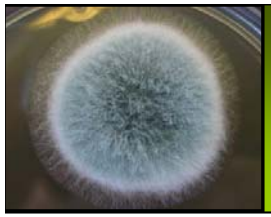
[1]Department of Medical Microbiology, and [2]Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Centre, [3]Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, [4]Department of Medical Microbiology, VU–University Medical Centre, Amsterdam, [5]Department of Medical Microbiology, University Medical Centre Utrecht, [6]Department of Internal Medicine, Division of Infectious Diseases, Erasmus Medical Centre, Rotterdam, [7]Department of Medical Microbiology, Leiden University Medical Centre, and [8]Department of Medical Microbiology, University Medical Centre Maastricht, The Netherlands; [9]Department of Microbiology, University of Szeged, Hungary; and [10]LeadParma Medicine B.V., Nijmegen, The Netherlands

Van Der Linden *et al*., Clin Infect Dis 2013

Table 2. Recovery of Azole-Resistant *Aspergillus fumigatus* Isolates Through Environmental Air Sampling of 14 000 L per Location

Site	City	Location	No. of Resistant Colonies	TR_{46}/ Y121F/ T289A	TR_{34}/ L98H
1	Nijmegen	Hospital restaurant	3	. . .	3
		Hospital pediatrics department	9	2	7
		Outside hospital	5	. . .	5
2	Schaijk	Living room	2	. . .	2
		Basement	3	. . .	3
		Back yard	7	. . .	7
3	Schaijk	Living room	1	. . .	1
		Conservatory	5	. . .	5
		Back yard	3	. . .	3
4	Overasselt	Kitchen	6	1	5
		Basement	7	. . .	7
		Back yard	7	. . .	7
5	Schaijk	Living room	6	. . .	6
		Basement	3	. . .	3
		Back yard	3	. . .	3
6	Berghem	Living room	. . .	. . .	. . .
7	Schaijk	Hall	13	3	10
		Balcony	6	. . .	6
8	Groningen	Hospital entrance[a]	. . .	. . .	. . .
		Outside hospital	29	2	27
9	Scharmer	Living room	4	1	3
		Back yard	6	3	3
10	Garmerwolde	Living room[a]	. . .	. . .	. . .
		Back yard	12	2	10

[a] For these sites the agar plates, supplemented with azoles, were rapidly overgrown with zygomycetes, thereby precluding the opportunity to select suspected azole-resistant *A. fumigatus* colonies.



La face cachée de l'iceberg ?

High-frequency Triazole Resistance Found In Nonculturable *Aspergillus fumigatus* from Lungs of Patients with Chronic Fungal Disease

MAJOR ARTICLE

David W. Denning,[1,2,3] Steven Park,[4] Cornelia Lass-Florl,[5] Marcin G. Fraczek,[2,3] Marie Kirwan,[1,2] Robin Gore,[2] Jaclyn Smith,[2] Ahmed Bueid,[2] Caroline B. Moore,[3] Paul Bowyer,[2] and David S. Perlin[2,4]

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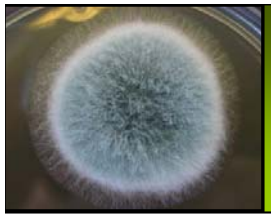
Table 1. ***Aspergillus*** **Culture, qPCR, and** ***A fumigatus*** **Resistance Mutation Detection in 4 Study Populations**

Laboratory result	ABPA	CPA	IPA	Normals
Culture positive for *Aspergillus* spp.	0/19	7/42 (16.7%)	20/22 (90.9%)	0/11
Culture positive for *A fumigatus*	0/19	7/42 (16.7%)	10/22 (45.5%)	0/11
qPCR positive for *Aspergillus* spp	15/19 (78.9%)	30/42 (71.4%)	21/22 (95.5%)	4/11 (36.4%)
A. fumigatus CYP51A mutation detected directly from qPCR-positive sample	6/8 (75%)	12/24 (50%)	NT[a]	NT[a]

Cultures mycologiques souvent négatives au cours des aspergilloses chroniques mais prévalence élevée de souches mutées sur le gène *CYP51A* (jusqu'à 75 % !!)

Denning DW *et al.*, Clin Infect Dis 2011 May;52(9):1123-9.



Origine environnementale de la résistance aux azolés: news

Figure 1. Use of triazoles in agriculture in the United Kingdom, the Netherlands and Denmark, 2008 (logarithmic scale)

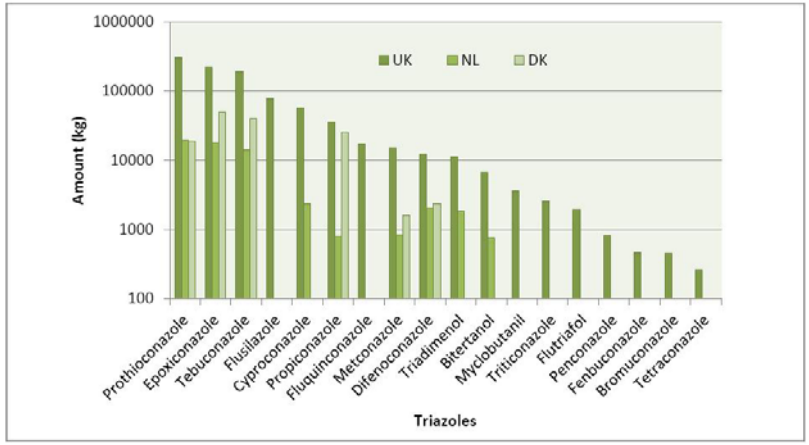


Table 4. Use of triazole fungicides in the production of selected crops/commodities in the United Kingdom

Crop	Year[a]	Production area (ha)	Key triazole fungicide products[b]	Area treated (ha)[c]	Amount used (kg)	Average spray number[d]	Average dose rate[e]
Beans	2008	118 462	Tebuconazole	65 691	10 222	1.32	0.62
			Cyproconazole/chlorothalonil	24 899	14 938	1.19	0.72
Carrots	2007	15 380	Tebuconazole	11 131	2 328	1.56	0.84
Wheat	2008	2 068 104	Epoxiconazole	1 242 366	75 598	1.46	0.49
			Prothioconazole/tebuconazole	571 313	89 840	1.57	0.54
Dessert apples	2008	5 577	Myclobutanil	22 356	1 309	4.70	0.64
			Penconazole	11 987	386	3.30	0.65
Field roses	2009	324	Myclobutanil	446	20	2.47	volumetric
Herbaceous plants	2009	910	Prochloraz/propiconazole	163	39	4.00	0.39
Hops	2008	1 071	Myclobutanil	4 139	162	4.86	volumetric
Oilseed rape	2008	597 706	Flusilazole/carbendazim	286 508	44 283	1.26	0.52
			Prothioconazole	225 120	22 877	1.24	0.58
			Metconazole	180 784	7 442	1.15	0.57
Outdoor bulbs	2009	4 875	Tebuconazole	6 311	966	1.65	0.61
Strawberries	2006	4 480	Myclobutanil	8 084	634	2.35	0.85
Vines	2006	856	Myclobutanil	1 577	80	2.62	1.02

[a] Year when crops were surveyed by the Food and Environment Research Agency (FERA) and the Scottish Agricultural Science Agency (SASA). Wheat and beans includes winter and spring grown varieties.

[b] Triazoles were applied as solo or in mixtures with other sterol demethylation inhibitors or fungicides with different modes of action.

[c] Area treated is the total number of hectares sprayed.

[d] Average spray number is the average number of applications of the product on the area treated.

[e] Average dose rate is defined as the average proportion of full label rate.

ECDC, Niels Kleinkauf, Paul E. Verweij, Maiken C. Arendrup, Peter J. Donnelly, Manuel Cuenca-Estrella, Bart Fraaije, Willem J.G. Melchers, Niels Adriaenssens, Gert H.J. Kema, Andrew Ullmann, Paul Bowyer, David W. Denning

En résumé…

- Problématique d'actualité et **phénomène dynamique**
- **L'antifongigramme ne doit pas être systématique**
- Intérêt de tester la CMI des espèces rares
- Emergence récente de la résistance des *Candida* aux échinocandines
- Problématique croissante de la **résistance d' *A. fumigatus* aux azolés, d'origine environnementale** (fongicides): **TR_{34}/L98H**, **TR_{46}/Y121F, T289A**
- Résistance d' *A. fumigatus* aux azolés, variable selon les centres :0.8 à 9.5 %
- Enjeu de **santé publique**
- Beaucoup de progrès de standardisation mais expertise requise (**rôle des laboratoires spécialisés**)

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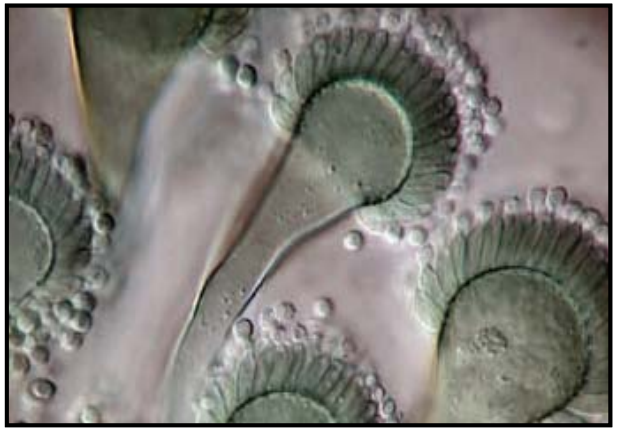
International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*

Paul E. Verweij[a,*], Michelle Ananda-Rajah[b], David Andes[c], Maiken C. Arendrup[d], Roger J. Brüggemann[e], Anuradha Chowdhary[f], Oliver A. Cornely[g], David W. Denning[h], Andreas H. Groll[i], Koichi Izumikawa[j], Bart Jan Kullberg[k], Katrien Lagrou[l], Johan Maertens[m], Jacques F. Meis[a,n], Pippa Newton[h], Iain Page[h], Seyedmojtaba Seyedmousavi[a], Donald C. Sheppard[o], Claudio Viscoli[p], Adilia Warris[q], J. Peter Donnelly[r]

Verweij *et al.*, Drug Resist Updates 2015 21-22, July, 30-40

Détermination de la sensibilité aux antifongiques:

Pourquoi ? Quand ? Comment ?



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- 44ème Colloque National des Biologistes des Hôpitaux, Nantes, 23-25 Septembre 2015-