

Quoi de neuf en Mycologie ?

Aspects épidémiologiques et diagnostiques Conséquences thérapeutiques

Pr. Jean-Pierre GANGNEUX

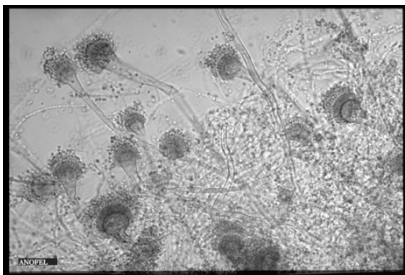
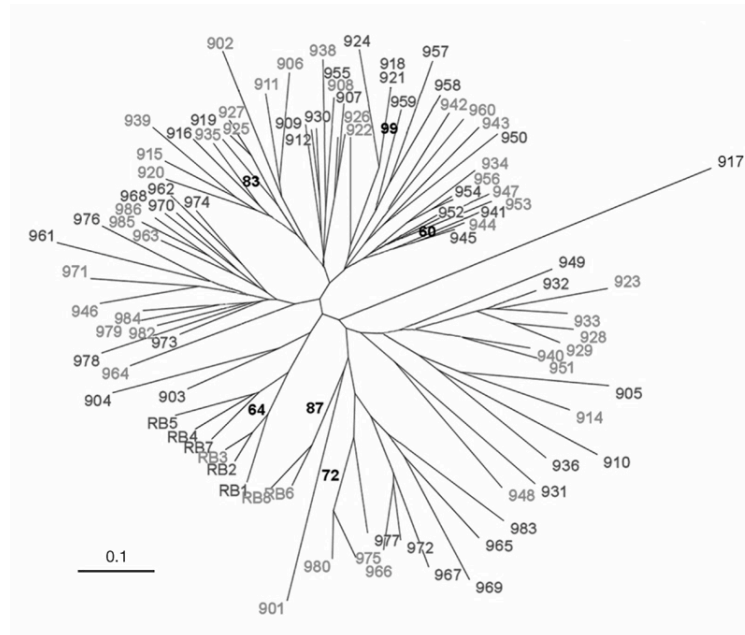
Service de Parasitologie-Mycologie
CHU et Faculté de Médecine de Rennes
INSERM U 1085 : IRSET

Institut de Recherche Santé Environnement Travail , Université Rennes 1

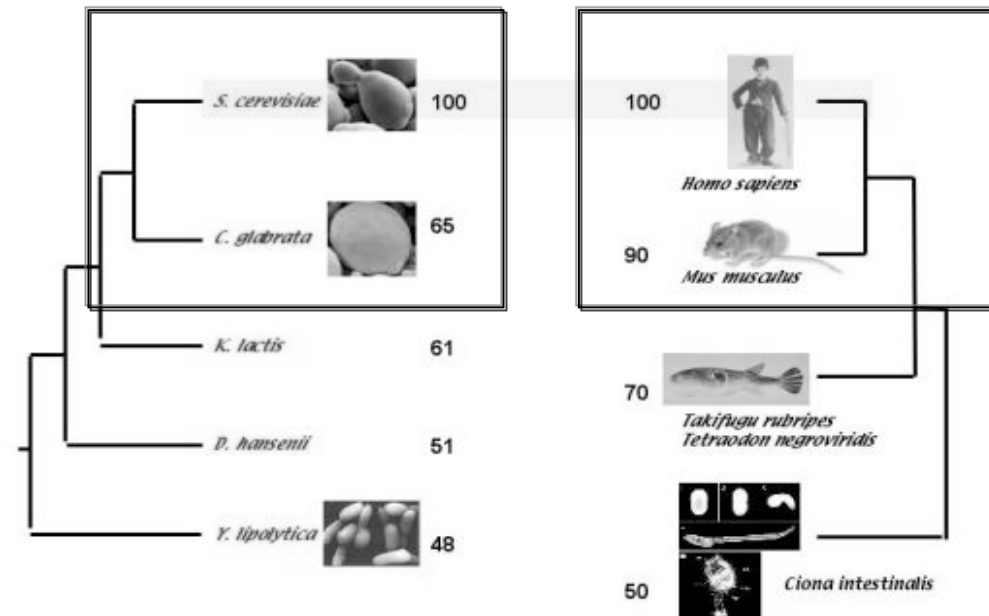
Jean-pierre.gangneux@chu-rennes.fr

Le défi de la biodiversité

Aspergillus fumigatus



Levures



Méthodes nouvelles d'identification : quel apport en pratique?

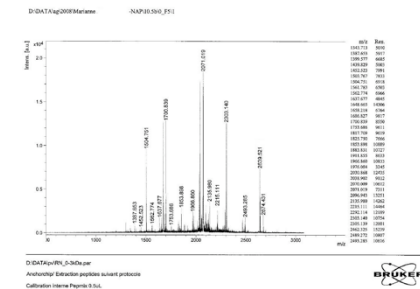
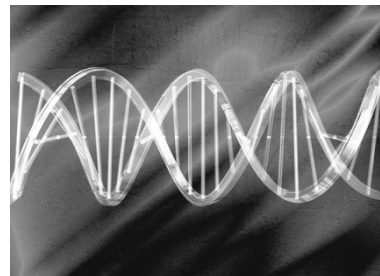
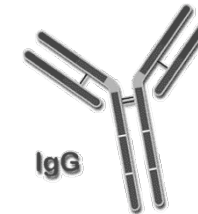
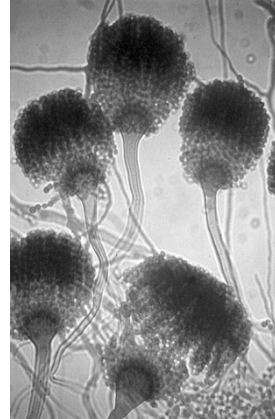
-Examen microscopique et cultures

-Histologie

-Immunologie : marqueurs solubles

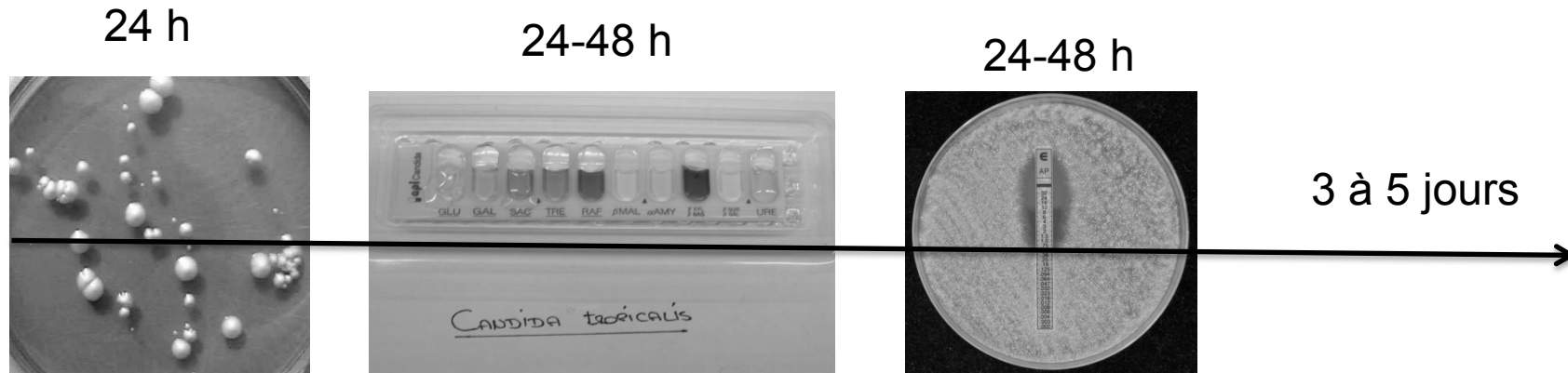
-Biologie moléculaire ?

-Spectrométrie de masse?

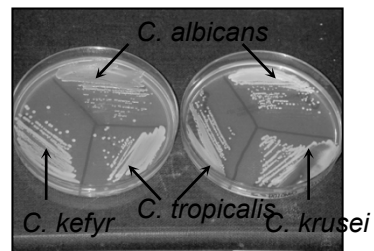


Limite du diagnostic mycologique

1. Délai de culture : Levures



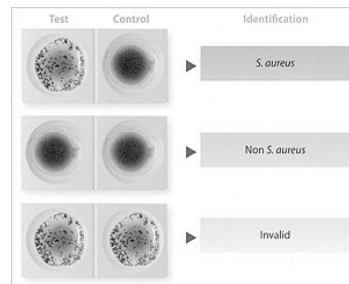
Chromogenic media



24 h

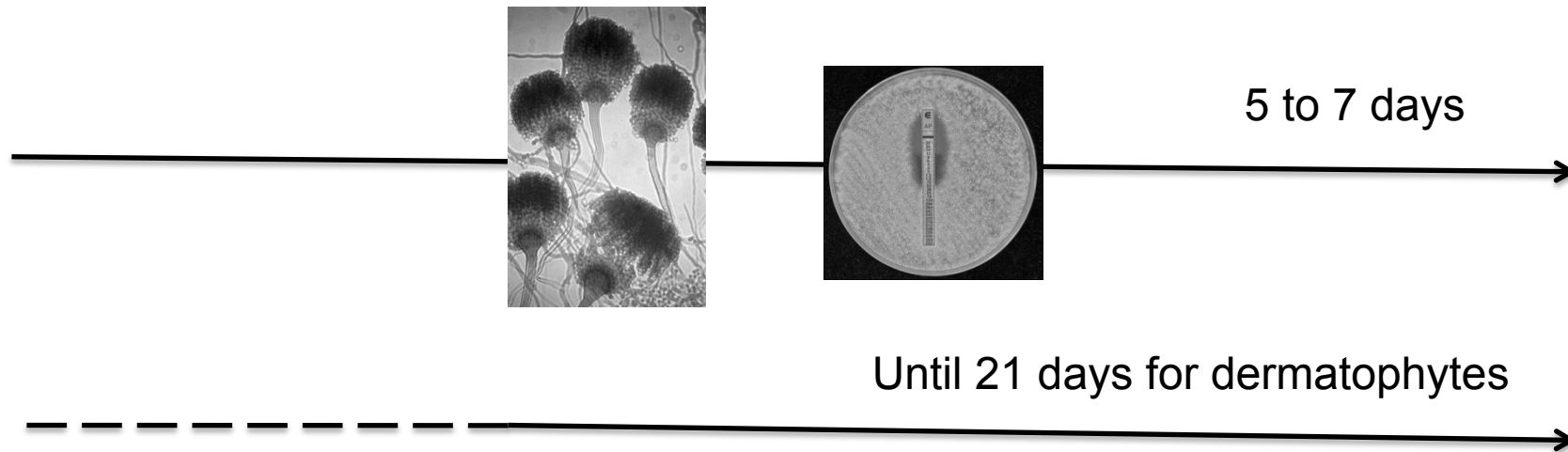


Agglutination tests

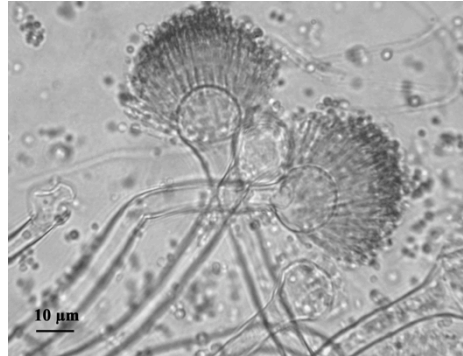


Limite du diagnostic mycologique

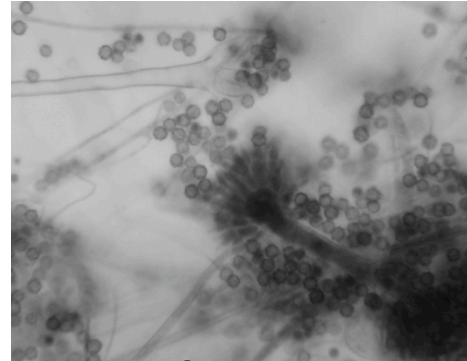
1. Délai de culture : champignons filamenteux



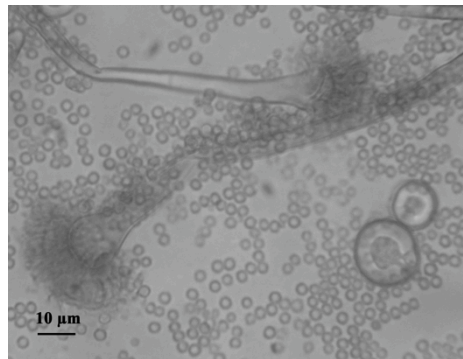
2. La mycologie et la morphologie ne permettent qu'une discrimination limitée entre espèces et entre isolats



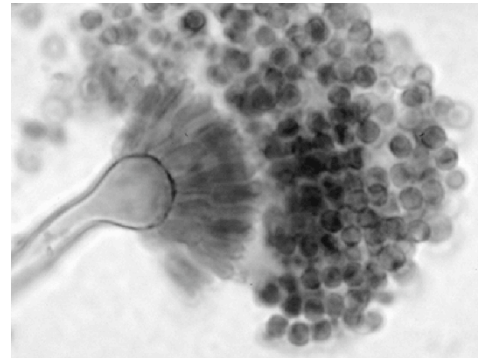
A. terreus



A. ustus



A. nidulans



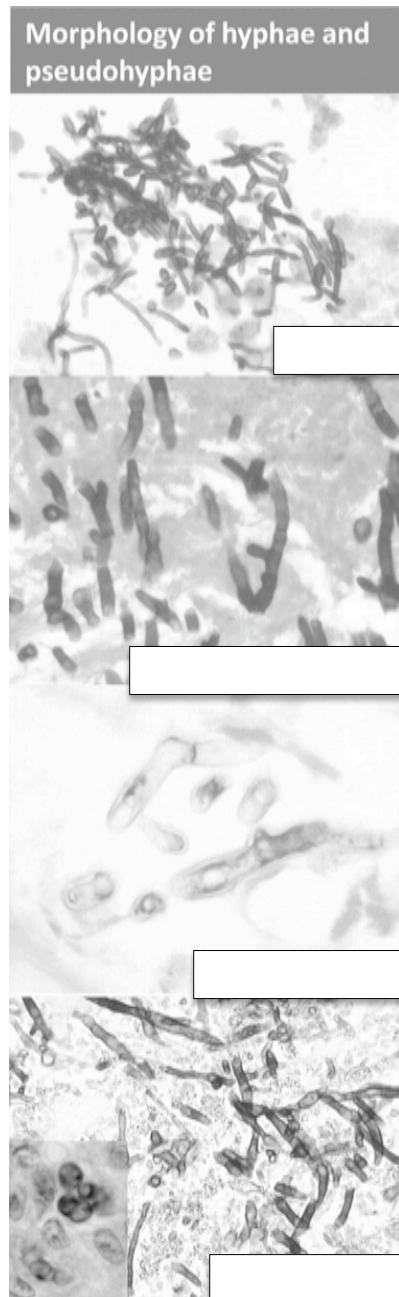
A. flavus

3. Variabilité de la sensibilité en fonction du terrain

4. Que veulent dire “hyphes ou pseudohyphes” dans les tissus?

**Morphology, description,
diagnosis, and comment for
fungal infections that present
with hyphae or pseudohyphae
in tissues**

**Guarner, J. et al. 2011. Clin.
Microbiol. Rev. 24(2):247-280**

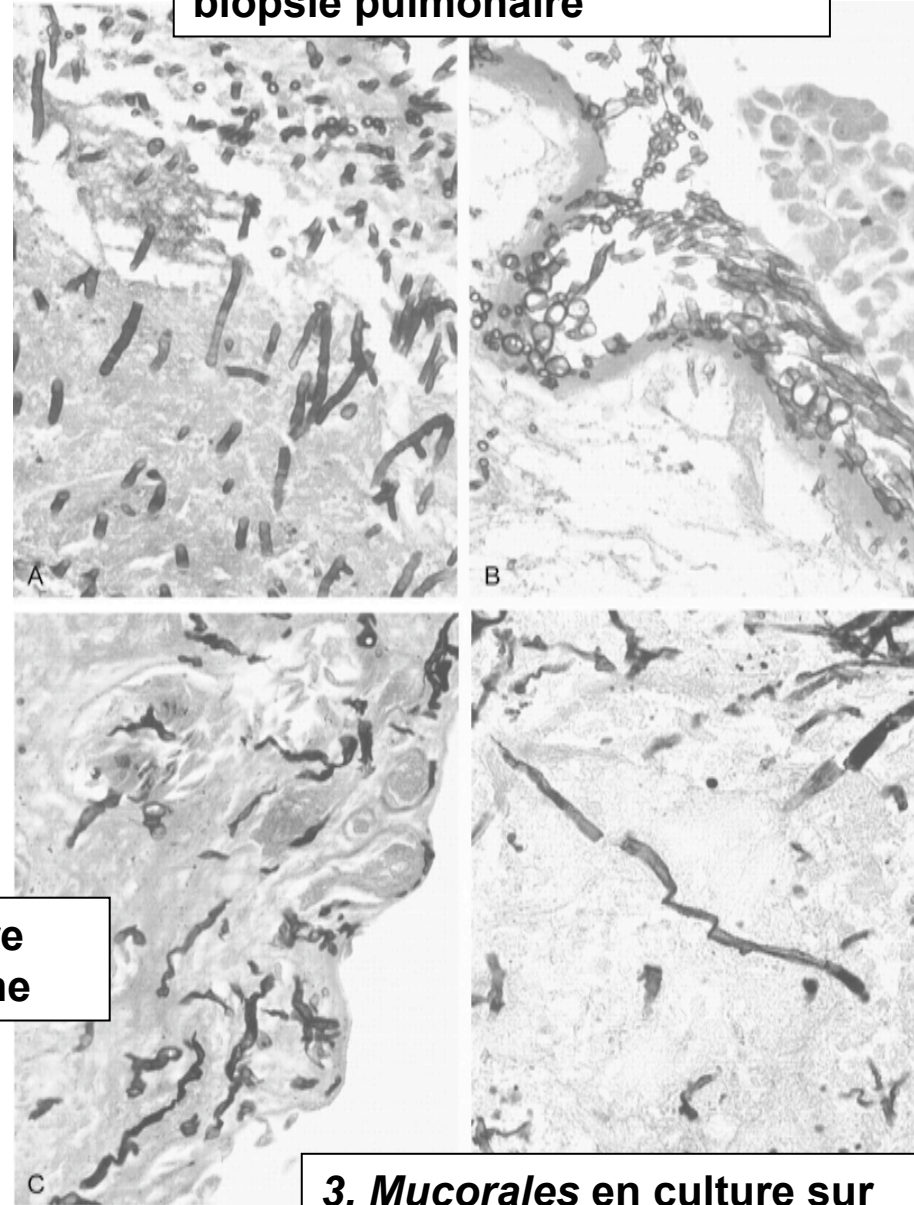


5. Problème des co-infections

**Sequential specimens stained with
GMS (magnification, x120)
showing mold infections in a
neutropenic patient with chronic
lymphocytic leukemia**

Guarner, J. et al. 2011. Clin.
Microbiol. Rev. 24(2):247-280

**1. *Aspergillus* en culture sur
biopsie pulmonaire**



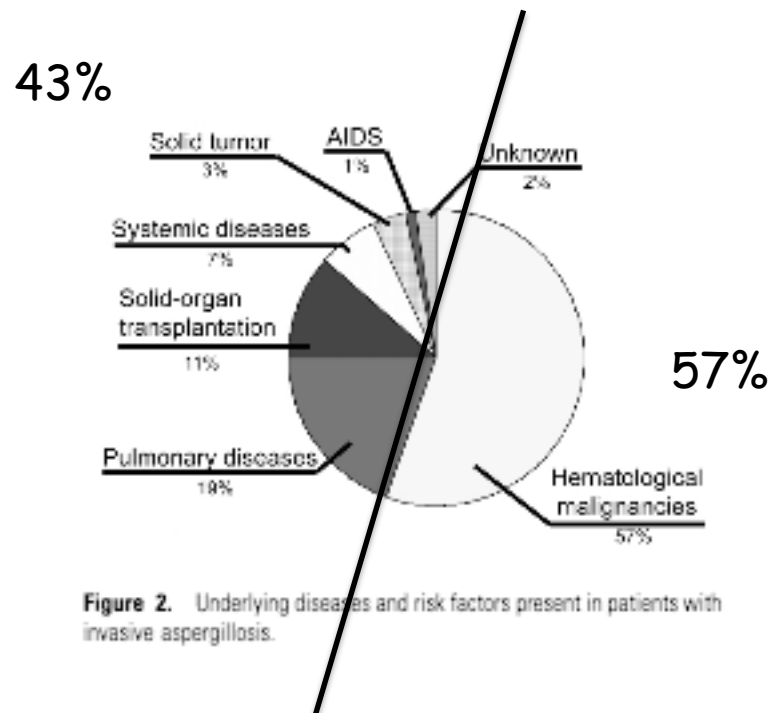
**2. *Fusarium* en culture
sur biopsie sinusienne**

**3. *Mucorales* en culture sur
pièce autopsique pulmonaire**

Comparison of Epidemiological, Clinical, and Biological Features of Invasive Aspergillosis in Neutropenic and Nonneutropenic Patients: A 6-Year Survey

A. Cornillet,¹ C. Camus,² S. Nimubona,³ V. Gandemer,⁴ P. Tattevin,² C. Belleguic,⁵ S. Chevrier,¹ C. Meunier,⁶ C. Lebert,⁷ M. Aupée,⁸ S. Caulet-Maugendre,⁹ M. Faucheux,¹⁰ B. Lelong,¹¹ E. Leray,¹² C. Guiguen,¹ and J.-P. Gangneux¹

Clinical Infectious Diseases 2006;43:577-84



	Sensibilité ED/culture
Neutropéniques (n=52)	58%
Non-neutropéniques (n=36)	85%* p<0,05
Total (n=88)	69%

Maladies sous-jacentes

Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005–2007)

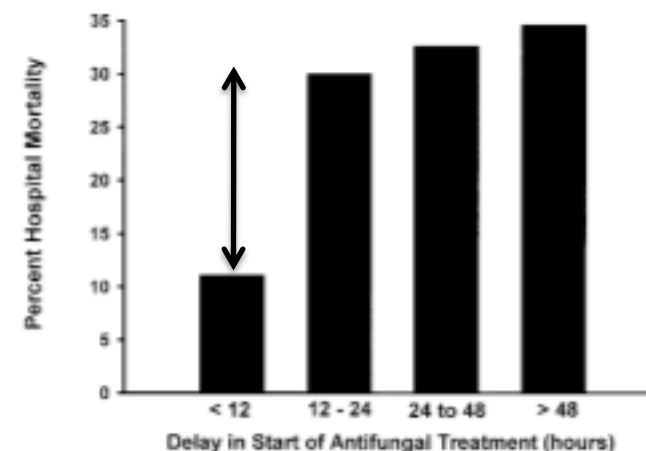
O. Lortholary^{1,2,3}, J.-P. Gangneux^{4,5}, K. Sitbon^{1,2}, B. Lebeau⁶, F. de Monbrison⁷, Y. Le Strat⁸, B. Coignard⁸, F. Dromer^{1,2} and S. Bretagne^{1,9,10}, for the French Mycosis Study Group*

393 Adults	Direct examination	Culture
Acute leukemia (n=136)	48,4%	52,6%
Allo-Hsct (n=84)	45,5%	68,2%
Chronic lymphoproliferative disorders (n=85)	52,6%	88,2%
Solid Organ Transplantation (n=17)	68%	94%
Systemic inflammatory diseases(n=18)	78%	100%
Chronic respiratory diseases (n=9)	78%	100%

Delaying the Empiric Treatment of *Candida* Bloodstream Infection until Positive Blood Culture Results Are Obtained: a Potential Risk Factor for Hospital Mortality

Matthew Morrell,¹ Victoria J. Fraser,² and Marin H. Kollef^{1*}

Pulmonary and Critical Care Division¹ and Division of Infectious Diseases,² Washington University School of Medicine, St. Louis, Missouri 63110



Treatment-related risk factors for hospital mortality in *Candida* bloodstream infections*

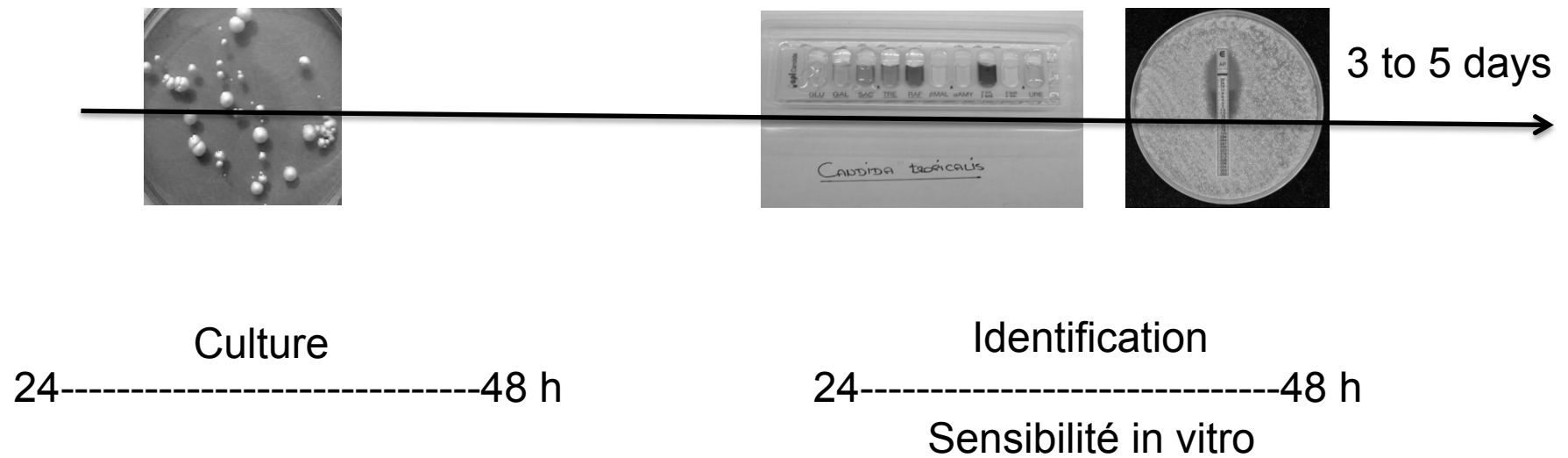
Andrew J. Labelle, MD; Scott T. Micek, PharmD; Nareg Roubinian, MD; Marin H. Kollef, MD

Table 4. Multivariate analysis of risk factors for hospital mortality

	Hospital Cohort ^a			Intensive Care Unit Cohort ^b		
	Adjusted Odds Ratio	95% Confidence Interval	<i>p</i>	Adjusted Odds Ratio	95% Confidence Interval	<i>p</i>
APACHE II score (1-point increments)	1.18	1.11–1.25	0.003	1.21	1.14–1.29	0.001
Central vein catheter retention	4.85	2.54–9.29	0.015	6.21	3.02–12.77	0.011
Corticosteroid use	3.41	1.96–5.93	0.027	—	—	—
Inadequate initial fluconazole dosing	3.31	1.83–6.00	0.044	9.22	2.15–19.79	0.004



Nouvelles approches diagnostiques et impact sur les données épidémiologiques



Alternatives :

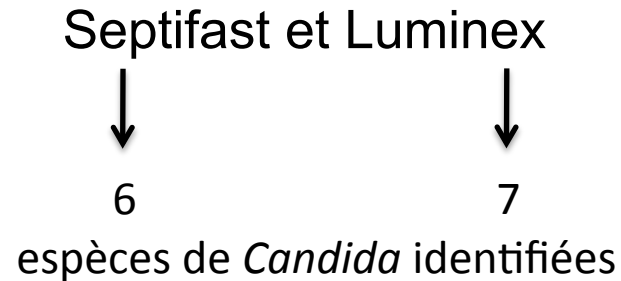
- Marqueurs solubles d'infection
(GM, Mannane, β -glucanes)
- **PCR**

Alternatives :

- **Identification moléculaire**
- **Spectrometrie de masse**
- PNA-FISH

PCR sur prélèvement sanguin

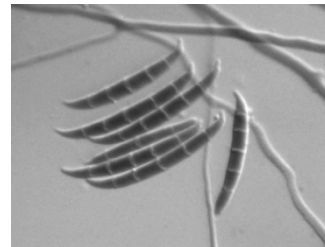
-2 méthodes « multicibles » commercialisées :



Limites de détection :

⇒ autres espèces de *Candida*, Cryptocoques, *Trichosporon*, *Geotrichum*, *Saccharomyces*, *Malassezia*

⇒ Filamenteux : *Fusarium*



SITUATION ACTUELLE POUR LA PCR SUR SANG :

- Difficultés pour trouver des cibles « universelles »
- PCR ciblée la plus évaluée : PCR *Aspergillus* en hématologie
 - ⇒ absence de standardisation
 - ⇒ sang total? serum? Plasma?
 - ⇒ méthode d'extraction? Volume?
 - ⇒ 1 ou 2 déterminations/semaine
 - ⇒ faux-positifs? Infection/colonisation
 - ⇒ critère non classant pour l'EORTC/MSG 2008
- ⇒ Mais volonté de standardisation des protocoles

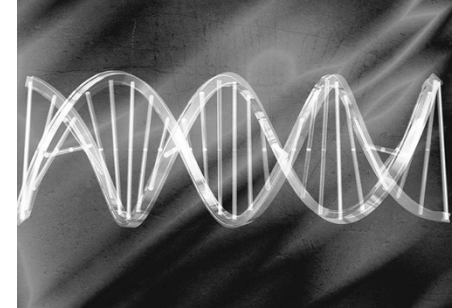
J Clin Microbiol. 2010 April; 48(4): 1231–1240.

***Aspergillus* PCR: One Step Closer to Standardization**

P. Lewis White et al. and on behalf of the European *Aspergillus* PCR Initiative

Intérêts non discutés de la biologie moléculaire :

- PCR sur biopsies, LBA
- Identification moléculaire précise
- Génotypes de résistance aux antifongiques

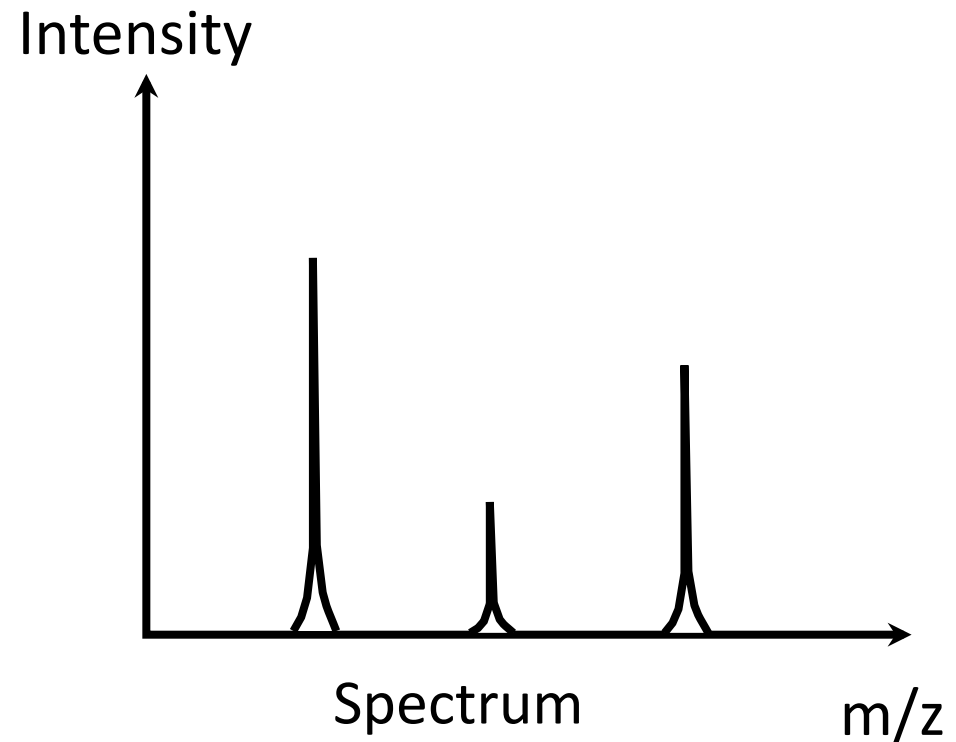
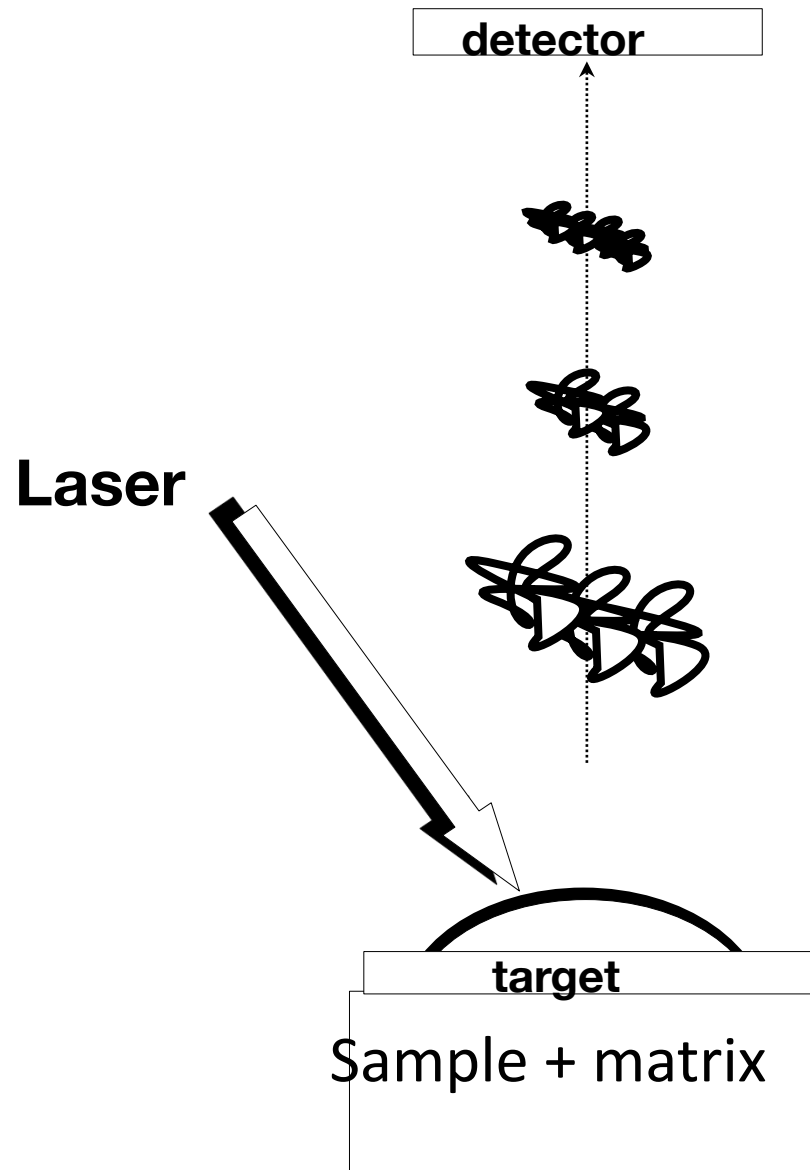


Clin Infect Dis. 2011 May;52(10):1218-26.

Comparison of an Aspergillus real-time polymerase chain reaction assay with galactomannan testing of bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. Luong ML, et al.

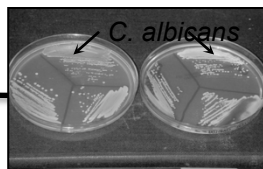
=> Sensibilité PCR (100%)>sensibilité GM LBA (85%)

Spectrométrie de masse MALDI-TOF

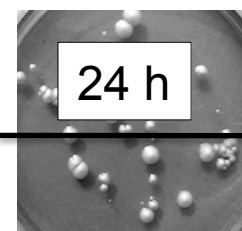


Une technique rapide, simple, robuste et flexible, accès à l'identification de nombreux champignons, selon la base de données

C. albicans : 24 h



C. non albicans : 72 h



+ Séquençage d'ADN
Identification 24 h



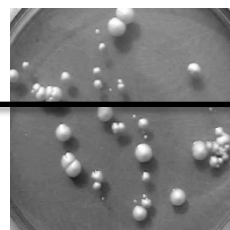
48 h

+ SM MALDI-TOF
Identification ¼ h

24 h

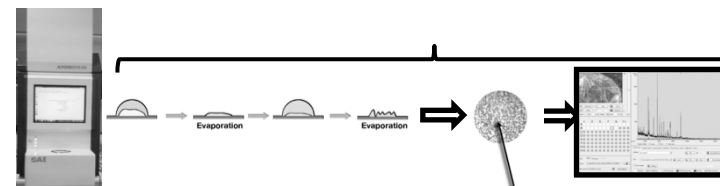


Hémoculture
positive



Biochimie
Identification moléculaire
48-72 h

SM MALDI-TOF
Identification ¼ h

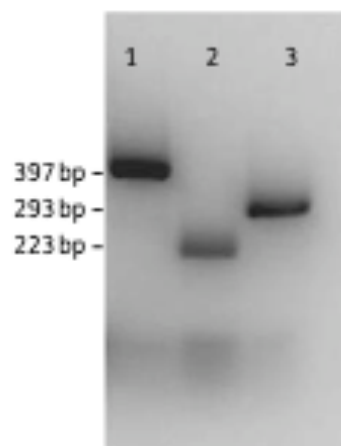


Prospective Multicenter Study of the Epidemiology, Molecular
Identification, and Antifungal Susceptibility of
Candida parapsilosis, *Candida orthopsilosis*,
and *Candida metapsilosis* Isolated from
Patients with Candidemia[†]

Emilia Cantón,^{1*} Javier Pemán,² Guillermo Quindós,³ Elena Eraso,³ Ilargi Miranda-Zapico,³
 María Álvarez,⁴ Paloma Merino,⁵ Isolina Campos-Herrero,⁶ Francesc Marco,⁷
 Elia Gomez G. de la Pedrosa,⁸ Genoveva Yagüe,⁹ Remedios Guna,¹⁰
 Carmen Rubio,¹¹ Consuelo Miranda,¹² Carmen Pazos,¹³
 David Velasco,¹⁴ and the FUNGEMYCA Study Group[†]

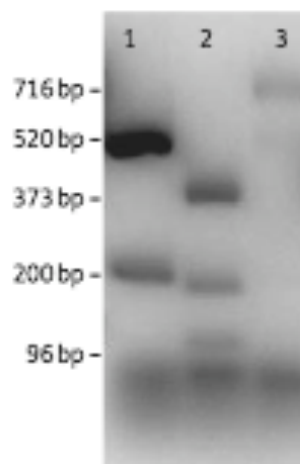
Dec 2011, n = 364

C. glabrata
cryptic species



Cg Cb Cn

C. parapsilosis
cryptic species



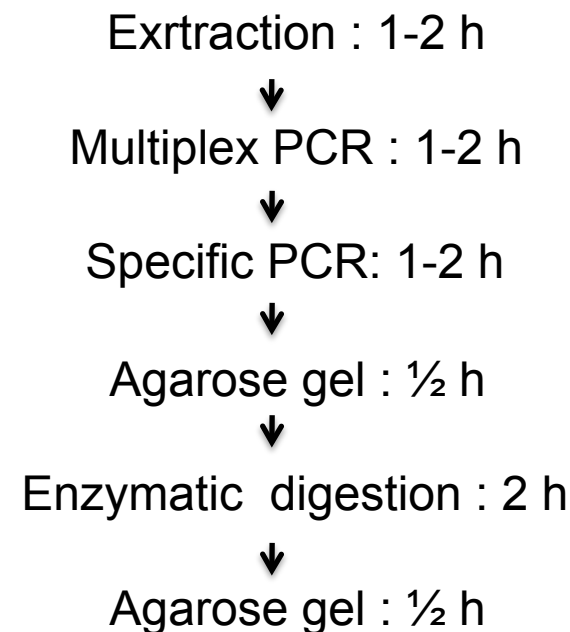
Cp Cm Co

J Antimicrob Chemother 2011; 66: 2315-2322
 doi:10.1093/jac/dkr298 Advance Access publication 26 July 2011

Prevalence and antifungal susceptibility patterns of new cryptic
species inside the species complexes *Candida parapsilosis* and
Candida glabrata among blood isolates from a Spanish tertiary hospital

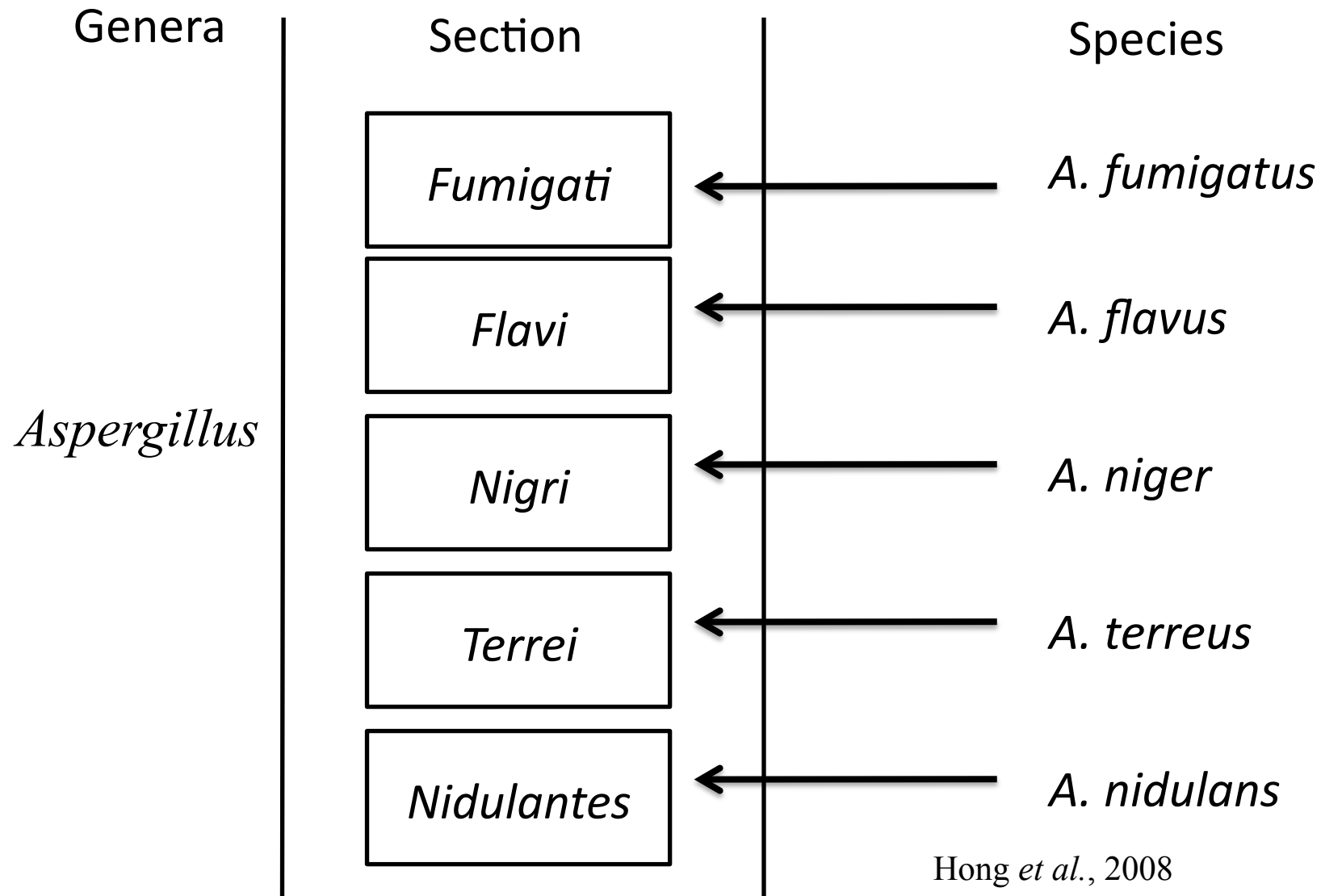
Ilargi Miranda-Zapico¹, Elena Eraso¹, José Luis Hernández-Almaraz², Leyre Mónica López-Soria²,
 Alfonso Javier Carrillo-Muñoz³, Juan Manuel Hernández-Molina⁴ and Guillermo Quindós^{1*}

March 2011, n = 691



=> Tedious protocol

Champignons filamenteux:
L'exemple d'*Aspergillus* et de *Scedosporium*



Identification moléculaire d'*Aspergillus fumigatus*

35 species

Genera

Section

Fumigati

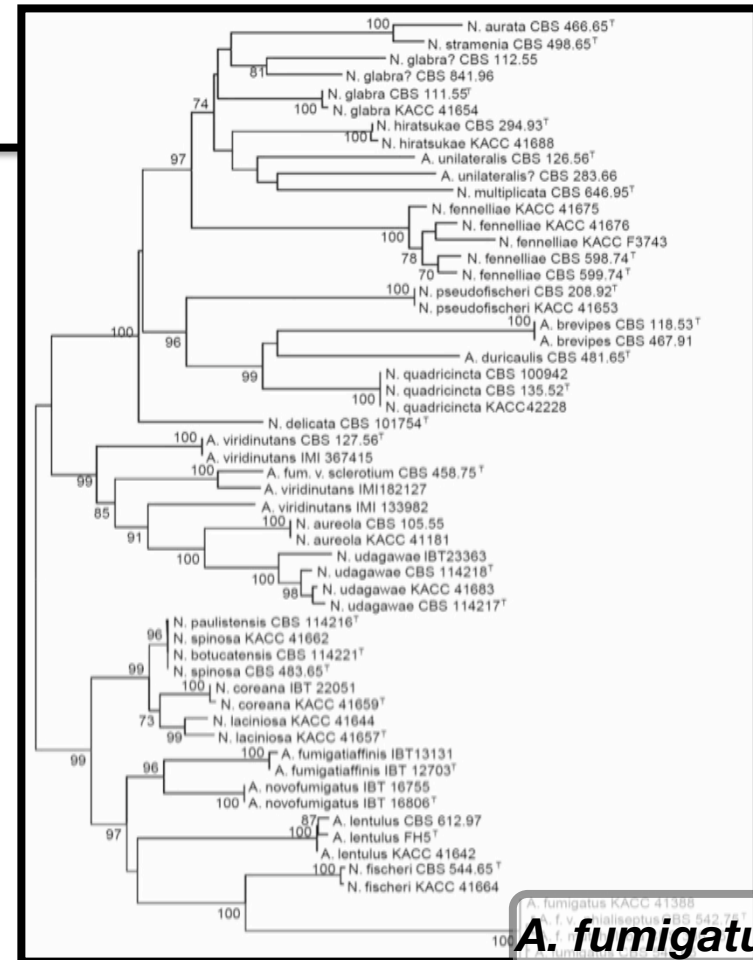
Flavi

Nigri

Terrei

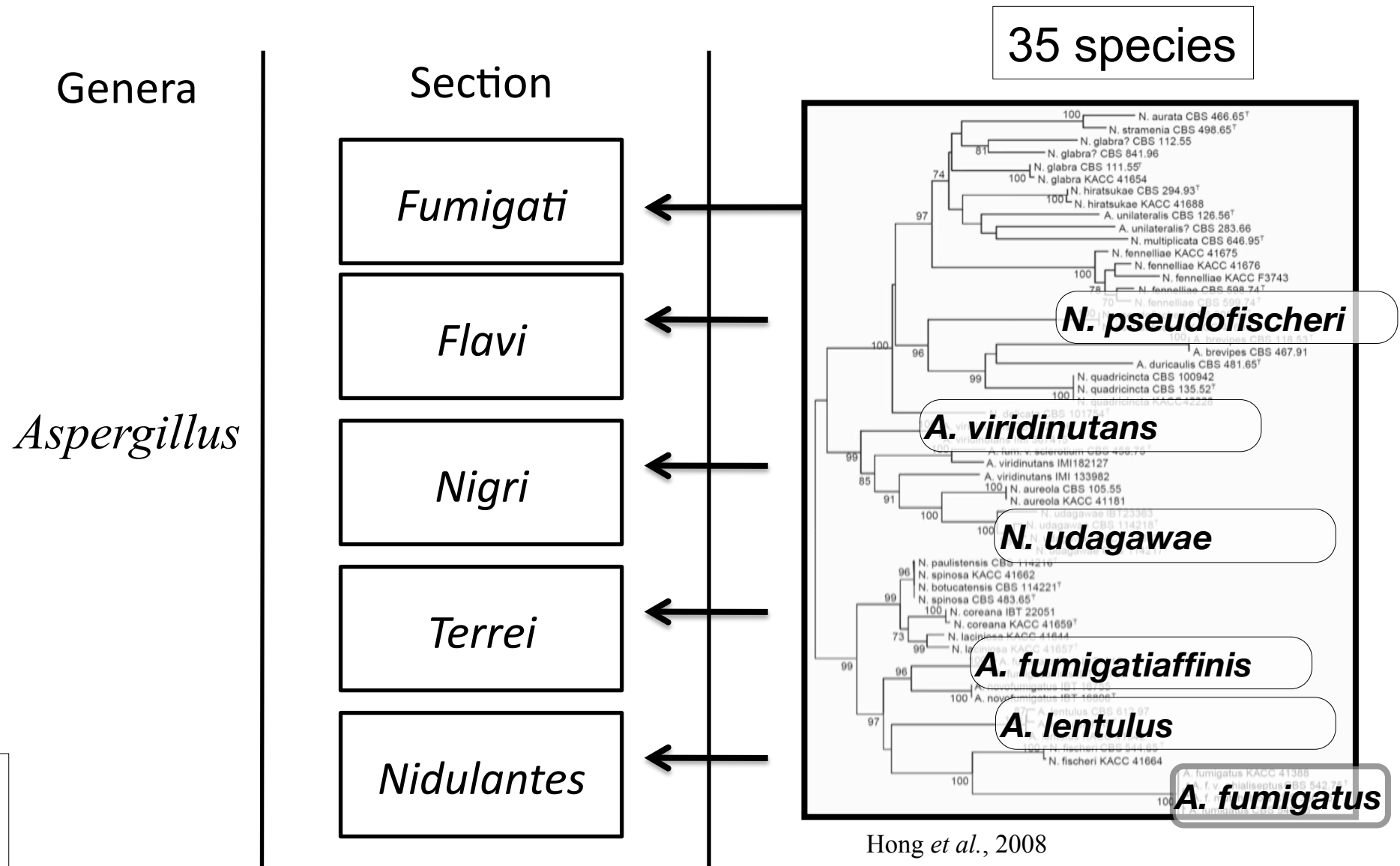
Nidulantes

Aspergillus



Hong *et al.*, 2008

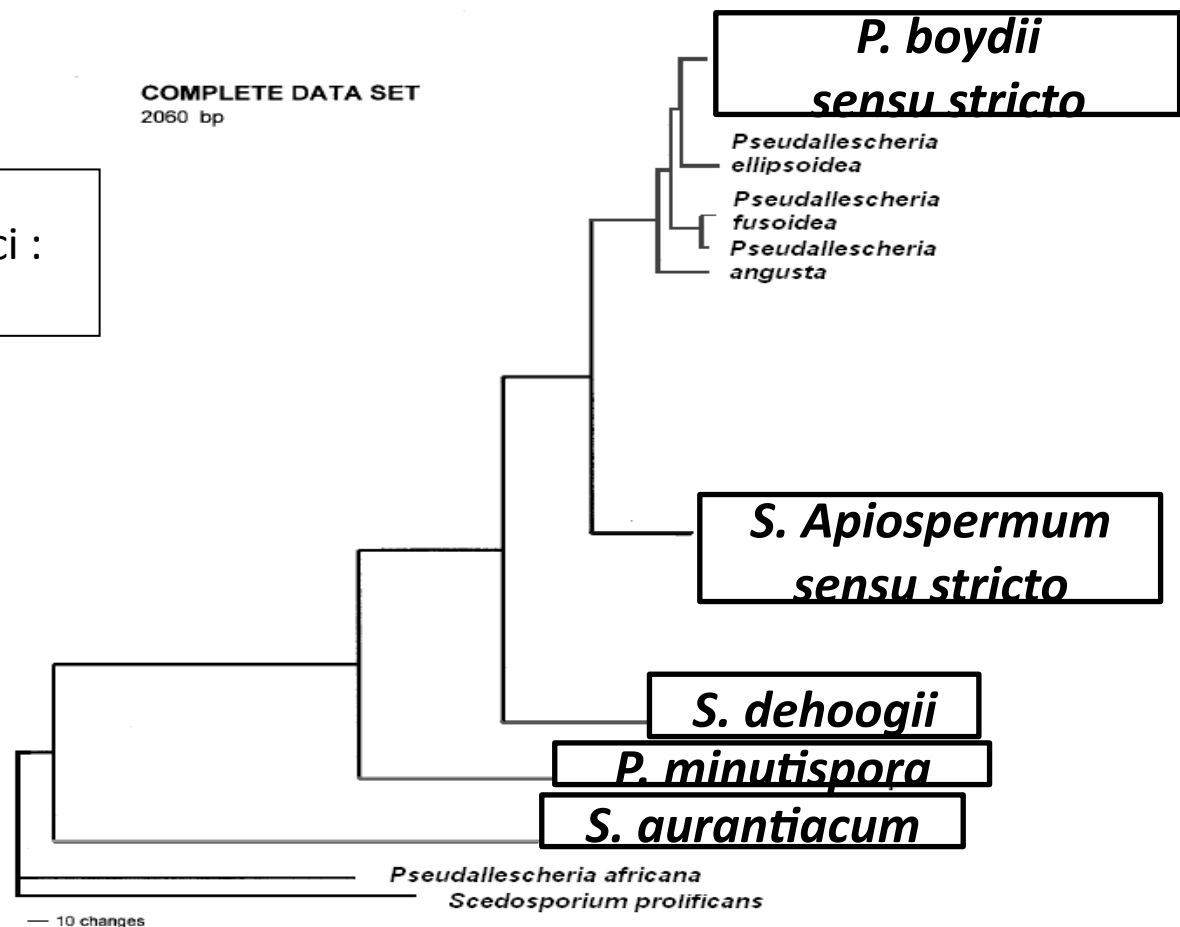
Identification moléculaire d'*Aspergillus*



Identification moléculaire de *Scedosporium*

Based on the sequencing of 3 loci :
ITS, β -tubuline, and calmoduline

Felix Gilgado et coll. JCM 2005,
JCM 2008
Kalseis J. et coll., Med Mycol 2009;
Gilgado F. et coll., Med Mycol 2009



Identification d'*Aspergillus* et de *Scedosporium* par Maldi-Tof

1^{ère} méthode automatisée et standardisée pour l'identification d'*Aspergillus* et de *Scedosporium* :

→ identification précise des espèces

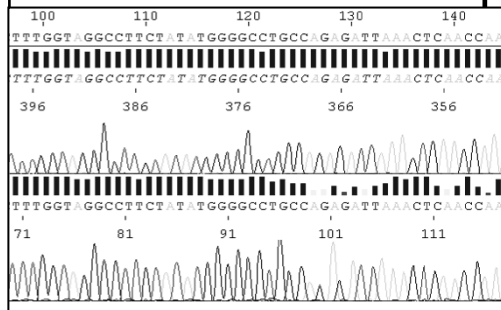
– fréquentes mais aussi rares

– discrimination entre espèces de morphologie identique

→ Protocole simple et rapide : quelques minutes *versus* quelques jours

- Nécessite mise à jour régulière des bases de données

L'identification et la SM Maldi-Tof sont complémentaires pour construire les bases de données



Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
U02201.1	Candida orthopositis strain CBS 10906 18S ribosomal RNA gene, parti	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain U02201 18S ribosomal RNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain ATCC 96141 18S ribosomal RNA gene, par	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain L7956 18S ribosomal RNA gene, partial se	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain 840 18S ribosomal RNA gene, partial sequ	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain L7786 18S ribosomal RNA gene, partial se	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain L6068A 18S ribosomal RNA gene, partial s	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain L7787 18S ribosomal RNA gene, partial se	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain L6786 18S ribosomal RNA gene, partial se	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 5S rRNA gene, 18S rRNA gene, ITS1, 5.8S rRNA	322	322	100%	0.0	99%	
U02201.1	Candida orthopositis strain L7805 18S ribosomal RNA gene, partial se	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain L3106A 18S ribosomal RNA gene, partial s	322	322	100%	0.0	99%	
U02201.1	Candida orthopositis strain L6785 18S ribosomal RNA gene, partial se	322	322	100%	0.0	99%	
U02201.1	Candida orthopositis strain L6201A 18S ribosomal RNA gene, partial s	322	322	100%	0.0	99%	
U02201.1	Candida orthopositis strain L7941 18S ribosomal RNA gene, partial se	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis strain MCO450 18S ribosomal RNA gene, partial	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis isolate HEMCAN138 18S ribosomal RNA gene, po	322	322	100%	0.0	100%	
U02201.1	Candida orthopositis isolate HEMC755 18S ribosomal RNA gene, parti	322	322	100%	0.0	100%	

Identification d'*Aspergillus* et de *Scedosporium* par Maldi-Tof

Amélioration majeure de la prise en charge des IFI

- différenciation des espèces environnementales des espèces pathogènes pour l'homme
- Etudes épidémiologiques de l'impact de la biodiversité sur la présentation clinique
- prescription d'un traitement antifongique approprié

***Aspergillus* = 28 espèces**

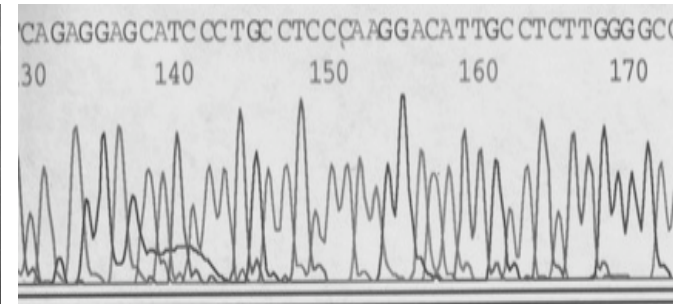
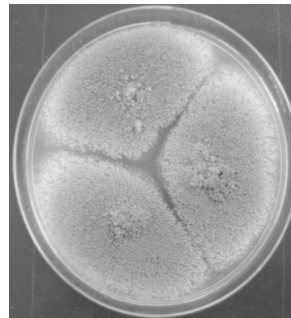
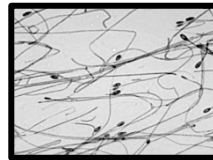
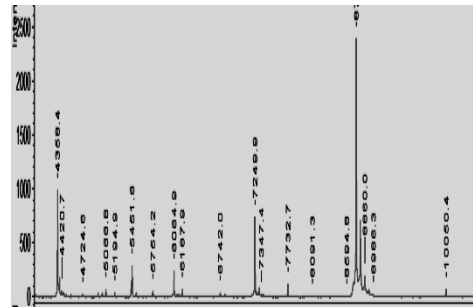
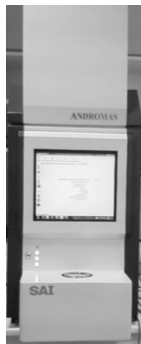
- **Frequent *Aspergillus* species (n=6)** : *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, *A. nidulans*, *A. versicolor*
- **Rare *Aspergillus* species (n=22)** : *A. lentulus*, *N. udagawae*, *N. pseudofischeri*, *A. viridinutans*, *A. fumigatiaffinis*, *A. fumisynematus*, *N. fischeri*, *N. hiratsukae*, *N. spinosa*, *N. fennelliae*, *A. oryzae*, *A. tamarii*, *A. parvisclerotigenus*, *P. alliaceus*, *A. tubengensis*, *A. foetidus*, *A. sydowii*, *E. quadrilineata*, *A. calidoustus*, *A. pseudodeflectus*, *A. insuetus*, *A. ochraceus*

***Scedosporium* = 6 espèces**

S. prolificans, *S. apiospermum*, *S. aurantiacum*, *S. dehoogii*, *P. boydi*, *P. minutispora*

ANDROMAS Database

Concordance entre Maldi Tof et identification moléculaire *Ad'spergillus* et de *Scedosporium*

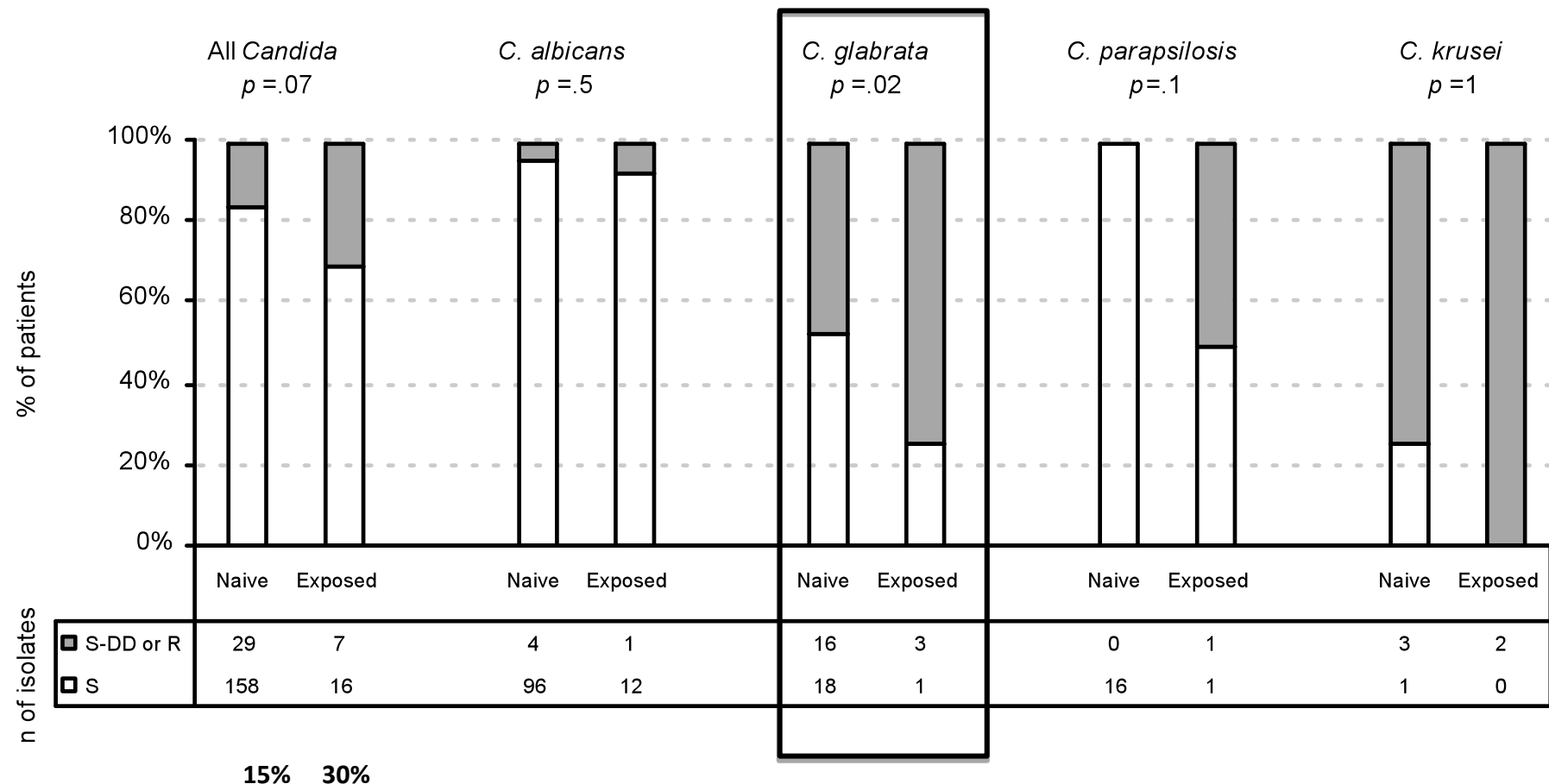


**134/136 (98,4%) pour *Aspergillus* sp.
61/61 pour *Scedosporium* sp.**

Impact de la biodiversité fongique sur la stratégie thérapeutique

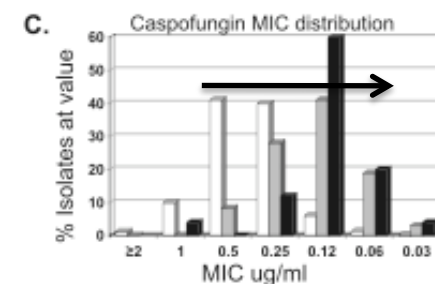
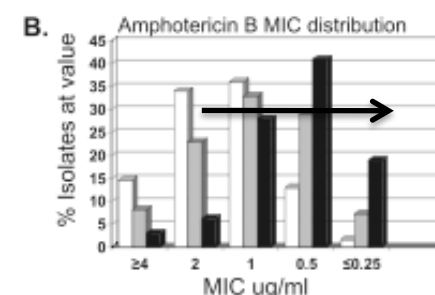
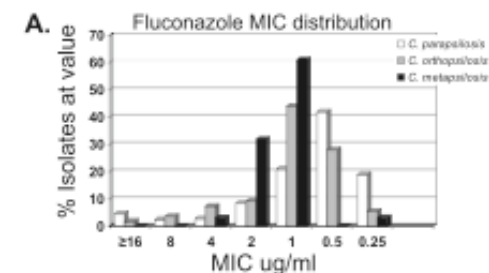
=> Biodiversité et sensibilité aux antifongiques

Enquête Amarcan : prospective, multicentrique, française
-300 épisodes de candidoses invasives en réanimation



Geographic Distribution and Antifungal Susceptibility of the Newly Described Species *Candida orthopsilosis* and *Candida metapsilosis* in Comparison to the Closely Related Species *Candida parapsilosis*[▽]

Shawn R. Lockhart,^{1*} Shawn A. Messer,¹ Michael A. Pfaller,¹ and Daniel J. Diekema^{1,2}
 Departments of Pathology¹ and Internal Medicine,² University of Iowa Carver College of Medicine, University of Iowa Hospitals and Clinics, Iowa City, Iowa



CMI 90 (microg/mL)					
	Fluconazole	Amphotéricine B	Caspofungine	Anidulafungine	Micafungine
<i>C. Nivariensis</i>	2	1	0,06	0,06	0,015
<i>C. Bracarensis</i> (1)	16	8	0,03	0,06	0,015
<i>C. Bracarensis</i> (2)	2	1	0,03	0,06	0,015

Résistance acquise chez *Candida*

	Azoles P450 déméthylase	Echinocandines Glucane synthase	Amphotéricine B Ergostérol
Mutation du gène cible	ERG11	FKS1 FKS2	-
Hyperexpression de la cible	Promoteur de ERG11	-	-
Pompes à efflux	MDR, CDR, CgSNQ2	-	-

Perspectives : biomarqueurs de résistance

Proteomics 2009, 9, 4627–4631

DOI 10.1002/pmic.200900152

4627

RAPID COMMUNICATION

MALDI-TOF MS-based drug susceptibility testing of pathogens: The example of *Candida albicans* and fluconazole

Carine Marinach^{1,2*}, Alexandre Alanio^{1,2,3*}, Martine Palous³, Stéphanie Kwasek^{1,2}, Arnaud Fekkar^{1,2,3}, Jean-Yves Brossas^{1,2,4}, Sophie Brun^{1,2,3}, Georges Snounou^{1,2,5}, Christophe Hennequin^{1,2}, Dominique Sanglard⁶, Annick Datry³, Jean-Louis Golmard^{7*} and Dominique Mazier^{1,2,3*}

¹ INSERM, UMRS 945 Paris, France

² Université Pierre et Marie Curie-Paris6, Paris, France

³ AP-HP, Groupe hospitalier Pitié-Salpêtrière, Service Parasitologie-Mycologie, Paris, France

⁴ Centre d'Investigation Biomédical, Groupe hospitalier Pitié-Salpêtrière, Paris, France

⁵ Department of Microbiology, National University of Singapore, Singapore

⁶ Institute of Microbiology, University of Lausanne and University Hospital Center, Lausanne, Switzerland

⁷ EA 3974, Modélisation en Recherche Clinique, Groupe hospitalier Pitié-Salpêtrière, Paris, France

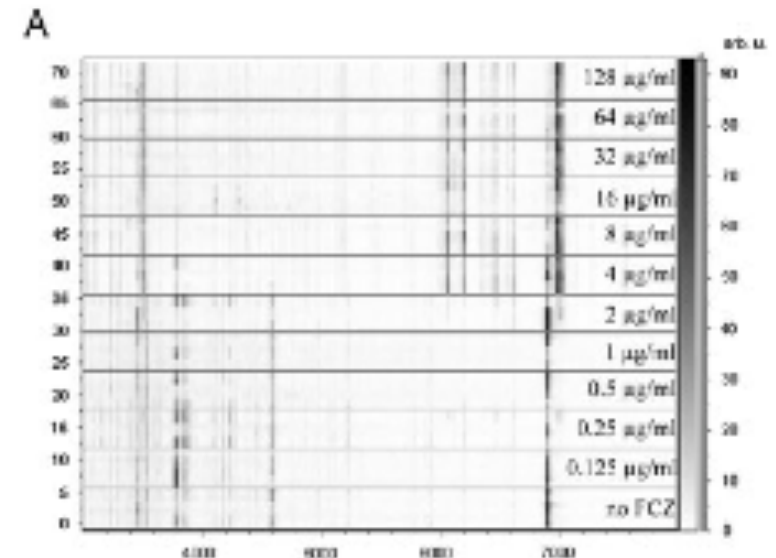


Figure 1. (A) Alterations in the mass spectra of the DSY226C *C. albicans* strain exposed to increasing FCZ concentrations (virtual gel). The x-axis represents m/z value, on the left the y-axis shows running spectrum number, whereas on the right, peak intensity is expressed in a gray color scale with arbitrary units (au). (B) A portion of the average mass spectra (range m/z

Révisions des breakpoints CLSI et EUCAST

	CLSI M27-S3	CLSI Revised 2010/11	EUCAST
AMB	≤ 1	≤ 1	≤ 1 ; > 1
Fluco	≤ 8 ; > 32	≤ 2 ; > 4 (<i>alb, para, trop</i>) SDD ≤ 32 ; R > 32 (<i>glab</i>) (<i>krus</i> poor target)	≤ 2 ; > 4 (<i>albi, trop, para</i>) (<i>glab</i> IE) (<i>krus</i> poor target)
Itra	≤ 0.125 ; > 0.5	≤ 0.125 ; > 0.5	-
Posa	-	-	≤ 0.06 ; > 0.06 (<i>alb, trop, para</i>) (<i>glab/krus</i> IE)
Vori	≤ 1 ; > 2	≤ 0.125 ; > 0.5 (<i>alb, para, trop</i>) ≤ 0.5 ; > 1 (<i>krus</i>) (<i>glab</i> IE)	≤ 0.125 ; > 0.125 (<i>alb, trop, para</i>) (<i>glab/krus</i> IE)

Révisions des breakpoints CLSI et EUCAST : Echinocandines

	CLSI M27-S3	CLSI Revised 2011	EUCAST
ANF	≤2	≤0.25; >0.5 (<i>alb, krus, trop</i>) <u>≤0.125; >0.25</u> (<i>glab</i>)	≤0.032; >0.032 (<i>alb</i>) ≤0.06; >0.06 (<i>glab, krus, trop</i>) (<i>para</i> not a good target) (<i>guillier</i> IE)
CSF	≤2	≤2; >4 (<i>para, guillier</i>)	-
MFG	≤2	≤0.25; >0.5 (<i>alb, krus, trop</i>) <u>≤0.06; >0.125</u> (<i>glab</i>) ≤2; >4 (<i>para, guillier</i>)	-

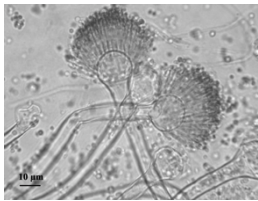
Révisions des breakpoints CLSI et EUCAST : Utilisation pratique avec l'E-test

EUCAST CLSI

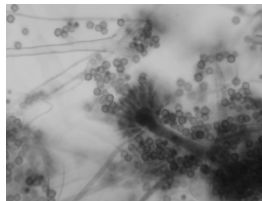
			0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16
Anidulafungin	<i>C. albicans</i> *	80	43	31	5	1										
	<i>C. glabrata</i>	81		8	67	6										
	<i>C. dublinensis</i>	14	2	11	1											
	<i>C. tropicalis</i>	8			2	5						1				
	<i>C. krusei</i>	5				5										
	<i>C. parapsilosis</i>	36						1				14	11	6	1	3
	<i>C. guilliermondii</i>	2												1	1	
	<i>C. lusitanae</i>	8				7	1									
Caspofungin	<i>C. albicans</i> *	80				17	29	26	6	2						
	<i>C. glabrata</i>	81						23	58							
	<i>C. dublinensis</i>	14				2	7	5								
	<i>C. tropicalis</i>	8						2	5						1	
	<i>C. krusei</i>	5								3	2					
	<i>C. parapsilosis</i>	36								16	20					
	<i>C. guilliermondii</i>	2									1	1				
	<i>C. lusitanae</i>	8								6	2					
Micafungin	<i>C. albicans</i> *	80		36	38	6										
	<i>C. glabrata</i>	81		4	74	3										
	<i>C. dublinensis</i>	14		6	7	1										
	<i>C. tropicalis</i>	8			2	5						1				
	<i>C. krusei</i>	5					1	4								
	<i>C. parapsilosis</i>	36							2	8	24	2				
	<i>C. guilliermondii</i>	2									2					
	<i>C. lusitanae</i>	8				1	6	1								

Biodiversité des champignons filamenteux et résistance ?

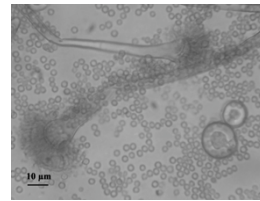
- *Aspergillus fumigatus* > 80% des infections
- Emergence d'*A. terreus*, *A. flavus* etc...
- Remaniements : *Aspergillus* sections *fumigati*, *flavi*, etc...



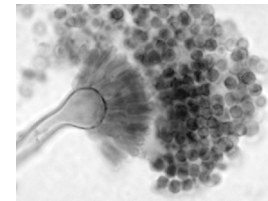
A. terreus



A. ustus



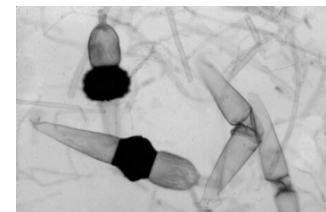
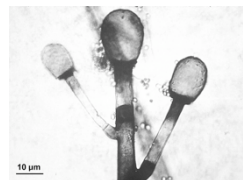
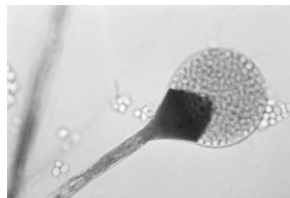
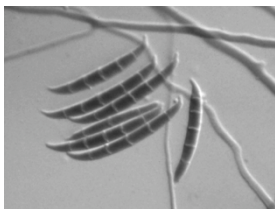
A. nidulans



A. flavus

⇒ IFI non aspergillaires

- *Fusarium* sp., mucormycètes...



=> Résistance naturelle ?

- Variabilité de sensibilité aux antifongiques entre les espèces
- Explication des différences de sensibilité observées au sein de la section *fumigati*

Espèces	Section	AmB	Azolés	Cas
<i>A. fumigatus</i>	<i>Fumigati</i>			
<i>A. lentulus</i>				
<i>A. fumigatiaffinis</i>				
<i>A. viridinutans</i>				
<i>A. fumisynnematus</i>				
<i>N. fischeri</i>				
<i>N. pseudofischeri</i>				
<i>N. udagawae</i>				
<i>N. fennelliae</i>				
<i>N. hiratsukae</i>				
<i>N. spinosa</i>				

⇒ Résistance acquise

-Liée à l'exposition aux azolés au long cours ?

#	Présentation clinique	Pré-exposition azolés	Traitement	Evolution
1	API prouvée (granulomatose chronique)	Prophylaxie itraconazole 6 ans	Voriconazole fortes doses	Survie
2	AI prouvée (mastoïdite)	-	Chirurgie + local	Survie
3	API prouvée (syndrome hyper-IgE)	TTT voriconazole 2 ans	Chirurgie + posaconazole	Survie
4	API possible (fibrose pulmonaire)	-	Voriconazole	Survie
5	API probable (granulomatose chronique)	Prophylaxie itraconazole 10 ans	Caspofungine + posaconazole	Survie
6	AI disséminée probable (LAM)	-	Voriconazole	décès
7	API possible (BPCO)	-	Voriconazole, AMB, posaconazole	Survie
8	AI osseuse prouvée (granulomatose chronique)	Prophylaxie itraconazole >2ans	Voriconazole, Caspofungine, posaconazole	Survie
9	AI sinusienne prouvée (greffé de moelle)	Prophylaxie itraconazole 4 sem	Posaconazole	Décès

Verweij et al.,
New Engl J Med, 2007

⇒ Résistance acquise

-A partir d'un réservoir environnemental ?

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);

OPEN ACCESS Freely available online

PLOS MEDICINE

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

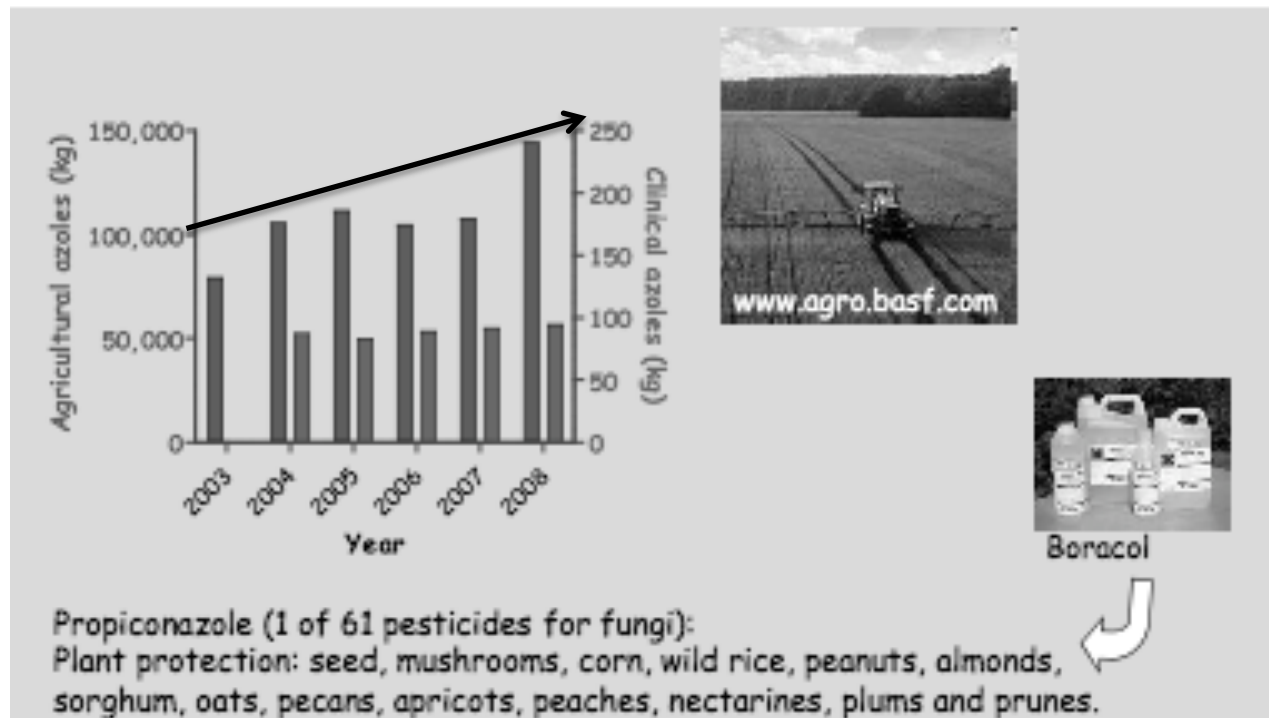
Eveline Snelders^{1,2}, Henrich A. L. van der Lee^{1,2}, Judith Kuijpers^{1,2}, Anthonius J. M. M. Rijs^{1,2}, János Varga^{3,4}, Robert A. Samson³, Emilia Mellado⁵, A. Rogier T. Donders⁶, Willem J. G. Melchers^{1,2}, Paul E. Verweij^{1,2*}

1 Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 2 Nijmegen Institute for Infectious Diseases, Inflammation and Immunity, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 3 Centraalbureau voor Schimmelcultures (CBS), Fungal Biodiversity Centre, Utrecht, The Netherlands, 4 Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary, 5 Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain, 6 Department of Epidemiology and Biostatistics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Diffusion clonale :
mutation TR/L98H

Mortensen et al., AAC 2010 ; Snelders E et al., Plos Med 2008; Verweij PE et al., Lancet Infect Dis 2009; Snelders E et al., Plos One 2012

Augmentation croissante de l'usage des azolés pesticides : l'expérience européenne



Danemark
Jusqu'à 7% de mutants TR/L98H

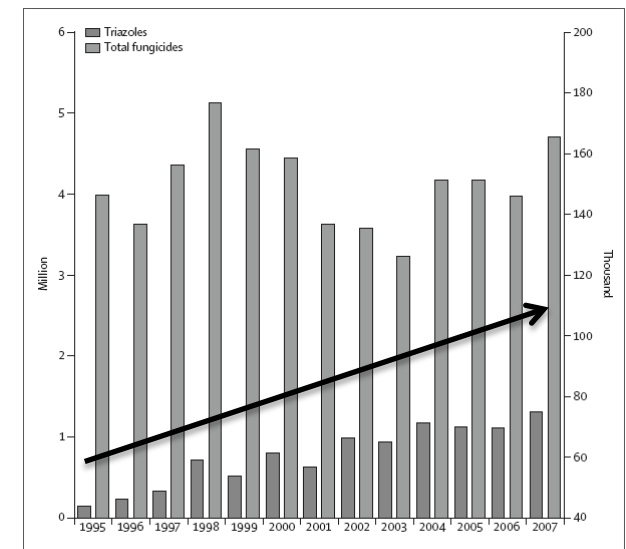


Figure 2: Total volume of fungicides and triazoles sold in the Netherlands between 1995 and 2007
Data from the Dutch Foundation for Phytofarmacy (Nefyto, Nederlandse Stichting voor Fytofarmacie).

Pays Bas
Jusqu'à 9% de mutants TR/L98H

Mortensen et al., AAC 2010 ; Snelders E et al., Plos Med 2008; Verweij PE et al., Lancet Infect Dis 2009;
Snelders E et al., Plos One 2012

Merci pour votre attention

Jean-pierre.gangneux@chu-rennes.fr

