

Molecular techniques versus MALDI-TOF mass spectrometry  
in fungal diseases : are they complementary or redundant ?

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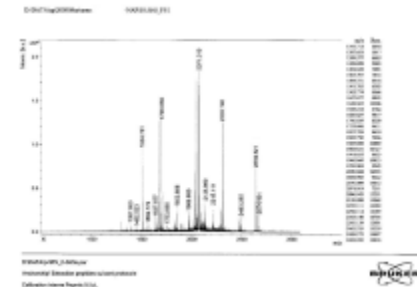
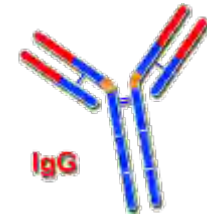
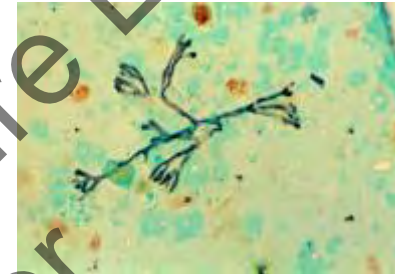
2. Paris-Necker Teaching Hospital and Institut Pasteur, Paris, FR



# Usual diagnostic tools for fungal infections

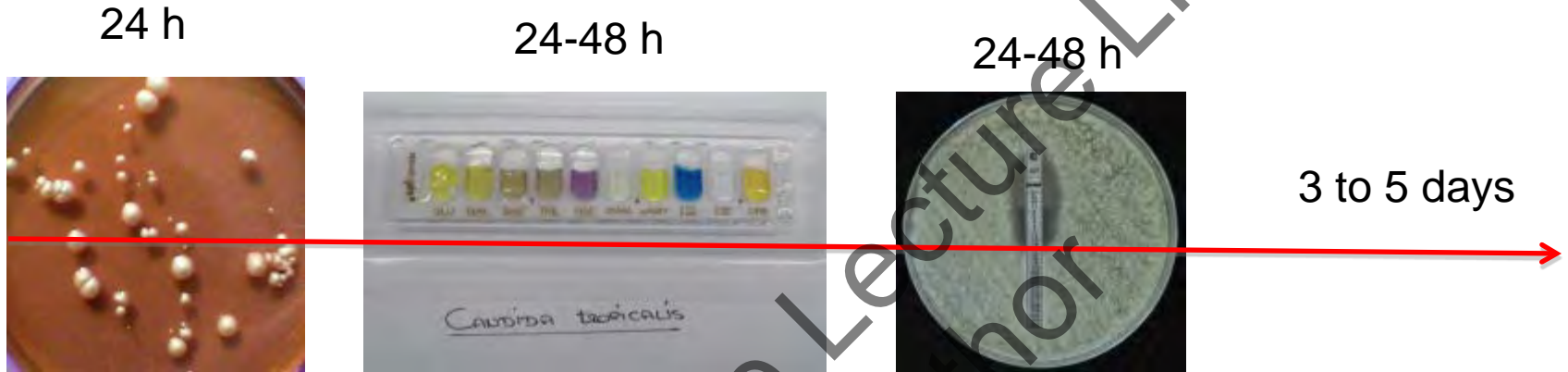
- Microscopic examination and culture
- Histology
- Immunology : soluble markers
- Molecular biology
- Mass spectrometry

Are they complementary or redundant ?

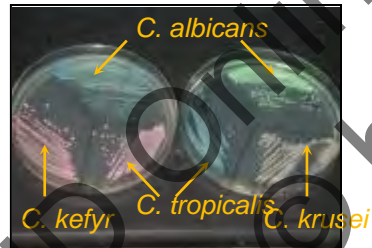


# Limitations of the mycological diagnosis

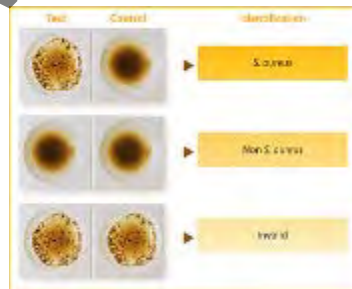
## 1. Time to get the final results : Yeasts



Chromogenic media

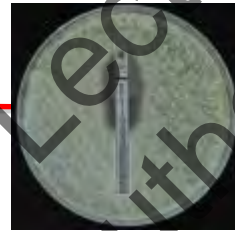
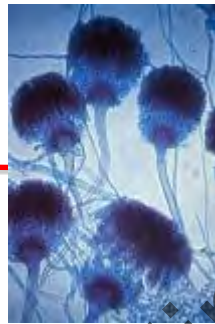


Agglutination tests



## Limitations of the mycological diagnosis

### 1. Time to get the final results : filamentous fungi



5 to 7 days

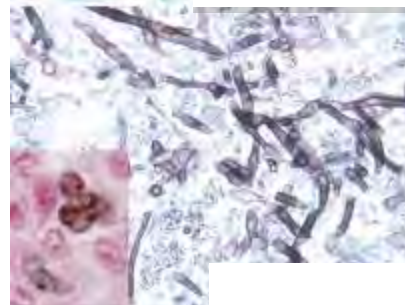
Until 21 days for dermatophytes

### 2. Mycology and morphology allow only limited discrimination between newly described species and between isolates

### 3. In tissues, what does mean “hyphae or pseudohyphae”?

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

Morphology of hyphae and  
pseudohyphae

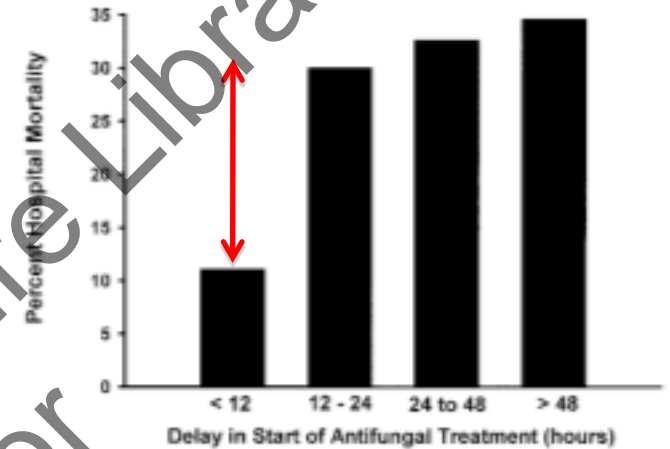


Guarner, J. et al. 2011. Clin.  
Microbiol. Rev. 24(2):247-280

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

Matthew Morrell,<sup>1</sup> Victoria J. Fraser,<sup>2</sup> and Marin H. Kollef<sup>1\*</sup>

Pulmonary and Critical Care Division<sup>1</sup> and Division of Infectious Diseases,<sup>2</sup> Washington University School of Medicine, St. Louis, Missouri 63110

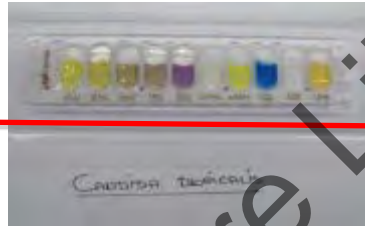


## Treatment-related risk factors for hospital mortality in *Candida* bloodstream infections\*

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

Table 4. Multivariate analysis of risk factors for hospital mortality

|                                       | Hospital Cohort <sup>a</sup> |                         |          | Intensive Care Unit Cohort <sup>b</sup> |                         |          |
|---------------------------------------|------------------------------|-------------------------|----------|---|-------------------------|----------|
|                                       | 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    |



3 to 5 days

Time of culture  
24-----48 h

Identification  
24-----48 h  
In vitro sensibility

### Alternatives :

- Soluble markers of the infection (GM, Mannan,  $\beta$ -glucans)
- DNA detection in samples

### Alternatives :

- Molecular identification
- Mass spectrometry
- PNA-FISH



# DNA detection from samples

## 1. Circulating DNA in blood

- Marketed solutions

Septifast and Luminex systems



6  
*Candida species identified*

7

No amplification:

⇒ Other *Candida species*, *Cryptococcus*, *Trichosporon*, *Geotrichum*,  
*Saccharomyces*, *Malassezia*

⇒ Filamentous fungi : *Fusarium*



-In-house solutions

- Difficulty to design « pan-fungal » assays

- Targeted PCR: the example of *Aspergillus* PCR

⇒ absence of standardisation

⇒ total blood? serum? Plasma?

⇒ extraction method? Volume?

⇒ 1 or 2 determinations/week

⇒ infection/colonisation

⇒ criteria not validated by the EORTC/MSG 2008 guidelines

⇒ However, multiple initiatives to standardize the assay

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**

## 2. DNA detection from other fluids and tissues

- BAL, biopsies etc...
- precise molecular identification
- molecular markers of virulence and antifungal resistance

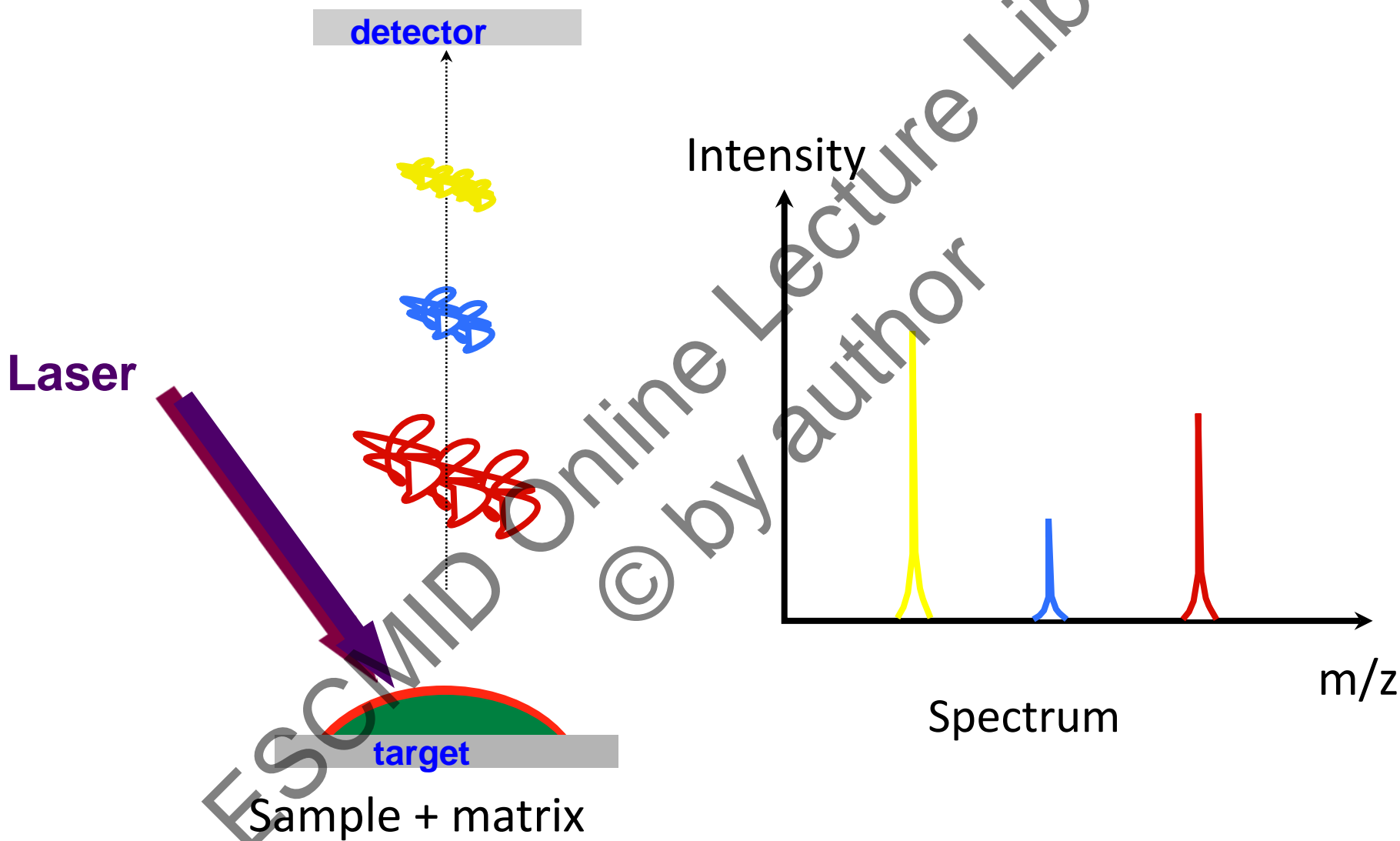


Clin Infect Dis. 2011 May;52(10):1218-26.

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.](#)

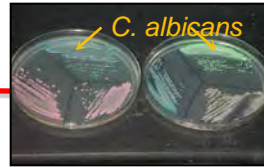
=> Sensitivity PCR (100%)>sensitivity GM (85%)

# MALDI-TOF mass spectrometry





C. albicans : 24 h



C. non albicans : 72 h



+ DNA sequencing  
Identification 24 h



48 h



+ MALDI-TOF-MS  
Identification ¼ h

24 h

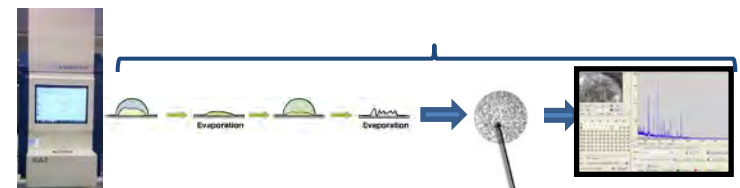


Biochemistry  
Molecular identification  
48-72 h

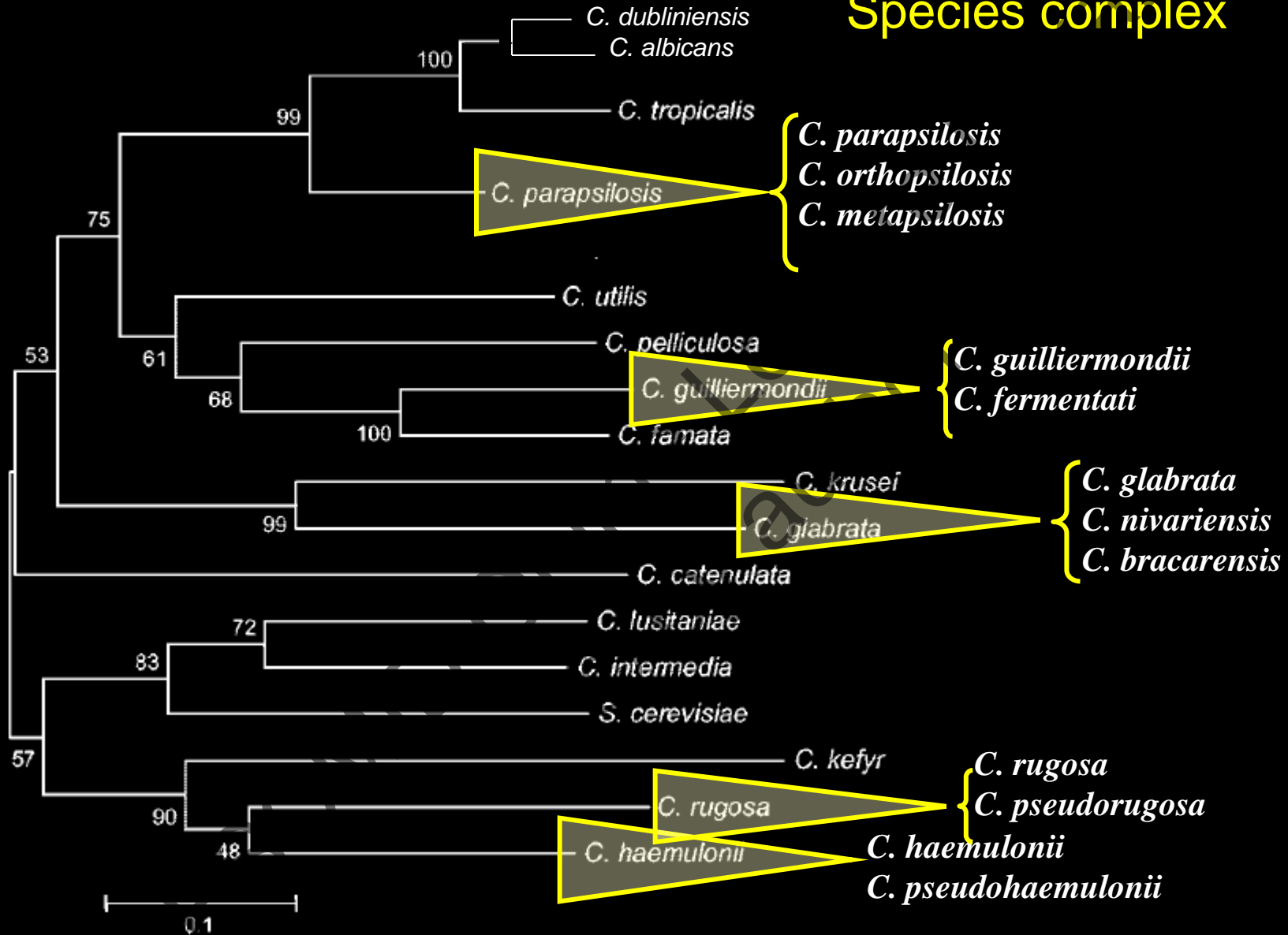


Positive blood culture

MALDI-TOF-MS  
Identification ¼ h

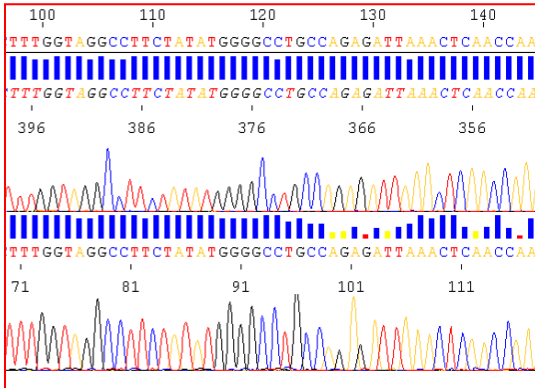


# Phylogenetic tree ( ITS1)



Tsui C. et coll.,  
FEMS Yeast res, 2008

# Molecular identification and MT-MS are complementary to update databases



Sequences providing significant alignments:

| Accession | Description   | Max score | Total score | Query coverage | E value | Max Ident | Links |
|-----------|---|-----------|-------------|----------------|---------|-----------|-------|
| U00212.1  | Candida orthoploides strain CBS 10096 18S ribosomal RNA gene, parti   | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain USA/HCFP 2161 18S ribosomal RNA gene,     | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain ATCC 96341 18S ribosomal RNA gene, par    | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain L7916 18S ribosomal RNA gene, partial se  | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain 849 18S ribosomal RNA gene, partial seque | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain L7208 18S ribosomal RNA gene, partial se  | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain L8088A 18S ribosomal RNA gene, partial s  | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain L7787 18S ribosomal RNA gene, partial se  | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain L6786 18S ribosomal RNA gene, partial se  | 333       | 369         | 100%           | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides 5S rRNA gene, 35S rRNA gene, ITS1, 5.8S rRNA     | 333       | 369         | 100%           | 0.0     | 99%       |       |
| U00212.1  | Candida orthoploides strain L7929 18S ribosomal RNA gene, partial se  | 333       | 369         | 100%           | 0.0     | 98%       |       |
| U00212.1  | Candida orthoploides strain L8196A 18S ribosomal RNA gene, partial s  | 333       | 369         | 100%           | 0.0     | 98%       |       |
| U00212.1  | Candida orthoploides strain L6735 18S ribosomal RNA gene, partial se  | 333       | 369         | 100%           | 0.0     | 99%       |       |
| U00212.1  | Candida orthoploides strain L4291A 18S ribosomal RNA gene, partial s  | 333       | 369         | 100%           | 0.0     | 99%       |       |
| U00212.1  | Candida orthoploides strain L7941 18S ribosomal RNA gene, partial se  | 333       | 369         | 100%           | 0.0     | 99%       |       |
| U00212.1  | Candida orthoploides strain AK0456 18S ribosomal RNA gene, partial    | 333       | 369         | 99%            | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,   | 331       | 368         | 97%            | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,   | 331       | 368         | 97%            | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,   | 331       | 368         | 97%            | 0.0     | 100%      |       |
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| U00212.1  | Candida orthoploides 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,   | 331       | 368         | 97%            | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides strain L7519 18S ribosomal RNA gene, partial se  | 331       | 368         | 97%            | 0.0     | 99%       |       |
| U00212.1  | Candida orthoploides 18S rRNA gene (partial), ITS1, 5.8S rRNA gene,   | 331       | 368         | 97%            | 0.0     | 99%       |       |
| U00212.1  | Candida orthoploides strain 5315 18S ribosomal RNA gene, partial seq  | 331       | 368         | 95%            | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides isolate HSMCAN120 18S ribosomal RNA gene, pa     | 331       | 368         | 95%            | 0.0     | 100%      |       |
| U00212.1  | Candida orthoploides isolate HSMC755 18S ribosomal RNA gene, parti    | 331       | 368         | 95%            | 0.0     | 100%      |       |

- ⇒ but MT-MS is far more rapid
- ⇒ cost saving regarding lab technicians
- ⇒ cost saving regarding treatments

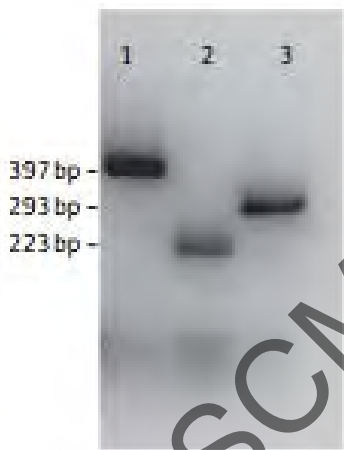


### Prospective Multicenter Study of the Epidemiology, Molecular Identification, and Antifungal Susceptibility of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* Isolated from Patients with Candidemia<sup>V</sup>

Emilia Cantón,<sup>1,4</sup> Javier Pemán,<sup>2</sup> Guillermo Quindós,<sup>3</sup> Elena Eraso,<sup>3</sup> Ilargi Miranda-Zapico,<sup>3</sup> María Álvarez,<sup>4</sup> Paloma Merino,<sup>5</sup> Isolina Campos-Herrero,<sup>6</sup> Francesc Marco,<sup>7</sup> Elia Gomez G. de la Pedrosa,<sup>8</sup> Genoveva Yagüe,<sup>9</sup> Remedios Guna,<sup>10</sup> Carmen Rubio,<sup>11</sup> Consuelo Miranda,<sup>12</sup> Carmen Pazos,<sup>13</sup> David Velasco,<sup>1,4</sup> 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-Zapico<sup>1</sup>, Elena Eraso<sup>1</sup>, José Luis Hernández-Almaraz<sup>2</sup>, Leyre Mónica López-Soria<sup>2</sup>, Alfonso Javier Carrillo-Muñoz<sup>3</sup>, Juan Manuel Hernández-Molina<sup>4</sup> and Guillermo Quindós<sup>1\*</sup>

March 2011, n = 691

Extraction : 1-2 h



Multiplex PCR : 1-2 h



Specific PCR: 1-2 h



Agarose gel : ½ h



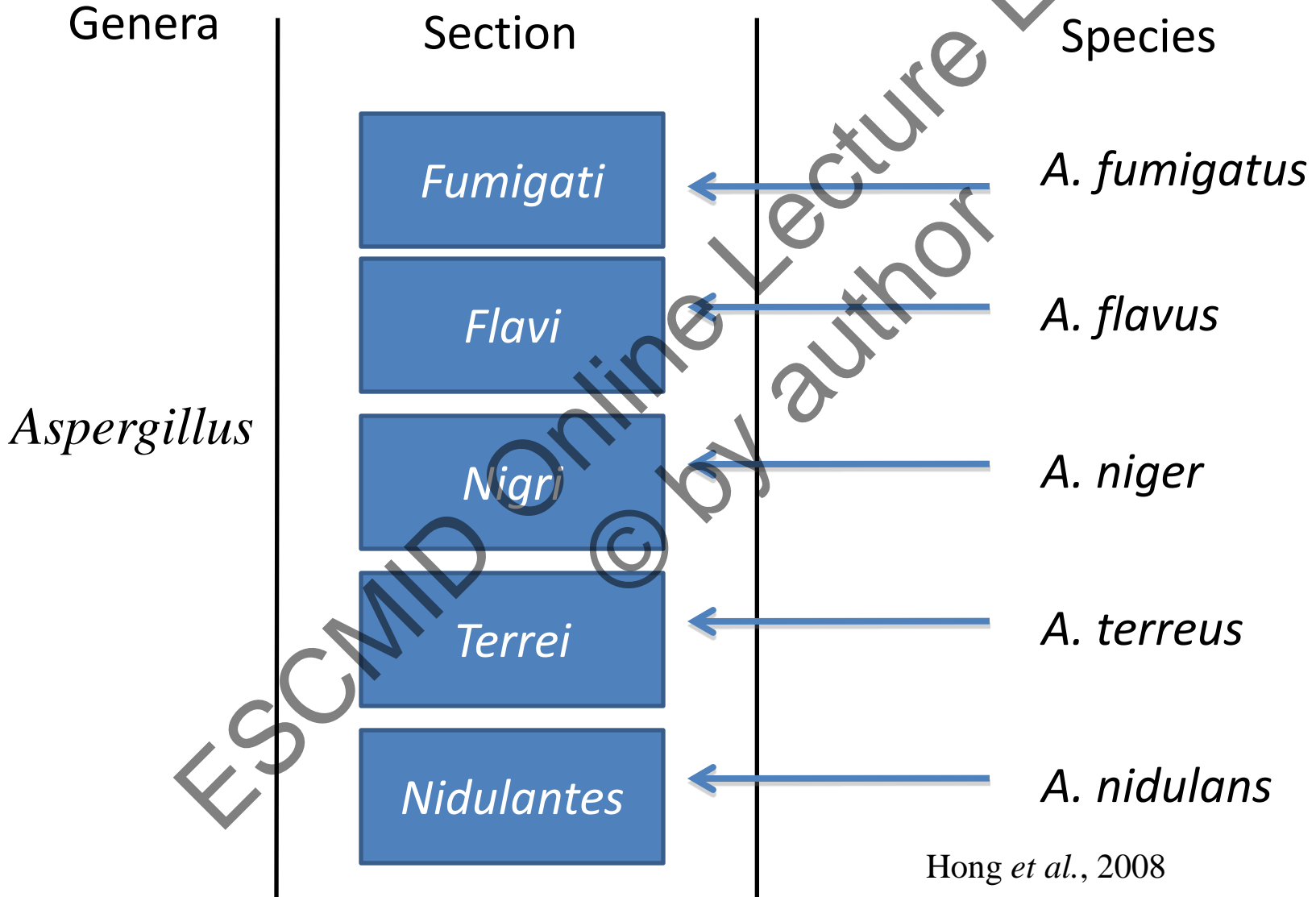
Enzymatic digestion : 2 h



Agarose gel : ½ h

**=> Tedious protocol**

The particular situation for filamentous fungi :  
The examples of *Aspergillus* and *Scedosporium*



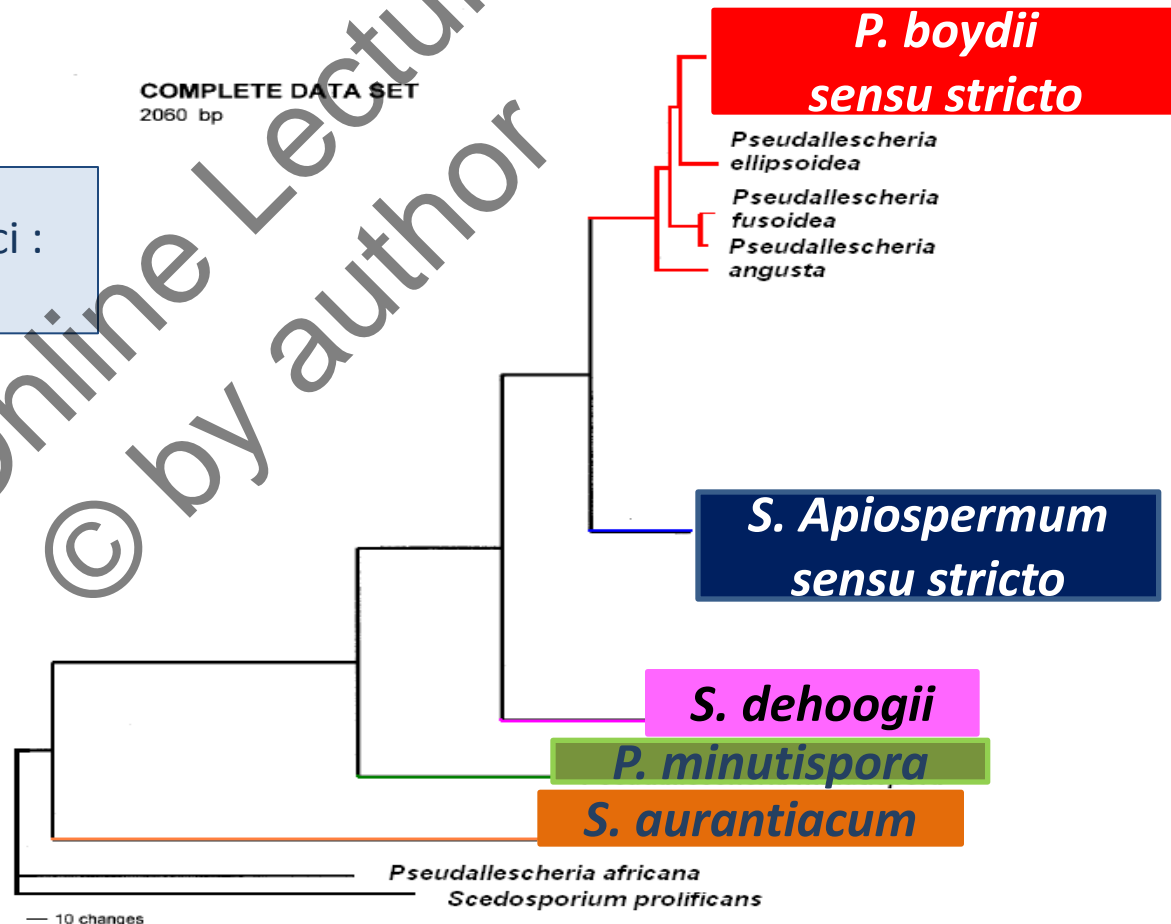




# Molecular identification of *Scedosporium*

Based on the sequencing of 3 loci :  
ITS,  $\beta$ -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 of *Aspergillus* and *Scedosporium* by MT-MS

1st quick automated and standardized technique for *Aspergillus* and *Scedosporium* identification :

- precise identification of species
  - including frequent and rare species
  - discrimination of species with similar morphology
- simple and quick experimental protocol : few min versus few days
- databases easily and regularly updated

## ***Aspergillus* = 28 species**

- **Common *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. viridinitans*, *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*

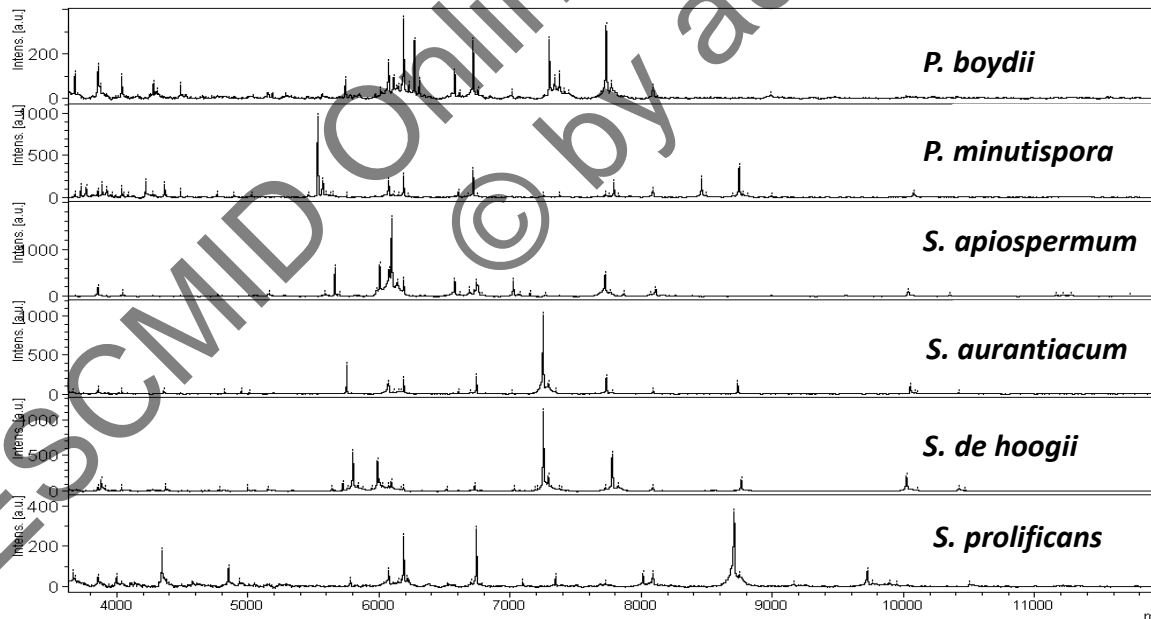
## ***Scedosporium* = 6 species**

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

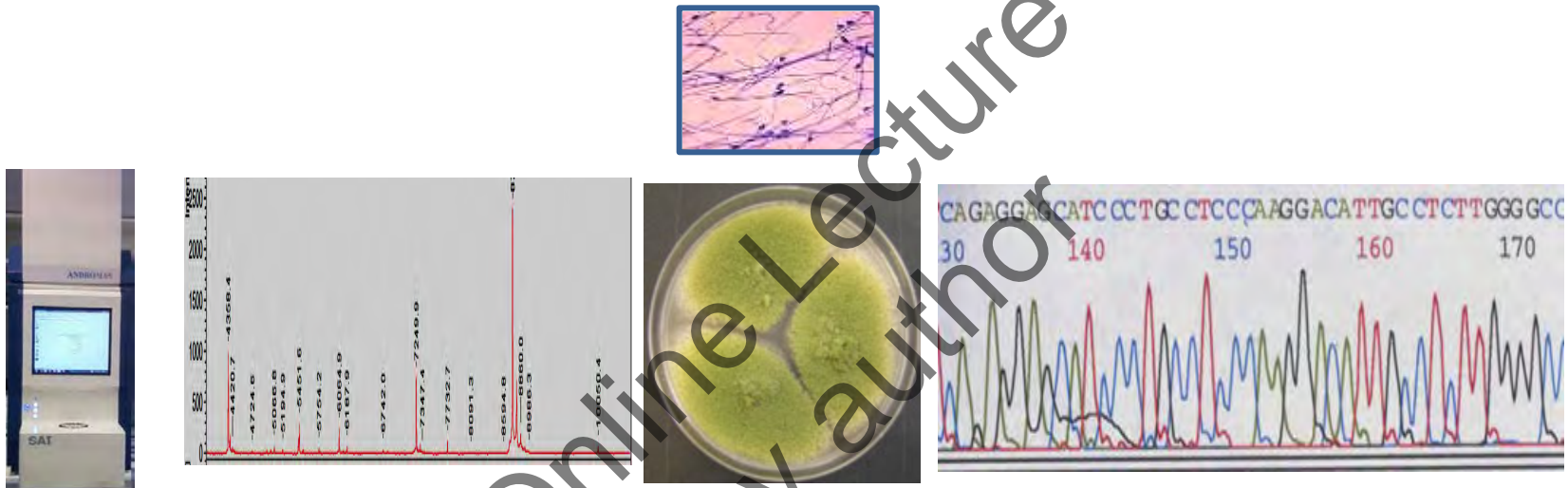
# Identification of *Aspergillus* and *Scedosporium* by MT-MS

## Dramatic improvement of the patient management

- differentiation of environmental and pathogenic species for humans
- Epidemiological studies on the impact of biodiversity on clinical presentations
- appropriate antifungal therapy



# Concordance between MT-MS and molecular identification for *Aspergillus* and *Scedosporium*



**134/136 (98.4%) for *Aspergillus* sp.**  
**61/61 for *Scedosporium* sp.**

Alanio A et coll. CMI 2010

Bougnoux ME et coll. 2<sup>nd</sup> meeting ECMM/ISHAM Fri-CF, 2011



Rapid results = cost savings

High cost of the management of IFI (Hematology settings-France)

-Invasive candidiasis : 35,000

-Invasive aspergillosis : 50,000 €

Rapid adaptation of the antifungal therapy to the species

= Prescription of an efficient drug in case of fluconazole-resistant yeast or voriconazole-resistant *Aspergillus*

⇒ decreased mortality

⇒ cost-effectiveness strategies : Ex. de-escalation in case of sensitivity

-Fluconazole ≈ 10 €

-Echinocandins ≈ 400 €

Michallet, Gangneux, Lafuma et al, J Med Econ 2011



# => Biodiversity impacts on antifungal sensitivity

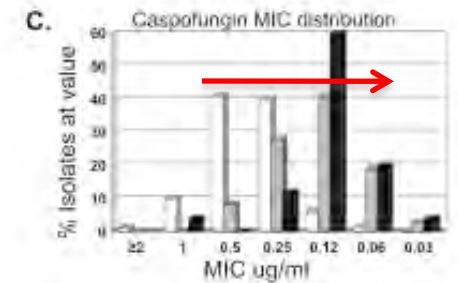
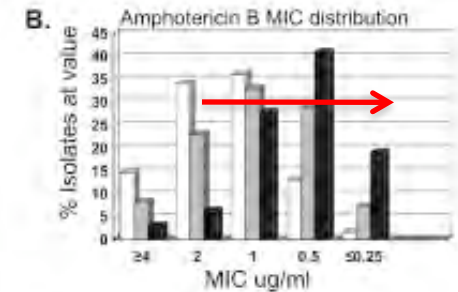
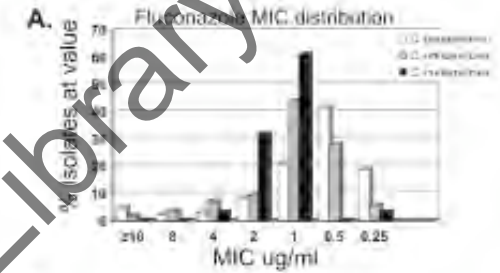
JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2008, p. 2659-2664  
 0095-1137/08/\$08.00+0 doi:10.1128/JCM.00803-08  
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Vol. 46, No. 8

## Geographic Distribution and Antifungal Susceptibility of the Newly Described Species *Candida orthopsilosis* and *Candida metapsilosis* in Comparison to the Closely Related Species *Candida parapsilosis*<sup>∇</sup>

Shawn R. Lockhart,<sup>1\*</sup> Shawn A. Messer,<sup>1</sup> Michael A. Pfaller,<sup>1</sup> and Daniel J. Diekema<sup>1,2</sup>

Departments of Pathology<sup>1</sup> and Internal Medicine,<sup>2</sup> University of Iowa Carver College of Medicine, University of Iowa Hospitals and Clinics, Iowa City, Iowa



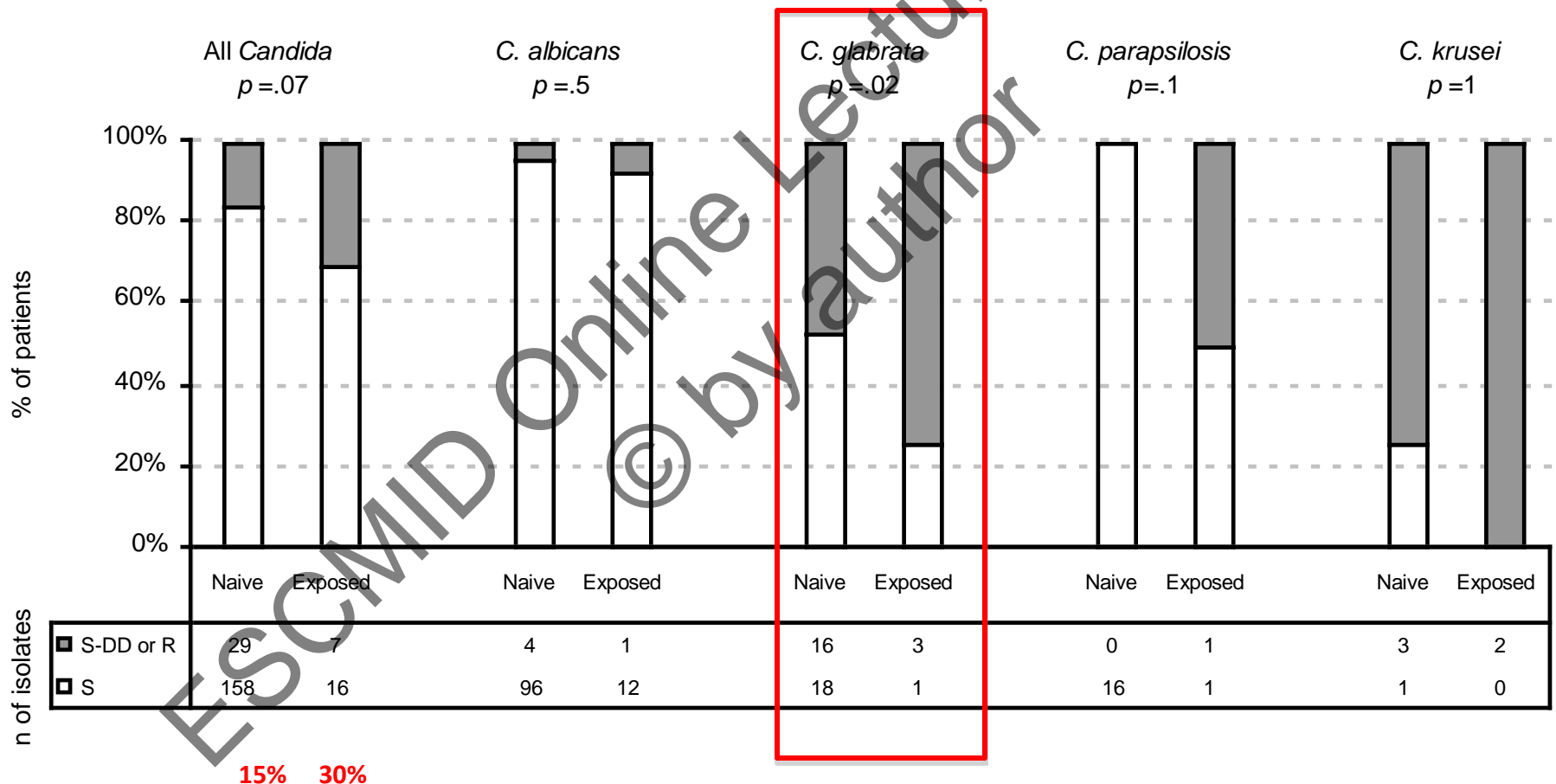
|                           | CMI 90 (microg/mL) |                |              |                |             |
|---------------------------|--------------------|----------------|--------------|----------------|-------------|
|                           | Fluconazole        | Amphotericin B | Caspofungine | 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       |

## => Biodiversity impacts on antifungal sensitivity

Amarcand study : French prospective multicentric study,

300 episodes of invasive candidiasis in intensive care units

=> Low sensitivity of *Candida glabrata* isolates to fluconazole, particularly in case of pre-exposure to fluconazole



## Perspectives : biomarkers of antifungal resistance

= Prescription of an efficient drug in case of fluconazole-resistant yeast or voriconazole-resistant *Aspergillus*

Proteomics 2009, 9, 4627-4631

DOI 10.1092/proteic.200900152

4627

RAPID COMMUNICATION

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

Carine Marinach<sup>1,2\*</sup>, Alexandre Alanio<sup>1,2,3\*</sup>, Martine Palous<sup>3</sup>, Stéphanie Kwasek<sup>1,2</sup>, Arnaud Fekkar<sup>1,2,3</sup>, Jean-Yves Brossas<sup>1,2,4</sup>, Sophie Brun<sup>1,2,3</sup>, Georges Snounou<sup>1,2,5</sup>, Christophe Hennequin<sup>1,2</sup>, Dominique Sanglard<sup>6</sup>, Annick Datry<sup>3</sup>, Jean-Louis Golmard<sup>7\*</sup> and Dominique Mazier<sup>1,2,3\*</sup>

<sup>1</sup>INSERM, UMRS 945 Paris, France

<sup>2</sup>Université Pierre et Marie Curie-Paris6, Paris, France

<sup>3</sup>AP-HP, Groupe hospitalier Pitié-Salpêtrière, Service Parasitologie-Mycologie, Paris, France

<sup>4</sup>Centre d'investigation Biomédical, Groupe hospitalier Pitié-Salpêtrière, Paris, France

<sup>5</sup>Department of Microbiology, National University of Singapore, Singapore

<sup>6</sup>Institute of Microbiology, University of Lausanne and University Hospital Center, Lausanne, Switzerland

<sup>7</sup>EA 3974, Modélisation en Recherche Clinique, Groupe hospitalier Pitié-Salpêtrière, Paris, France

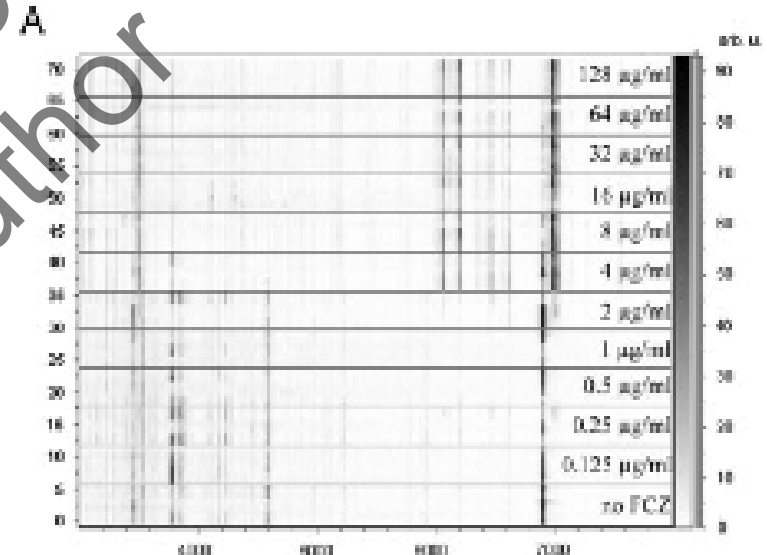


Figure 1. (A) Alterations in the mass spectra of the D5Y226C *C. albicans* strain exposed to increasing FCZ concentrations (virtual gel). The x-axis represents *m/z* value, on the left the y-axis shows running spectrum number, whereas on the right peak intensity is expressed in a gray color scale with arbitrary units (au). (B) A portion of the average mass spectra (range *m/z*

## Complementarity of MT-MS and molecular tools : For the management and the Treatment of iFI

- PCR on samples is standardized and helpful, except in blood
- Maldi-Tof MS is powerful for identification : faster than molecular identification and concordant with molecular databases
- DNA sequencing remains the gold standard for taxonomy and epidemiological studies thanks to its high discrimination power

### -Maldi-Tof MS : a potential to extend applications ?

- identification of antifungal resistance,
- identification of virulence markers,
- discrimination between isolates/amplicons
- SNP genotyping
- etc...

| Cost savings |           |
|--------------|-----------|
| Lab tech     | Treatment |
|              | X         |
| X            | X         |
|              | X         |

**Thank you for your  
attention**

[Jean-pierre.gangneux@chu-rennes.fr](mailto:Jean-pierre.gangneux@chu-rennes.fr)  
[mbougnoux6@gmail.com](mailto:mbougnoux6@gmail.com)

Necker Enfants Malades Hospital, Assistance Publique-Hôpitaux de Paris



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