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Molecular identification of yeasts causing fungaemia: are cryptic species frequent?

P. Escribano [1,2,3], S. Recio [1,2], T. Peláez [1,2,3,4], C. Sánchez-Carrillo [1,2,3,4], M. Rodríguez-Créixems [1,2,3], P. Muñoz [1,2,3,4], E. Bouza [1,2,3,4], *J. Guinea [1,2,3,4]

[1] Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain. [2] Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain. [3] CIBER Enfermedades Respiratorias-CIBERES (CD06/06/0058), Palma de Mallorca, Spain. [4] Medicine Department, Faculty of Medicine, Universidad Complutense de Madrid, Madrid, Spain

Doctor Esquerdo, 46
28007 Madrid, Spain
Phone: +34-915867163
Fax: +34-915044906
E-mail: jguineaortega@yahoo.es

ABSTRACT

Objectives: The study of the epidemiology of fungaemia is necessary to optimize empirical and proven treatment. However, morphological and biochemical procedures are unable to detect closely related species (cryptic species) in complexes of *Candida parapsilosis*, *Candida guilliermondii*, and *Candida glabrata*. We used molecular techniques to confirm identification of species causing fungaemia in patients admitted to a tertiary hospital.

Methods: We studied 445 fungal isolates from the blood cultures of 401 patients with fungaemia admitted to our hospital between January 2007 and August 2011. Each isolate represented 1 fungaemia episode. Multiple episodes were defined as isolation of the same fungal species in additional blood cultures taken ≥7 days after the last isolation in blood culture. Isolates were identified after amplification and sequencing of the ITS1-5.8S-ITS2 region. A phylogenetic tree based on the sequenced ITS1-5.8S-ITS2 region was constructed to detect the presence of cryptic species within the *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata* complexes.

Results: Only 9 (2%) fungaemia episodes were caused by a mixture of 2 different yeast species. The distribution of species involved in the fungaemia episodes was as follows: *Candida albicans* (n=217, 48.7%), *C. parapsilosis* (n=129, 29%), *C. glabrata* (n=45, 10.1%), *Candida tropicalis* (n=28, 6.3%), *C. guilliermondii* (n=6, 1.3%), *Candida krusei* (n=6, 1.3%), *Candida dubliniensis* (n=4, 0.9%), *Candida kefyr* (n=2, 0.5%), *Candida lusitaniae* (n=2, 0.5%), *Candida pelliculosa* (n=1, 0.25%), and other non-*Candida* yeasts (n=14, 3.1%). Only 3 isolates from the *C. parapsilosis* complex were cryptic species. One adult patient who underwent cardiac surgery developed candidemia by *Candida metapsilosis*. Two adult patients with digestive cancer developed candidemia by *Candida orthopsilosis*. Only 1 of the isolates identified as *C. guilliermondii* was confirmed as *Pichia caribbica*. (The isolate was from a patient with digestive cancer.) No cryptic species were found in the isolates identified as *C. glabrata.*

Conclusion: In our hospital, most episodes of fungaemia were caused by *C. albicans*, followed by *C. parapsilosis*. Cryptic species were uncommon within the *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata* complexes and represented only 2% of isolates.

INTRODUCTION

Several species of yeasts causing fungaemia show significant differences in antifungal susceptibility. Therefore, the isolates causing fungaemia must be indentified accurately to optimize antifungal treatment.

In most clinical microbiology departments, yeasts are generally identified by studying the biochemical and morphological characteristics of the isolates.

However, these procedures are unable to detect closely related species (cryptic species) in complexes of *Candida parapsilosis*, *Candida guilliermondii*, and *Candida glabrata*.

Without molecular identification of the isolates, information on the prevalence and clinical relevance of cryptic *Candida* species is scarce.

PURPOSE

We used molecular techniques to identify yeast isolates causing fungaemia in patients admitted to a large tertiary hospital, with emphasis on the presence of cryptic species of *Candida*.

METHODS

We studied 445 fungal isolates from the blood cultures of 401 patients with fungaemia admitted to our hospital between January 2007 and August 2011.

Each isolate represented 1 fungaemia episode. Multiple episodes were defined as isolation of the same fungal species in additional blood cultures taken ≥7 days after the last isolation in blood culture.

Blood samples were cultured in the BACTEC 9240 system with continuous shaking.

Strains were primarily identified using API 32C (bioMérieux).

Identification was further refined using amplification and sequencing of the ITS1-5.8S-ITS2 region and a BLAST search. Identification was confirmed when the homology between the isolate and the reference sequence was ≥99%.

A phylogenetic tree based on the sequenced ITS1-5.8S-ITS2 region was constructed to detect the presence of cryptic species within the *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata* complexes. Reference sequences of each complex retrieved from GenBank were included. *Aspergillus fumigatus* was also included as an outgroup.

RESULTS

The morphological species identification of the isolates causing fungaemia is shown in Figure 1.

Most of the episodes (98%) were monofungal, and only 9 (2%) episodes were caused by a mixture of 2 different yeast species. *C. albicans* was involved in 88.8% of the polyfungal episodes.

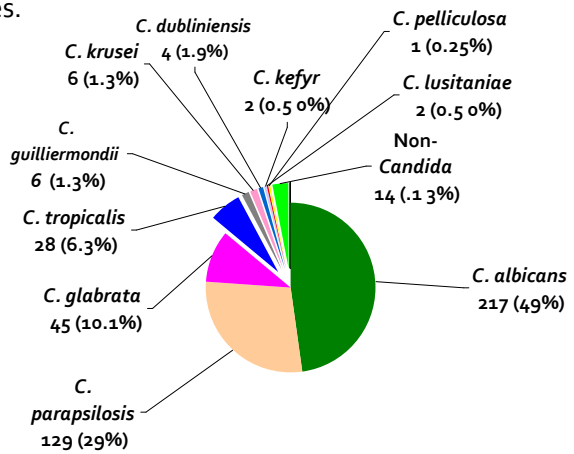

Figure 1. Distribution of the 445 isolates from the blood cultures of 401 patients with fungaemia

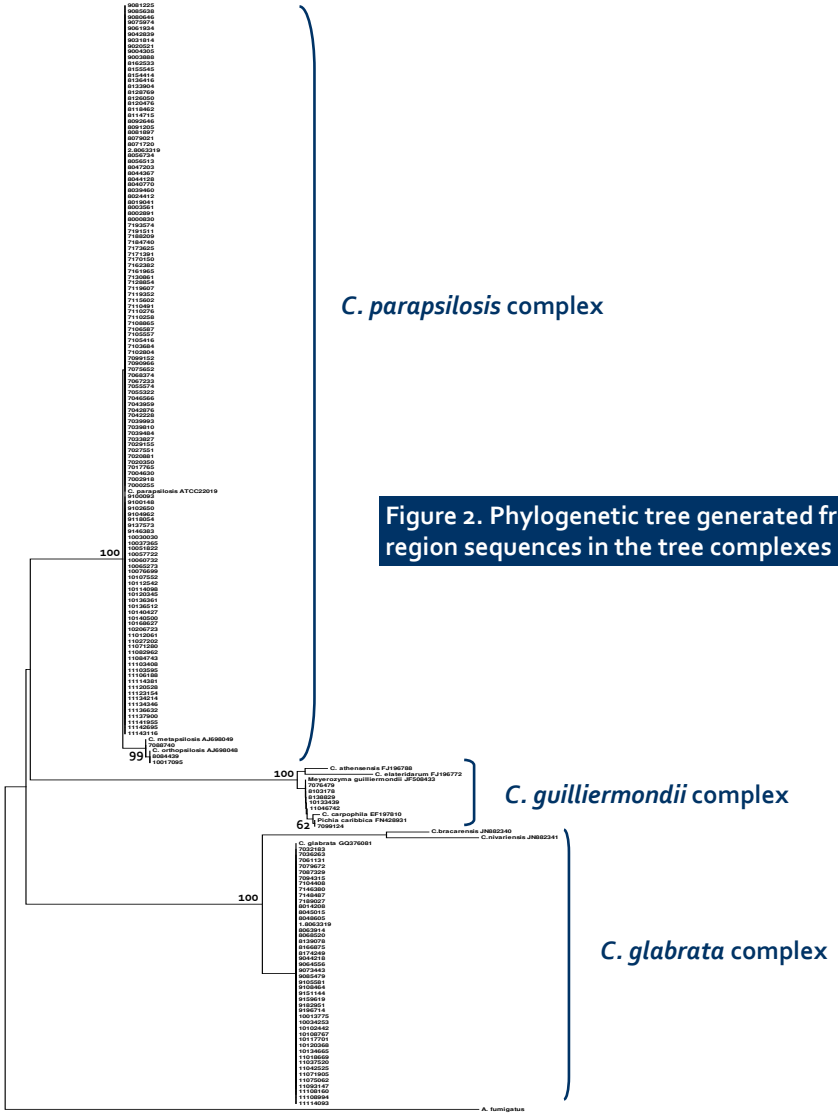

Figure 2. Phylogenetic tree generated from ITS region sequences in the tree complexes

Molecular identification revealed that only 3/129 isolates of the *C. parapsilosis* complex were cryptic species (Figure 2). One adult patient who underwent cardiac surgery developed candidemia by *Candida metapsilosis* (no. 7088740). Two adult patients with digestive cancer developed candidemia by *Candida orthopsilosis* (no. 8084439 and no. 10017095).

Only 1 of the 6 isolates (no. 7099124) previously identified as *C. guilliermondii* was confirmed as *Pichia caribbica* (Figure 2). The isolate was from a patient with digestive cancer.

No cryptic species were found in the isolates identified as *C. glabrata* (Figure 2).

CONCLUSIONS

- **In our hospital, cryptic species within the *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata* complexes were uncommon and accounted for only 2% of isolates.**
- **Concordance between molecular and conventional identification was very high.**
- **The clinical relevance of cryptic species is unknown.**

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