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Short communication

Looking for *Candida nivariensis* and *C. bracarensis* among a large Italian collection of *C. glabrata* isolates: results of the FIMUA working group

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Summary

Two recently described pathogenic *Candida* species, *C. nivariensis* and *C. bracarensis*, share many phenotypic characteristics with *C. glabrata* and are easily misidentified as such. The aim of this study was to determine the occurrence of these cryptic species in Italy. One thousand yeast isolates collected in 14 Italian regions and identified as *C. glabrata* by phenotypic and biochemical methods were included in this study: 928 were screened on CHROMagar and 72 were analysed by a multiplex PCR. None of these cryptic species was identified despite the nationwide distribution and the variety of biological origin of the isolates.

Key words: *Candida nivariensis*, *Candida bracarensis*, *Candida glabrata*.

Introduction

Although *Candida albicans* remains the predominant agent of superficial as well as deep-seated candidosis, non-*albicans Candida* species have emerged in the recent years as significant opportunistic pathogens, and especially *C. glabrata* characterised by a rapidly acquired resistance to fluconazole.[1]

Two new species, *C. nivariensis* and *C. bracarensis*, were recently identified molecularly within the *C. glabrata* clade.[2,3] These species are difficult to separate by the use of conventional identification methods due to the several overlapping traits. All the three species assimilate a narrow range of carbon compounds including trehalose.[2,3] However, they yield white colonies on CHROMagar in contrast with the pink colonies usually exhibited by *C. glabrata.*[2,4]

Different molecular approaches for the detection of these closely related species were applied: sequencing the ITS region and the D1–D2 region of the 26S rRNA gene,[2,3,5] fingerprinting profiles using GTG5 and M13 primers,[6] species-specific peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH),[7] and pyrosequencing of the ITS2 region.[8] Recently was developed a multiplex PCR protocol for the rapid identification of *C. glabrata* and its phylogenetically related species *C. nivariensis* and *C. bracarensis*.[9]

Following the description of these new potentially pathogenic *Candida* species,[2,3] cases of infection have been anecdotally reported[6,10,11] and their occurrence in collections of clinical isolates investigated. A total of 16 isolates of *C. nivariensis* were received at the United Kingdom Mycology Reference Laboratory over a 12-month period.[8] Three of 137, initially identified as *C. glabrata* isolates, were positive with the *C. bracarensis* probe and none with the *C. nivariensis* probe at the Johns Hopkins Hospital of Baltimora, USA.[7] Three of 143 *C. glabrata* clinical strains sent to the Spanish Reference Laboratory in 2008–2009 were identified as

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C. bracarensis by DNA sequencing while none as *C. nivariensis.* In addition, none of the 31 isolates from a Spanish population-based surveillance study of candidemia were found to belong to these cryptic species.[12] The largest study analyses isolates collected as a part of the ARTEMIS antifungal surveillance program in 28 countries on six continents: PNA FISH identified only two *C. bracarensis* and one *C. nivariensis* among the 1598 isolates phenotypically identified as *C. glabrata*.[13] The aim of the present study was to determine the occurrence of these two cryptic species in Italy.

Materials and methods

A total of 1000 yeast isolates, collected between January 2009 and November 2011 from 18 medical centres in 14 Italian regions, were included in this study. These isolates, from biological samples cultured on Sabouraud dextrose agar added of chloramphenicol in the routine activity of laboratories of clinical microbiology, had been identified as *C. glabrata* by API ID32C (bioMerieux, Firenze, Italy).

A total of 928 isolates were screened on CHROMagar™ *Candida* medium (PBI International, Milan, Italy) and colony colour scored as either pink or white. The remaining 72 isolates were analysed by a multiplex PCR using four primers targeting the ITS1 region and the 5.8S ribosomal RNA gene, as previously reported.[8] The combination of these primers allows discrimination among *C. glabrata*, *C. nivariensis* and *C. bracarensis.*[9] *Candida nivariensis* (CN 5907–63) and *C. bracarensis* (NCYC3133) were used as control isolates in both the screenings.

Results and discussion

The characteristics of the analysed isolates are reported in Table 1. A total of 645 isolates were isolated in centres of Northern Italy [total population 27 568 435; centres of Bolzano, Mestre, Milano (3), Modena, Torino, Trieste, Verona], 146 and 209 in centres of Central [population 11 890 464; centres of Ancona, Firenze, Perugia, Pisa, Roma] and Southern Italy [population 20 881 429; centres of Bari, Cagliari, Catania, Messina] respectively.

Table 1 Characteristics of the 1000 analysed isolates.

Body origin of isolates	
Blood	177
Other sterile sites	57
Vaginal exudate	229
Other	531
Unknown	6
Demographic characteristics	
Gender	
Female/male	1.7/1
Age	
Mean	63.5 year
Range	2 days–100 years

All the tested isolates screened on CHROMagar yielded pink colonies, while the *C. nivariensis* and *C. bracarensis* used as control isolates yielded white colonies. All the 72 isolates submitted to multiplex PCR using the four primers targeting the ITS1 region and the 5.8S ribosomal RNA gene were identified as *Candida glabrata* as a 397 bp fragment was amplified. Amplification products of 293 and 223 bp were obtained in the *C. nivariensis* and *C. bracarensis* control isolates respectively.

In conclusion, among the 1000 isolates examined, none was identified as *C. nivariensis* or *C. bracarensis*, despite the nationwide distribution and the variety of biological origin of the isolates. These results are consistent with the results of a recent analysis of a global collection of 1598 isolates reporting a prevalence of 0.2%.[13] However, because of the documented increase in these cryptic species in some European countries and their propensity to exhibit antifungal resistance, it would be prudent to continue monitoring these emerging pathogens.

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Conflict of interest

No conflict of interest.

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