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**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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# UNIVERSITÀ DEGLI STUDI DI TORINO

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1    **RESEARCH NOTE**

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

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13    **Running title:** *Candida nivariensis* and *C. bracarensis* in an Italian collection

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**Abstract**

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.

**Keywords:** *Candida nivariensis*, *Candida bracarensis*, *Candida glabrata*

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

41 Two new species, *C. nivariensis* and *C. bracarensis*, were recently identified molecularly  
42 within the *C. glabrata* clade [2,3]. These species are difficult to separate by the use of the  
43 conventional phenotypic identification methods due to the several overlapping traits.  
44 However they yield white colonies on CHROMagar in contrast to the pink colonies usually  
45 exhibited by *C. glabrata*.

46 Different molecular approaches for the detection of these closely related species were  
47 applied: sequencing the ITS region and the D1-D2 region of the 26S rRNA gene [2,4],  
48 fingerprinting profiles using GTG5 and M13 primers [5], species-specific peptide nucleic  
49 acid fluorescence in situ hybridization (PNA FISH) [6], pyrosequencing of the ITS2 region  
50 [7]. Recently was developed a multiplex PCR protocol for the rapid identification of *C.*  
51 *glabrata* and its phylogenetically related species *C. nivariensis* and *C. bracarensis* [8].

52 Following the description of these new potentially pathogenic *Candida* species [2,9], cases  
53 of infection have been anecdotally reported [5,10,11] and their occurrence in collections of  
54 clinical isolates investigated. A total of 16 isolates of *C. nivariensis* were received at the  
55 United Kingdom Mycology Reference Laboratory over a 12-month period [7] Three of 137  
56 initially identified as *C. glabrata* isolates were positive with the *C. bracarensis* probe and  
57 none with the *C. nivariensis* probe at the Johns Hopkins Hospital of Baltimore, USA [6].  
58 Three of 143 *C. glabrata* clinical strains sent to the Spanish Reference Laboratory in 2008-  
59 2009 were identified as *C. bracarensis* by DNA sequencing while none as *C. nivariensis*.

60 In addition none of the 31 isolates from a Spanish population-based surveillance study of  
61 candidaemia were found to belong to these cryptic species [12]. The largest study  
62 analyses isolates collected as a part of the ARTEMIS antifungal surveillance program in 28

63 countries on six continents: PNA-FISH identified only two *C. bracarensis* and one *C.*  
64 *nivariensis* among the 1598 isolates phenotypically identified as *C. glabrata* [13].

65 The aim of the present study was to determine the occurrence of these two cryptic species  
66 in Italy.

67 A total of 1000 yeast isolates, collected between January 2009 and November 2011 from  
68 18 medical centres in 14 Italian regions, were included in this study. These isolates had  
69 been identified as *C. glabrata* by phenotypic and biochemical methods.

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

77 The characteristics of the analysed isolates are reported in Table 1. A total of 645 isolates  
78 were isolated in centres of Northern Italy (total population 27 568 435), 146 and 209 in  
79 centres of Central (population 11 890 464) and Southern Italy (population 20 881 429),  
80 respectively.

81 All the tested isolates screened on CHROMagar yielded pink colonies and all the 72  
82 isolates submitted to multiplex PCR using the four primers targeting the ITS1 region and  
83 the 5,8 S ribosomal RNA gene were identified as *Candida glabrata*.

84 In conclusion, among the 1000 isolates examined none was identified as *C. nivariensis* or  
85 *C. bracarensis*, despite the nationwide distribution and the variety of biological origin of the  
86 isolates. These results are consistent with the results of a recent analysis of a global  
87 collection of 1598 isolates reporting a prevalence of 0.2% [13]. However, because of the  
88 documented increase of these cryptic species in some European countries and their

89 propensity to exhibit antifungal resistance, it would be prudent to continue monitoring  
90 these emerging pathogens.

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151 **TABLE 1. Characteristics of the 1000 analysed isolates.**  
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154	Body origin of isolates	
155	blood	177
156	other sterile sites	57
157	vaginal exudate	229
158	other	531
159	unknown	6
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161	Demographic characteristics	
162	Sex	
163	female/male	1.7/1
164	Age	
165	mean	63.5 year
166	range	2 days-100 years

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