Prevalence of Candida bracarensis and Candida nivariensis in a Spanish collection of yeasts: comparison of results from a reference centre and from a population-based surveillance study of candidemia


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Two new species related to Candida glabrata, i.e., Candida nivariensis and Candida bracarensis, have been proposed. The occurrence of these species among isolates collected in a Spanish mycology reference laboratory in 2008–2009 was reviewed. In addition, strains recovered as part of an active population-based surveillance of candidemia conducted in Barcelona between 2002 and 2003 were also analyzed. Among 143 clinical isolates received in 2008–2009, three (2%) were identified as C. bracarensis and none as C. nivariensis through sequencing of their ribosomal DNA. Of the 31 strains initially identified as C. glabrata in the 2002–2003 population-based study (0.38 cases/100,000 population), none were found to belong to these related new species. Results from in vitro susceptibility studies of C. bracarensis isolates were comparable to those found with C. glabrata. Since new and cryptic species have been described, periodic surveillance including the use of molecular identification methods seems to be necessary in order to determine their frequency, geographical distribution and susceptibility profile.

Keywords emerging species, antifungal susceptibility, Candida spp.

Introduction

Two new species related to Candida glabrata, Candida nivariensis and Candida bracarensis have been recently proposed [1,2]. As stated in several reports, conventional methods of classification based on morphological, biochemical and physiological features have proven ineffective in accurately identifying such species [3,4]. Although the three species are related phylogenetically, PCR-based procedures and DNA sequencing have shown that C. glabrata, C. nivariensis and C. bracarensis are sufficiently genotypically different to justify their assignment as separate species. In addition, some authors have pointed out that the two new species could be more resistant than C. glabrata to antifungal agents [5–7].

C. nivariensis was described in 2005 from clinical samples (bronchoalveolar lavage, blood culture and urine) collected from three Spanish patients in a single institution in the Canary Islands [1]. Since that time, this organism has seldom been described in clinical reports. Lockhart et al. discussed one clinical isolate of C. nivariensis among a collection of 1,598 isolates of C. glabrata included in the global ARTEMIS antifungal surveillance programme conducted between 2001 and 2006. That isolate was obtained from the pleural fluid of a patient in Australia [8]. This species was also identified in clinical samples (blood culture and sputum) from two different patients which had been maintained in a collection of 363 (0.5%) yeast isolates in India [9]. It has also been reported in clinical cases such as a catheter-related fungemia in Japan and an oropharyngeal infection in an Indonesian HIV patient [10,11]. In addition, the United Kingdom

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Mycology Reference Laboratory identified a total of 16 clinical isolates of C. nivariensis between 2005 and 2006. They were initially isolated in 12 different hospitals from various clinical specimens, including deep, usually sterile sites [6]. Lastly, Bishop et al. failed to find any isolates of C. nivariensis in a panel of 137 clinical strains molecularly analyzed at a single institution in Baltimore [5].

C. bracarensis was proposed as a new species in 2006. Isolates were recovered from a vaginal exudate of a Portuguese patient and from blood cultures of a patient admitted to a UK hospital [2]. The ARTEMIS programme has reported two C. bracarensis isolates among 1,598 clinical strains of C. glabrata. These strains originated in the United States in a single institution, where one was isolated from sputum in 2002 and the other from a bloodstream infection in 2004 [8]. Bishop et al. found three C. bracarensis strains in a collection of 137 C. glabrata (2.2%) isolates. One of these was recovered from a pelvic abscess in a patient with perforated diverticulitis and the other two were found to be colonizing two adult oncology patients [5].

We describe the occurrence of these new species among C. glabrata isolates in a Spanish Reference Laboratory between 2008 and 2009 and in those collected in an active population-based surveillance of candidemia that was carried out in Barcelona over a period of two years (2002–2003).

Material and methods

Organisms

A total of 143 clinical isolates initially identified as C. glabrata were sent to the Mycology Laboratory of the Spanish National Centre for Microbiology for confirmation and susceptibility testing. The strains were collected from 56 Spanish hospitals over a period of two years, from 2008–2009, with each strain representing a unique isolate from a patient. The isolates were recovered from blood (61; 43.6%), biopsies and other deep sites, (46; 32.3%), and other locations (34; 23.7%).

In addition, a total of 31 strains identified as C. glabrata in a prospective population-based surveillance study of candidemia conducted in Barcelona (Spain) between 2002 and 2003 were also included in this study [12].

Candida albicans (CBS 562), C. glabrata (CBS 138), C. bracarensis (CBS10154), and C. nivariensis (CBS9984) obtained from the Centraalbureau voor Schimmelculures (CBS; Utrecht, The Netherlands) were included for molecular comparison as type and reference strains. Sequences of C. bracarensis (GeneBank accession number AY589573.2) and C. nivariensis (GeneBank accession number GU199443.1) were employed as references in the analysis.

Species identification

Isolate identification was confirmed at the Spanish Reference Mycology Laboratory by morphological, physiological and molecular methods according to routine procedures. Briefly, for molecular identification purposes, genomic DNA was directly prepared from a single yeast colony. DNA segments comprising the D1/D2 domains of the 26S ribosomal DNA and ITS1/ITS2 regions were amplified and sequenced using universal primers. Further analysis was performed by comparison with ITS sequences of type and reference isolates and with those included in the database of the Mycology Department of the Spanish National Centre for Microbiology, a restricted data base including more than 6,000 sequenced microorganisms. Analyses were conducted with InfoQuest FP 4.50 software (BIO-RAD Laboratories, Madrid, Spain), using the neighbour-joining method based on the Kimura two-parameter model. C. albicans (CBS562T) was used as an out-group to root the phylogenograms [4,7,13].

In-vitro susceptibility testing

Susceptibility studies were performed according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) reference method [14]. The antifungal agents used were amphotericin B (Sigma Aldrich Quimica S.A., Madrid, Spain), flucytosine (Sigma-Aldrich), fluconazole (Pfizer S.A., Madrid, Spain), itraconazole (Janssen S.A., Madrid, Spain), posaconazole (Schering-Plough, Kenilworth, NJ, USA), and voriconazole (Pfizer S.A.), anidulafungin (Pfizer S.A.), micafungin (Astellas Pharma Inc, Tokyo, Japan), and caspofungin (Merck & Co., Inc., Rahway, NJ, USA).

Results

A total of 3 (2%) isolates out of the 143 C. glabrata clinical strains sent to the Reference Laboratory in 2008 and 2009 were identified as C. bracarensis by DNA sequencing. One strain was isolated in 2008 from a cather exudate of a critically ill patient (CNM-CL-7030, Yeast Collection of Spanish National Centre for Microbiology) [4,15]. The other two were recovered in 2009, one from a sample of pleural liquid of a patient who underwent thoracic surgery due to an oesophageal fistula (CNM-CL-7326), and the other from blood of a patient who had a haematological disease (CNM-CL-7380). Notably, none of 143 clinical isolates that were initially identified as C. glabrata were found through molecular methods to be C. nivariensis.

Results of the 2002–2003 population-based surveillance included 31 episodes of fungemia were identified as © 2011 ISHAM, Medical Mycology, 49, 525–529
C. glabrata on the basis of conventional identification procedures. This was 9% of the total of 345 episodes of candidemia that were analyzed in the study period which is equal to an average annual incidence of 0.38 cases/100,000 population [2]. Based on molecular criteria, all the isolates were identified as C. glabrata as their ITS sequences completely matched that of the type strain of this species (C. glabrata CBS 138, ATCC 2001). Neither C. nivariensis nor C. bracarensis were detected among these strains. The ITS analysis showed three main clusters, with all the clinical strains studied found to be in the same cluster (>99% identity between them). The other two clusters included the reference strains of C. nivariensis and C. bracarensis which were part of these investigations for comparative purposes. Antifungal susceptibility data were consistent with those of C. glabrata reported in other studies (Table 1) [12,15]. The MIC values found with C. bracarensis isolates were comparable with those of C. glabrata (Table 2), but the limited number precludes any definitive conclusions as to its susceptibility.

**Discussion**

Candidemia remains a major cause of morbidity and mortality in the health care setting. Of particular concern is the reported increase in the proportion of such infections caused by C. glabrata, a species which is associated with reduced susceptibility to azole antifungal agents [15,16]. The occurrence of candidemia due to this species ranges between 10 and 35% according to different publications [17–20]. Recently, data from the prospective antifungal therapy Alliance registry has been published [21] and included a total of 2,019 patients with candidemia from 23 medical centres in North America. While C. albicans was the most commonly identified etiologic agent (45.6%), collectively, non-C. albicans Candida species appeared to be more frequent agents (54.4%), with 520 (26%) cases due to C. glabrata. In Spain, C. glabrata has emerged as an important nosocomial pathogen, being the fourth most common cause of candidemia [12]. It appears that marked differences exist in species distributions and antifungal drug susceptibilities among different countries, underscoring the need for continued surveillance to monitor trends in pathogen distribution and drug susceptibilities.

The two new C. glabrata-related species appear to be rarely recovered from clinical samples. Unlike the United Kingdom, the Spanish Reference Mycology Laboratory has not reported any isolates of C. nivariensis among 1,096 yeast analyzed by molecular methods between 2008 and 2009 [4,15] and in agreement with previous studies, about 2% of the isolates were C. bracarensis [4]. The susceptibility profile of C. bracarensis and C. nivariensis is similar to that of C. glabrata although a reduced susceptibility to azole agents and resistance *in vitro* to fluconazole have been described within the limited number of isolates whose susceptibility profiles have been reported [5,6].

However, results found through passive epidemiological surveillance by reference centres may be biased, as they receive uncommon species and microorganisms that are often difficult to identify. Results from active population-based surveillance studies should be considered as more significant as it identifies all cases of the disease being studied regardless of the health-care setting in which it occurs. It minimizes bias resulting from the selection of only a subset of hospitals, and it enables newly-affected patient groups to be identified. Results from the 2002–2003 population-based surveillance study of candidemia in Spain showed that the two C. glabrata-related new species did not cause fungemia.

In light of their minimal occurrence, the use of molecular methods on a routine basis to distinguish the two new species from C. glabrata is not recommended for use in clinical laboratories. However, susceptibility testing must be performed, since some of the antifungal agents available may prove to be less active against these species. Periodical surveillance including molecular methods of characterization seems to be advisable, since new and cryptic species

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<th>Table 1</th>
<th>In vitro susceptibility results in μg/ml of the 171 clinical strains of Candida glabrata analyzed. MIC&lt;sub&gt;50&lt;/sub&gt; is the MIC value including 50% of isolates and MIC&lt;sub&gt;90&lt;/sub&gt; is the MIC value including 90% of isolates.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antifungal agent</td>
<td>Range</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.03–0.50</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.01–4.0</td>
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<tr>
<td>Fluconazole</td>
<td>0.25–6.40</td>
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<tr>
<td>Itraconazole</td>
<td>0.80–6.0</td>
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<tr>
<td>Posaconazole</td>
<td>0.01–8.0</td>
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<tr>
<td>Anidulafungin</td>
<td>0.03–0.12</td>
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<tr>
<td>Micafungin</td>
<td>0.03–0.06</td>
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<tr>
<td>Caspofungin</td>
<td>0.03–1.0</td>
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have been described, in order to determine their frequency, geographical distribution and susceptibility profiles.

**The Barcelona Candidemia Project study group**

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**References**

Species related to Candida glabrata in Spain


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