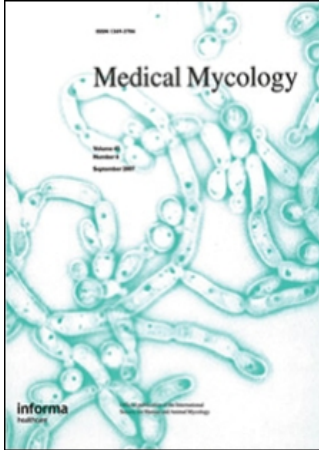


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Cholesterol dependent and Amphotericin B resistant isolates of a **Candida glabrata** strain from an Intensive Care Unit patient

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Case Report

Cholesterol dependent and Amphotericin B resistant isolates of a *Candida glabrata* strain from an Intensive Care Unit patient

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Here we report on two isolates of *Candida glabrata* recovered from urine samples collected from of an Intensive Care Unit patient. D1/D2 and ITS 1+2 rDNA sequence analysis confirmed its identification. The isolates were cholesterol dependent and resistant to Amphotericin B.

Keywords *Candida glabrata*, cholesterol, amphotericin B

Introduction

Here we report on two isolates of *Candida glabrata* recovered from urine samples collected from an intensive care unit (ICU) patient. D1/D2 and ITS 1+2 rDNA sequence analysis confirmed its identification. The isolates were cholesterol dependent and resistant to amphotericin B.

Case report

A 60-year-old female with an APACHE II score and antecedents of arterial hypertension, gallstones, diabetes mellitus II and depression was admitted to the hospital with acute pancreatitis and cholelithiasis. During the evolution of the disease she presented acute necrotizing pancreatitis, gallbladder perforation and choleperitoneum. The patient underwent a cholecystectomy and pancreatic drainage. She was then treated empirically with imipenem, amikacin, teicoplanin, ceftazidime, tobramycin and fluconazole. As a result

of the fungemia caused by *C. glabrata* (see identification procedures below), the patient was treated with 5 mg/kg/day of liposomal amphotericin B (Ambisome). Over a period of five months she was admitted four times to the ICU. Fortunately, the patient survived and was released from hospital.

During this period, four haemocultures and cultures inoculated with one sample from a venous catheter and one from a bronchial aspirate were positive for *C. glabrata*. These isolates were cultured on Sabouraud dextrose agar (SDA) and their identification was determined using CHROMagar medium, microscopic features and with the API 20 AUX system. All these isolates were able to assimilate glucose and trehalose and were discarded.

Ten days after the last *C. glabrata* isolate was recovered, the patient was febrile and with leucocytosis. Cultures were inoculated with blood, catheter, urine and bronchial aspirate specimens. Numerous (> 100,000 colony forming units [CFU]/ml) small, brilliant colonies were recovered on blood agar from urine samples. No growth was found with these samples when grown on SDA and CHROMagar media. However, subcultures on Leeming & Notman agar (LNA) [1] and modified Dixon (mDixon) medium [2] yielded colonies of a *C. glabrata*-like yeast after 24 hours incubating at 35°C. One month later a similar strain

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was isolated from urine and both isolates were analysed simultaneously. The amphotericin B minimum inhibitory concentrations (MIC) for each isolate from the positive urine samples (CG1 and CG2), determined using Mueller Hinton blood agar and based on AB Biodisk (Solna, Sweden) criteria were $>32\mu\text{g/ml}$. The quality control isolates used in the MIC investigations were *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 and were found to have MICs of $0.5\mu\text{g/ml}$ and $0.25\mu\text{g/ml}$, respectively.

Both isolates grew on SDA supplemented with chloramphenicol (Oxoid, Basingstoke, Hampshire, UK) and 2% oxbile (Oxoid) (not shown), bile esculine agar (Oxoid), chocolate agar (Oxoid) and LNA (Fig. 1). A suspension of the yeast cells in sterile saline, corresponding to 0.5 McFarland, was inoculated on three different areas of the surface of a SDA Petri dish containing a small amount of one of the following cholesterol-containing substrates; human serum, bovine fetal serum and sheep blood (Fig. 2). The isolates grew only at the three points of reference.

The D1/D2 domains and the ITS 1+2 regions of the ribosomal RNA (rRNA) genes of strains CG1 and CG2 were sequenced as described elsewhere [3] and compared with those in the GenBank database using a BLAST search [4]. The sequences of both isolates were identical, indicating that they probably belonged to a single phylogenetic strain. The ITS and D1/D2 sequences of both isolates showed a 98.2% and a 99.8% similarity respectively, to those of the type strain of *C. glabrata* CBS 138 (AY046165 and AY048154, respectively). *Candida nivariensis* and *C. bracarensis*, two recently described species [5,6] were also closely related to our strains (Fig. 3A and 3B). The D1/D2 and

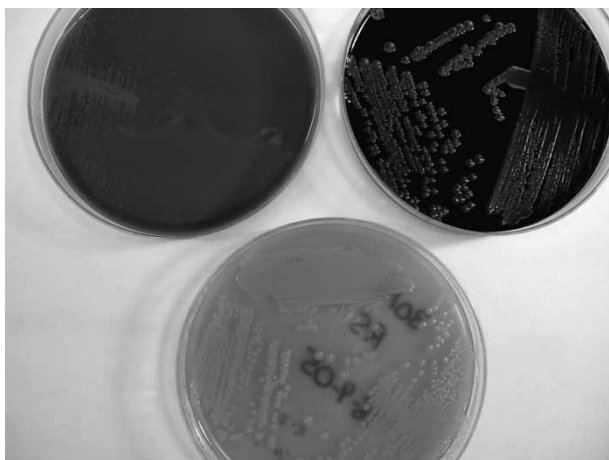


Fig. 1 Growth of a cholesterol-dependent *C. glabrata*-like isolate on chocolate agar (above left), bile-esculine agar (above right), and Leeming and Notman medium (below).



Fig. 2 Growth of a cholesterol-dependent *C. glabrata* isolate on Sabouraud chloramphenicol agar with (above left) sheep blood, (above centre) human serum and (above right) bovine foetal serum.

the ITS sequences of *C. nivariensis* showed 95.4% and 72.5% similarity with the lipid dependent strains, and those of *C. bracarensis* 94.7% and 74.7% similarity, respectively.

Discussion

Candida glabrata is an important agent of urinary tract infections. Although *C. albicans* is the species most frequently recovered from urine samples of ICU patients, over 40% of cases of candiduria are caused by non-*C. albicans* *Candida* species, especially *C. glabrata* (8.2%–21.7%) [7,8]. Previous use of antifungal agents seems to be one risk factor for the selection of non-*C. albicans* candiduria [7], and the presence of *C. glabrata* isolates was found to be related to the use of fluconazole and quinolones [9]. In addition, as in the case report presented here, recent studies focusing on the relevance of *Candida* infection in patients with pancreatitis demonstrated that *C. albicans* and *C. glabrata* were the species most commonly isolated. *Candida* infections are associated with higher mortality and caused more systemic complications in patients with infected pancreatic necrosis [10,11].

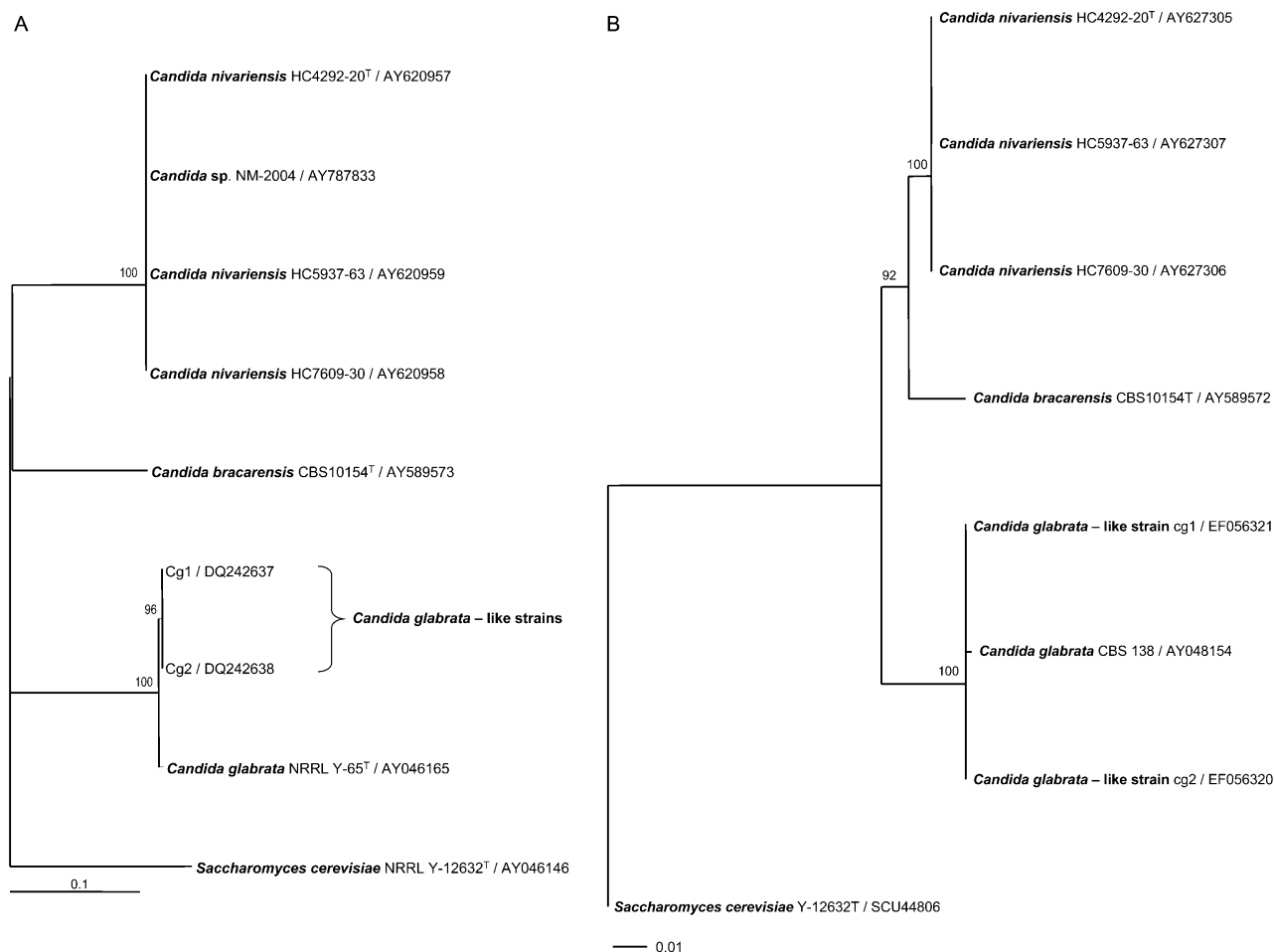


Fig. 3 Phylogenetic position of the *Candida glabrata* isolates (CG1, CG2) described in this study using neighbour joining clustering. Figures on the branches represent bootstrap values (100 bootstrap replicates). (A) The phylogenetic tree using ITS 1+2 sequences. (B) The phylogenetic tree using the D1/D2 domains of the 26S rDNA (LSU).

Although amphotericin B therapy may be effective in clearing *Candida* infection [10], new azoles, echinocandins -or high-dose polyenes- are preferred for the treatment of infections caused by *C. glabrata* [12]. High-dose liposomal amphotericin (Ambisome, 5 mg/kg/day) may be more effective and was administered to our patient during her episodes of fungemia [13].

The *C. glabrata*-like isolates did not grow on routine media but grew well on those media normally used to recover strains of *Malassezia* spp. To our knowledge, this is the second report of the occurrence of nutritionally distinct *C. glabrata*-like strains [14]. Recently, Hazen *et al.* [14] described similar isolates of *C. glabrata* recovered in the UK and the USA that required bile for growth. Bile is a component of media used for recovering lipophilic fungi, such as mDixon and LNA, which are used to grow lipid-dependent *Malassezia* species [2].

In agreement with Hazen *et al.* [14] and Bard *et al.* [15], the addition of Tweens 20, 40, 60 and 80 on Sabouraud agar was not sufficient to allow growth of our yeast isolates (data not shown).

Our patient received liposomal amphotericin B and Propofol, an anaesthetic containing a high lipid concentration. These treatments may have resulted in the selection of a lipophilic *C. glabrata* strain that could have escaped detection if only SDA or CHROMagar were used for the isolation and identification of potential yeast-like pathogens in our study.

Similar to the isolates studied by Hazen *et al.* [14], ours were resistant to amphotericin B. Further studies are needed to fully understand the nature of the cholesterol dependency of these *C. glabrata* strains. The resistance to amphotericin B and their role in human infections, such as urinary tract infections, also requires further attention.

Acknowledgements

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References

- 1 Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987; **25**: 2017–2019.
- 2 Gueho E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek* 1996; **69**: 337–355.
- 3 Gupta AK, Boekhout T, Theelen B, Summerbell R, Batra R. Identification and typing of *Malassezia* species by Amplified Fragment Length Polymorphism (AFLP) and sequence analysis of the internal transcribed spacer (ITS) and large subunit (LSU) regions of ribosomal DNA. *J Clin Microbiol* 2004; **42**: 4253–4260.
- 4 Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 1997; **25**: 3389–3402.
- 5 Alcoba-Flórez J, Méndez-Álvarez S, Cano J, et al. Phenotypic and molecular characterization of *Candida nivariensis* sp. nov., a possible new opportunistic fungus. *J Clin Microbiol* 2005; **43**: 4107–4111.
- 6 Correia A, Sampaio P, James S, Pais C. *Candida bracarenensis* sp. nov., a novel anamorphic yeast species phenotypically similar to *Candida glabrata*. *Int J Syst Evol Microbiol* 2006; **56**: 313–317.
- 7 Alvarez-Lerma F, Nolla-Salas J, Leon C, et al. EPCAN Study Group. Candiduria in critically ill patients admitted to intensive care medical units. *Intens Care Med* 2003; **29**: 1069–1076.
- 8 Morera Y, Torres-Rodriguez JM, Catalan I, Granadero A, Josic Z, Alvarez-Lerma F; Grup de Recerca en Micologia Experimental i Clinica (GREMEC). Candiduria in patients with urethral catheter admitted in Intensive Care Unit. Etiology and *in vitro* susceptibility to fluconazole. *Med Clin (Barc)* 2002; **118**: 580–582.
- 9 Harris AD, Castro J, Sheppard DC, Carmeli Y, Samore MH. Risk factors for nosocomial candiduria due to *Candida glabrata* and *Candida albicans*. *Clin Infect Dis* 1999; **29**: 926–928.
- 10 Aloia T, Solomkin J, Fink AS, et al. *Candida* in pancreatic infection: a clinical experience. *Am Surg* 1994; **60**: 793–796.
- 11 Robbins EG, Stollman NH, Bierman P, Grauer L, Barkin JS. Pancreatic fungal infections: a case report and review of the literature. *Pancreas* 1996; **12**: 308–312.
- 12 Pfaller MA, Messer SA, Boyken L, et al. Variation in the susceptibilities of invasive isolates of *Candida glabrata* to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. *J Clin Microbiol* 2004; **42**: 3142–3146.
- 13 Spellberg BJ, Filler SG, Edwards JE. Current treatment strategies for disseminated candidiasis. *Clin Infect Dis* 2006; **42**: 244–251.
- 14 Hazen KC, Stei J, Darracott C, et al. Isolation of cholesterol-dependent *Candida glabrata* from clinical specimens. *Diagn Microbiol Infect Dis* 2005; **52**: 35–37.
- 15 Bard M, Sturm AM, Pierson CA, et al. Sterol uptake in *Candida glabrata*: Rescue of sterol auxotrophic strains. *Diagn Microbiol Infect Dis* 2005; **52**: 285–293.