Mycopathologia (2016) 181:871–878
DOI 10.1007/s11046-016-0052-1

Candida glabrata species complex prevalence and antifungal susceptibility testing in a culture collection: First description of *Candida nivariensis* in Argentina

Soraya Eugenia Morales-López · Constanza G. Taverna · María Eugenia Bosco-Borgeat · Ivana Maldonado · Walter Vivot · Wanda Szusz · Guillermo Garcia-Effron · Susana B. Córdoba

Received: 27 February 2016 / Accepted: 18 August 2016 / Published online: 28 September 2016

Abstract The presence of the cryptic species belonging to the *Candida glabrata* complex has not been studied in Argentina. We analyzed a collection of 117 clinical isolates of *C. glabrata* complex belonging to a National Culture Collection of Instituto Nacional de Microbiología "Dr. Carlos G. Malbrán" from Argentina (40 isolates from blood samples, 18 from other normally sterile sites, 20 from vagina, 14 from urine, 7 from oral cavity, 3 from catheter, 1 from a stool sample and 14 isolates whose clinical origin was not recorded). The aims of this work were to determine the prevalence of the cryptic species *Candida nivariensis* and *Candida bracarensis* and to evaluate the susceptibility profile of isolates against nine antifungal drugs. Identification was carried out by using classical phenotypic tests, CHROMagar™ Candida, PCR and MALDI-TOF. The minimal inhibitory concentrations of amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, voriconazole, ketoconazole, posaconazole, caspofungin and anidulafungin were determined according to the EDef 7.3 (EUCAST) reference document. Of the 117 isolates, 114 were identified as *C. glabrata* and three as *C. nivariensis* by using PCR and MALDI-TOF. There were no major differences between *C. nivariensis* and *C. glabrata* susceptibility profiles. No resistant strains were found to echinocandins. We have found that the percentage of *C. nivariensis* in our culture collection was 2.56. This is the first description of *C. nivariensis* in Argentina, and data obtained could contribute to the knowledge of the epidemiology of this cryptic species.

Keywords *Candida glabrata* complex · *Candida nivariensis* · *Candida bracarensis* · MALDI-TOF · Antifungal susceptibility testing · Yeast identification

S. E. Morales-López · C. G. Taverna · M. E. Bosco-Borgeat · W. Vivot · W. Szusz · S. B. Córdoba (✉)
Departamento de Micología, Instituto Nacional de Enfermedades Infecciosas "Dr. Carlos G. Malbrán", Avenida Vélez Sarsfield 563, Ciudad Autónoma de Buenos Aires, Argentina
e-mail: sorayaeugeniam@hotmail.com

I. Maldonado
Laboratorios Domeq y Lafage, Ciudad Autónoma de Buenos Aires, Argentina

G. Garcia-Effron
Laboratorio de Micología y Diagnóstico Molecular, Cátedra de Parasitología y Micología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina

G. Garcia-Effron
Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Santa Fe, Argentina

Introduction

Candida glabrata is considered a species complex since the description of two separate cryptic species named *Candida nivariensis* and *Candida bracarensis*

in 2005 and 2006, respectively [1, 2]. *C. glabrata* sensu stricto together with this two newly described species are the only three human pathogens of the *Nakaseomyces* clade and have been associated with bloodstream infections, invasive and oral candidiasis, urinary tract infections and vulvovaginitis [3–6]. Despite its clinical importance, data about the epidemiology of these species are scanty partly due to the inability of classical identification methods to differentiate them. As the identification methods shifted to molecular-based techniques [7], reports about the low prevalence of these species in North American, European and Asian culture collections (between 0.06 and 2.1 %) started to be published [8–10]. *C. glabrata* sensu lato is the third and fourth causative agent of fungal bloodstream infections in South America and in Argentina, respectively [11, 12]. Recently, Figueiredo-Carvalho reported the first identification of *C. nivariensis* in Brazil [13], however until now, little is known about the prevalence of the *C. glabrata* cryptic species in Latin America.

The aims of this study were to determine the percentage of *C. bracarensis* and *C. nivariensis* in an Argentinean culture collection of *C. glabrata* complex isolates using phenotypic, polymerase chain reaction (PCR) and matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) methods and to determine the in vitro activity of nine antifungal agents by using microbroth dilution method.

Materials and Methods

Isolates

A total of 117 clinical isolates phenotypically identified as *C. glabrata* and conserved in the Culture Collection of the Department of Mycology (DMic) of the Instituto Nacional de Enfermedades Infecciosas "Dr. Carlos G. Malbrán" (Argentina) were included in this study. All the isolates represented unique clinical isolates obtained from different patients and were randomly derived between 1984 and 2013 by the laboratories enrolled in the Argentinean National Mycology Laboratory Network. The collection included 40 isolates from blood samples, 18 from other normally sterile sites, 20 from vagina, 14 from urine, 7 from oral cavity, 3 from catheter, 1 from a stool sample and 14 isolates whose clinical origin was not recorded. *C. glabrata* sensu stricto ATCC 2001 (CBS 138) and ATCC 90030 were used as control strains.

Phenotypic Identification

Isolates were subcultured on CHROMagar™ *Candida* medium (CHROMagar *Candida*, Paris, France) and incubated for 48 h, and then colony colors were recorded. The classical phenotypic identification was performed to confirm previous results using standard procedures methods, including the assimilation of 19 carbons and two nitrogenous compounds by auxanographic method, fermentation of six carbohydrates, growth at 28 and 37 °C, urea hydrolysis and morphological features [14].

Genotypic Identification

Genotypic identification was achieved by PCR-based assay using a single primer pair targeting the RPL31 gene that allows discrimination between *C. glabrata*, *C. bracarensis* and *C. nivariensis* according to the size of the generated amplicon proposed by Enache-Angoulvant et al. [7]. The pair of primers used in this protocol (RPL31cgF [5'-GCCGGTTTGAAGGACGT TGTTACT-3'] and RPL31cgR [5'-GAACAATG GGTTCTTGGCGT-3']) allows to differentiate the species of the complex by the length of the fragments: 1,061 bp for *C. glabrata*, 902 bp for *C. bracarensis*, 665 bp for *C. nivariensis*. The conditions for the touchdown PCR were: 3 min at 95 °C; 3 cycles, with 1 cycle consisting of 30 s at 95 °C, 30 s at 62 °C, and 30 s at 72 °C; 3 cycles, with 1 cycle consisting of 30 s at 95 °C, 30 s at 58 °C, and 30 s at 72 °C; 3 cycles, with 1 cycle consisting of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C; 3 cycles, with 1 cycle consisting of 30 s at 95 °C, 30 s at 50 °C, and 30 s at 72 °C; 35 cycles, with 1 cycle consisting of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C; and a final extension step of 10 min at 72 °C.

The isolates identified as *C. nivariensis* were confirmed by amplification and sequencing of the internal transcribed spacer regions ITS1-5.8S-ITS2 and the D1/D2 domains of 26S rRNA by using the procedure described by Taverna et al. [15]. The amplification of ITS region was done using the primers ITS1 (5'-TCCGTAGGTGAA-CCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGAT-ATGC-

3') using the following parameters: 95 °C for 7 min, followed by 40 cycles at 95 °C for 1 min, 54 °C for 2 min, 72 °C for 1 min and a final extension at 72 °C for 10 min. The amplification of D1/D2 region of 26S rDNA, was done with the primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') using the parameters: 95 °C for 7 min, followed by 40 cycles at 95 °C for 1 min, 53 °C for 2 min, 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were sequenced in the forward and reverse directions using the initial amplification primers and an automated DNA sequencer (Genetic Analyzer 3500, Applied Biosystems). Sequence similarity was obtained using either the BLASTN tool of the National Center for Biotechnology Information (NCBI) website (ncbi.nlm.nih.gov/BLAST/). The identity of each isolate was determined based on the comparisons with the sequences of the type species of the complex.

MALDI-TOF Identification

Identification by MALDI-TOF was performed using on-plate protein extraction method with 70 % formic acid and α-cyano-4-hydroxycinnamic acid matrix. Each sample was tested in duplicate to ensure spectra reproducibility. The protein profile of each specimen with an *m*/*z* of 2.000–20.000 was generated based on a minimum 240 laser shot measurements. Analysis was performed using MALDI Biotyper™ 3.1 software, and the identifications were performed using the score value of ≥2.0 for highly probable species identification (Bruker Microflex LT system).

Antifungal Susceptibility Testing

Antifungal susceptibility tests were carried out by determining the minimal inhibitory concentration (MIC) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) EDef 7.3 reference document [16]. All isolates were tested against anidulafungin, fluconazole and voriconazole (Pfizer, Buenos Aires, Argentina), itraconazole, ketoconazole, 5-fluorocytosine and amphotericin B (Sigma-Aldrich, Buenos Aires, Argentina), caspofungin and posaconazole (Merck, Buenos Aires, Argentina).

The interpretative clinical breakpoints recently proposed for the EUCAST document were used: For fluconazole, isolates were classified as susceptible MIC ≤0.002 mg/L, intermediate MIC >0.002–32 mg/L or resistant MIC >32 mg/L; for anidulafungin, isolates were classified as susceptible MIC ≤0.06 mg/L or resistant MIC >0.06 mg/L; meanwhile, for amphotericin B, isolates were classified as susceptible MIC ≤1 mg/L or resistant MIC >1 mg/L [17]. Taking into account that the EDef 7.3 does not currently include breakpoints for all the antifungal drugs tested in our study, no categorical interpretation of results is available; thus, the MIC value obtained was showed. The range, mode, MIC_{50} and MIC_{90} were calculated. *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as control strains.

Results

Classical Phenotypic Tests and CHROMagar Candida

All the 117 isolates showed white, shiny, smooth, entire and butyrous colonies on Sabouraud dextrose agar. After growing 7 days in 5 % malt extract broth at 28 °C, cells were subglobose to ovoid and no production of hyphae or pseudohyphae was observed. All the isolates fermented and assimilated glucose and trehalose, grew at 28 and 37 °C and not hydrolyzed urea. Assimilations results for sucrose, L-sorbose, melibiose, raffinose, galactose, lactose, maltose, melezitose, cellobiose, L-rhamnose, D-xylose, L-arabinose, D-ribose, D-mannitol, inositol, erythritol, citrate and NO_3K and fermentations results for D-galactose, maltose, sucrose and lactose were negative.

On CHROMAgar™ Candida 114 (97.44 %) isolates produced mauve colonies, while three isolates (DMic 962066, DMic 962070 and DMic 134582) showed white colonies (2.56 %) (Fig. 1A).

Genotypic Identification

Using PCR assay targeting the RPL31 gene, 114 isolates were identified as C. glabrata sensu stricto and three isolates (DMic 962066, DMic 962070 and DMic 134582) were identified as C. nivariensis. (Fig. 1 B). No C. bracarensis were found. (Fig. 1B). The identification of these three isolates was confirmed by ITS1 5.8S and ITS2 regions and D1/D2 domains sequencing (Table 1). The sequences of the both regions of the

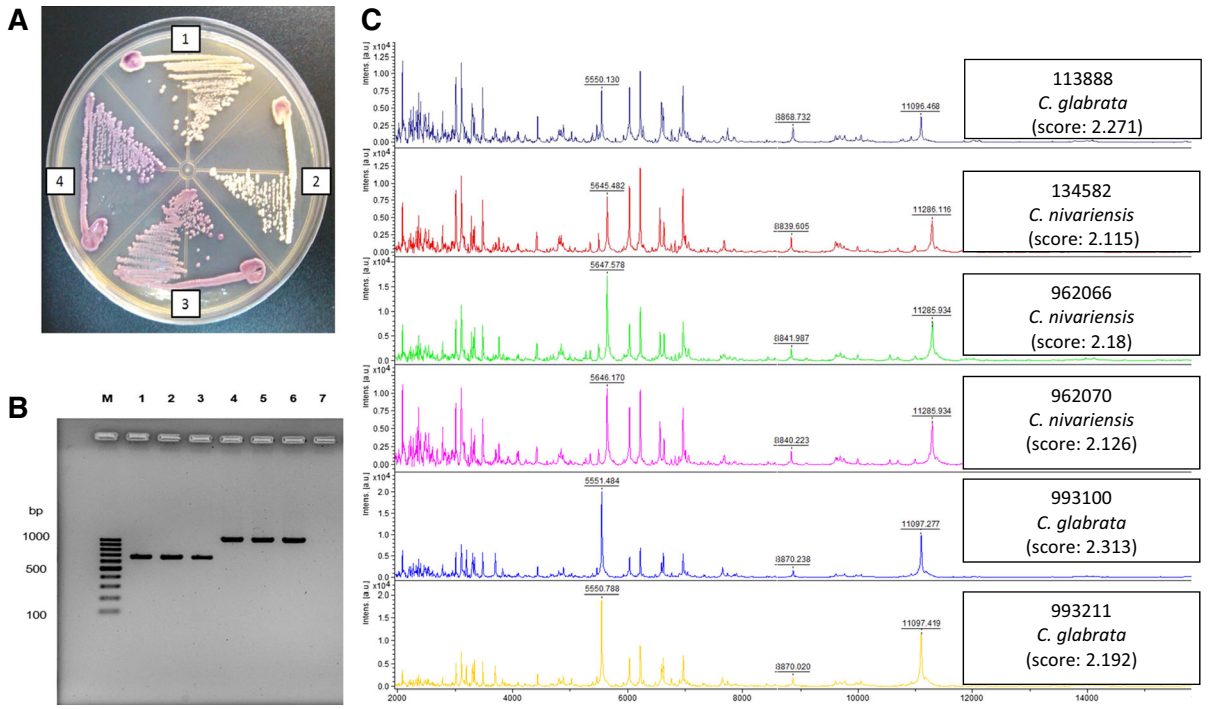

Fig. 1 **a** Colonies on CHROMagar Candida medium: 1–2 *C. nivariensis* (*white* colonies); 3–4 *C. glabrata* (*mauve* colonies). Protein profiles obtained for isolates of *C. nivariensis* (DMic 962066, 962070 and 134582) and isolates of *C. glabrata* (962064, 962069, 962072). **b** Agarose gel electrophoresis of PCR products obtained with the primers *RPL*31. *Lanes*: 1–3, *C. nivariensis* (665 bp) corresponding to DMic 962066,962070 and 134582, respectively; 4–6, *C. glabrata* (1061 bp) corresponding to DMic 993147, ATCC 90030 and ATCC 2001, respectively; 7, negative control; M, 100-bp DNA ladder. **c** Protein profiles obtained for isolates of *C. nivariensis* (DMic 962066, 962070 and 134582) and three isolates of *C. glabrata* (993211, 993100, 113888)

Table 1 Clinical origin and identification of the three *C. nivariensis* clinical isolates and *C. glabrata* control strains

Strain number	Year of isolation	Source	CHROM agar	Biochemical identification	MALDI-TOF identification (score)	PCR identification	Genbank accession number	
							D1/D2	ITS
DMic 962066	1996	Unknown	White	*C. glabrata*	*C. nivariensis* (2,18)	*C. nivariensis*	KP050696 (identical sequences)	KP050697 (identical sequences)
DMic 962070	1996	CAPBD	White	*C. glabrata*	*C. nivariensis* (2126)	*C. nivariensis*		
DMic 134582	2013	Double renal catheter	White	*C. glabrata*	*C. nivariensis* (2115)	*C. nivariensis*		
ATCC 2001	–	–	Mauve	*C. glabrata*	*C. glabrata* (2099)	*C. glabrata*		
ATCC 90030	–	–	Mauve	*C. glabrata*	*C. glabrata* (2209)	*C. glabrata*		

DMic Departamento Micología Collection; *CAPBD* continuous ambulatory peritoneal bag dialysis

Table 2 Minimal Inhibitory Concentration distribution of antifungal drugs tested against *Candida glabrata* (114) clinical isolates

Drugs MIC distribution (mg/L)	Accumulative percentage of isolates inhibited (number of isolates)												
	0.015	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64
Amphotericin B	0.87 (1)	7.01 (7)	13.15 (7)	33.33 (23)	**72.80 (45)**	99.12 (30)	100 (1)	–	–	–	–	–	–
5-Fluorocytosine	–	0.87 (1)	–	**96.49 (109)**	97.36 (1)	–	98.24 (1)	–	100 (2)	–	–	–	–
Fluconazole	3.50 (4)	–	–	**30.70 (31)**	35.08 (5)	46.49 (13)	67.54 (24)	82.45 (17)	92.98 (12)	96.49 (4)	100 (4)	–	–
Itraconazole	**41.22 (47)**	57.01 (18)	74.56 (20)	88.59 (16)	96.49 (9)	100 (4)	–	–	–	–	–	–	–
Voriconazole	**37.71 (43)**	60.52 (26)	85.08 (28)	93.85 (10)	96.49 (3)	100 (4)	–	–	–	–	–	–	–
Ketoconazole	17.54 (20)	47.36 (34)	**78.07 (35)**	90.35 (14)	93.85 (4)	100 (7)	–	–	–	–	–	–	–
Posaconazole	**32.45 (37)**	53.50 (24)	81.57 (32)	92.98 (13)	100 (8)	–	–	–	–	–	–	–	–
Anidulafungin	**45.61 (52)**	86.84 (47)	100 (15)	–	–	–	–	–	–	–	–	–	–
Caspofungin	8.77 (10)	12.28 (4)	19.29 (8)	31.57 (14)	**100 (78)**	–	–	–	–	–	–	–	–

Numbers in bold faces indicate the modal value

MIC minimal inhibitory concentration

isolates showed 100 % similarity to each other and to the sequences of C. nivariensis type strain CBS 9983 (GenBank accession number KP050697 (723bp) for ITS and KP050696 (591 bp) for D1/D2 domains).

Proteomic Identification

The MALDI-TOF methodology identified 114 *C. glabrata* isolate*s* and three (DMic 962066, DMic 962070 and DMic 134582) as *C. nivariensis*. All the isolates of *C. glabrata* and *C. nivariensis* were identified with scores higher than 2.0 (Fig. 1C). Different peaks at 5500 and 11000 Da are observed as subtle differences when comparing the profiles of *C. nivariensis* with those obtained for *C. glabrata* (Fig. 1C).

Antifungal Susceptibility Testing

All strains identified as *C. glabrata* showed intermediate MICs to fluconazole (0.015–16 mg/L), susceptible MICs to amphotericin B (≤1 mg/L) and susceptible MICs to anidulafungin (≤0.06 mg/L). For amphotericin B, we observed that 113 strains of *C. glabrata* showed MIC ≤0.13 mg/L and one strain yielded a value of MIC 1 mg/L.

The MIC values of *C. nivariensis* for antifungal agents were similar to those obtained for *C. glabrata*. All the strains had the same MIC value for fluconazole (4 mg/L), caspofungin and anidulafungin (0.06 mg/L). For the rest of antifungal, the values ranges were as follows: amphotericin B (0.5–1 mg/L), voriconazole (0.06–0–13 mg/L) and ketoconazole, itraconazole, posaconazole e itraconazole (0.13–0.25). The accumulative percentage of inhibition for each antifungal agent is showed in Table 2.

Discussion

The cryptic species of the *C. glabrata* species complex represent a small percentage among the isolates phenotypically identified as *C. glabrata* worldwide [8]. Our results show that Argentina is not an exception since *C. nivariensis* only signified a 2.56 % (3/117) of all the studied isolates. Comparing our results with those obtained for other culture collections, similar percentage of *C. nivariensis* were

described in a Chinese fungal collection (2.32 %) [4], while lower prevalence was reported in Malaysia and USA (1.08 % and 0.2 % respectively) [9, 10]. On the other hand, in Italian and Spanish culture collections no *C. nivariensis* isolates were found [8, 18].

In the present study it is worthy of mention that two of the *C. nivariensis* isolates were collected in 1996 and the third in 2013. Thus, these isolates in 1996 may be the oldest circulating strains that have been reported.

One of the *C. nivariensis* isolates in our study was recovered from a continuous ambulatory peritoneal dialysis bag and other from a double renal catheter, and there were no data about the origin of the third clinical isolate. The isolates of this species have been reported from vagina, blood, urine, oropharyngeal, stool and lower respiratory tract [1, 3, 4, 9, 10, 13, 19–21]. In 2008, Borman reported a strain isolated from an exit site swab of a patient with continuous ambulatory peritoneal dialysis (CAPD), while Fujita and Lopez-Soria also reported isolates of this species from catheter, but not renal type. [21–23].

No clinical data on patients with isolates in 1996. Isolation of 2013 came from a patient with urinary tract infection and breast cancer. With the exception of Sanata who described this agent in stool of asymptomatic patients, the findings of C. nivariensis always have been related to patients with patients with some degree of immunosuppression. [1, 3, 4, 9, 10, 13, 19–24].

In our study, no *C. bracarensis* were found in agreement with other studies in different countries that indicates that the prevalence of this species is even lower than *C. nivariensis* [4, 10, 25, 26].

The classical phenotypic tests were not able to differentiate between *C. glabrata* and *C. nivariensis* isolates. In addition, the three *C. nivariensis* isolates found were able to ferment trehalose within 5 days of incubation oppositely to what was published by Wahyuningsih [20], whose isolates showed clear fermentation of trehalose only at 6th–7th day, but in accordance with the biochemical profile of the type strain described by Alcoba-Florez [1] who reported that this species ferments trehalose.

On the other hand, CHROMagar ™ Candida proved to be a good screening method to differentiate *C. nivariensis* from *C. glabrata*. This characteristic was also observed by other authors [13, 19, 25, 27].

Proteomic and molecular methods easily differentiate *C. nivariensis* from *C. glabrata* (Fig. 1). In the case of MALDI-TOF, the reliable identification at the species level of yeasts has been proposed scores below 2.0 [28, 29]; however, although *C. nivariensis* and *C. glabrata* are genetically closely related species, we obtained good levels of identification allowing identification to species with score greater than 2.0. Our results are opposite to those found by Pinto who described the identification of both cryptic species, but in some cases with values lower than 1.7 [28].

For *C. glabrata* species complex, resistance to fluconazole and other azoles is an unresolved problem that limits the use of azoles in treatment [30–32]. To remark, in the present study no resistance to fluconazole was observed. Also all strains were susceptible to amphotericin B and anidulafungin.

Oppositely to what were described by Fujita [21], Figueiredo-Carvalho [13], and Borman [22], our *C. nivariensis* isolates showed low fluconazole and 5-fluorocytosine MIC values following EUCAST protocol. These results are similar to those described by others authors, including breakpoints values for amphotericin B and other azoles. [4, 10, 20, 23].

Candida nivariensis is considered an emerging pathogen highly difficult to identify by conventional methods. Molecular methods and/or MALDI-TOF are required for its identification. In this study, the MIC values obtained were similar to those obtained with *C. glabrata* sensu stricto. These three *C. nivariensis* isolates could be the first reported in Argentina, and data obtained contribute to the knowledge of the epidemiology of this cryptic species.

References

1. Alcoba-Florez J, Mendez-Alvarez S, Cano J, Guarro J, Perez-Roth E, del Pilar AM. Phenotypic and molecular characterization of *Candida nivariensis* sp. nov., a possible new opportunistic fungus. J Clin Microbiol. 2005;43:4107–11.
2. Correia A, Sampaio P, James S, Pais C. *Candida bracarensis* sp. nov., a novel anamorphic yeast species phenotypically similar to *Candida glabrata*. Int J Syst Evol Microbiol. 2006;56:313–7.
3. Gorton RL, Jones GL, Kibbler CC, Collier S. *Candida nivariensis* isolated from a renal transplant patient with persistent candiduria—molecular identification using ITS

PCR and MALDI-TOF. Med Mycol Case Rep. 2013;2:156–8.
4. Li J, Shan Y, Fan S, Liu X. Prevalence of *Candida nivariensis* and *Candida bracarensis* in vulvovaginal candidiasis. Mycopathologia. 2014;178:279–83.
5. Peman J, Canton E, Quindos G, Eraso E, Alcoba J, Guinea J, Merino P, Ruiz-Perez-de-Pipaon MT, Perez-del-Molino L, Linares-Sicilia MJ, Marco F, Garcia J, Rosello EM, de la Gomez G, Pedroza E, Borrell N, Porras A, Yague G. Epidemiology, species distribution and in vitro antifungal susceptibility of fungaemia in a Spanish multicentre prospective survey. J Antimicrob Chemother. 2012;67:1181–7.
6. Warren TA, McTaggart L, Richardson SE, Zhang SX. *Candida bracarensis* bloodstream infection in an immunocompromised patient. J Clin Microbiol. 2010;48:4677–9.
7. Enache-Angoulvant A, Guitard J, Grenouillet F, Martin T, Durrens P, Fairhead C, Hennequin C. Rapid discrimination between *Candida glabrata*, *Candida nivariensis*, and *Candida bracarensis* by use of a singleplex PCR. J Clin Microbiol. 2011;49:3375–9.
8. Cuenca-Estrella M, Gomez-Lopez A, Isla G, Rodriguez D, Almirante B, Pahissa A, Rodriguez-Tudela JL. 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. Med Mycol. 2011;49:525–9.
9. Lockhart SR, Messer SA, Gherna M, Bishop JA, Merz WG, Pfaller MA, Diekema DJ. Identification of *Candida nivariensis* and *Candida bracarensis* in a large global collection of *Candida glabrata* isolates: comparison to the literature. J Clin Microbiol. 2009;47:1216–7.
10. Tay ST, Lotfalikhani A, Sabet NS, Ponnampalavanar S, Sulaiman S, Na SL, Ng KP. Occurrence and Characterization of *Candida nivariensis* from a culture collection of *Candida glabrata* clinical isolates in Malaysia. Mycopathologia. 2014;178:307–14.
11. Cordoba S, Vivot W, Bosco-Borgeat ME, Taverna C, Szusz W, Murisengo O, Isla G, Davel G. Species distribution and susceptibility profile of yeasts isolated from blood cultures: results of a multicenter active laboratory-based surveillance study in Argentina. Rev Argent Microbiol. 2011;43:176–85.
12. Nucci M, Queiroz-Telles F, Alvarado-Matute T, Tiraboschi IN, Cortes J, Zurita J, Guzman-Blanco M, Santolaya ME, Thompson L, Sifuentes-Osornio J, Echevarria JI, Colombo AL. Epidemiology of candidemia in Latin America: a laboratory-based survey. PLoS ONE. 2013;8:e59373. doi:10.1371/journal.pone.0059373.
13. Figueiredo-carvalho MHG, Ramos LDS, Barbedo LS, Leal A, Muramoto IA, Luis A, Almeida-Paes R, Zancopé-oliveira RM. First description of Candida nivariensis in Brazil: antifungal susceptibility profile and potential virulence attributes. 2016;111:1–8. doi.10.1590/0074-02760150376
14. Kurtzman CP, Fell JW, Boekhout T, editors. The yeast, a taxonomic study. 5th ed. Amsterdan: Elsevier; 2011.
15. Taverna CG, Bosco-Borgeat ME, Murisengo OA, Davel G, Boité MC, Cupolillo E, Canteros CE. Comparative analyses of classical phenotypic method and ribosomal RNA gene sequencing for identification of medically relevant Candida species. Mem Inst Oswaldo Cruz. 2013;108:178–85.
16. Arendrup MC, Guinea J, Cuenca-Estrella M, Meletiadis J, Mouton JW, Lagrou K, Howard SJ and the Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). EUCAST DEFINITIVE DOCUMENT E.DEF 7.3. Method forthe determination of broth dilution minimum Inhibitory concentrations of antifungal agents for yeasts. www.eucast.org
17. European Committee on Antimicrobial Susceptibility Testing. Antifungal Agents. Breakpoint tables for interpretation of MICs Version 8.0 valid from 2015-11-16 www.eucast.org 2015;1–5.
18. Esposto MC, Prigitano A, Romeo O, Criseo G, Trovato L, Tullio V, Fadda ME, Tortorano AM. Looking for *Candida nivariensis* and *C. bracarensis* among a large Italian collection of *C. glabrata* isolates: results of the FIMUA working group. Mycoses. 2013;56:394–6.
19. Sharma C, Wankhede S, Muralidhar S, Prakash A, Singh PK, Kathuria S, Kumar DA, Khan N, Randhawa HS, Meis JF, Chowdhary A. *Candida nivariensis* as an etiologic agent of vulvovaginal candidiasis in a tertiary care hospital of New Delhi, India. Diagn Microbiol Infect Dis. 2013;76:46–50.
20. Wahyuningsih R, SahBandar IN, Theelen B, Hagen F, Poot G, Meis JF, Rozalyani A, Sjam R, Widodo D, Djauzi S, Boekhout T. *Candida nivariensis* isolated from an Indonesian human immunodeficiency virus-infected patient suffering from oropharyngeal candidiasis. J Clin Microbiol. 2008;46:388–91.
21. Fujita S, Senda Y, Okusi T, Ota Y, Takada H, Yamada K, Kawano M. Catheter-related fungemia due to fluconazole-resistant *Candida nivariensis*. J Clin Microbiol. 2007;45:3459–61.
22. Borman AM, Petch R, Linton CJ, Palmer MD, Bridge PD, Johnson EM. *Candida nivariensis*, an emerging pathogenic fungus with multidrug resistance to antifungal agents. J Clin Microbiol. 2008;46:933–8.
23. López-Soria LM, Bereciartua E, Santamaría M, Soria LM, Hernández-Almaraz JL, Mularoni A, Nieto J, Montejo M. First case report of catheter-related fungemia by Candida nivariensis in the Iberian Peninsula. Revista Iberoamericana de Micología. 2013;30(1):69–71. doi:10.1016/j.riam.2012.09.001
24. Sanata B, Salam OA, Ibrahim S, Adama Z, Mamoudou C, Simplice KD, Jacques S, Robert GT, Christophe H. Digestive fungal flora in asymptomatic subjects in Bobo-Dioulasso, Burkina Faso. Asian Pac J Trop Biomed. 2014;4(8):659–62. doi:10.12980/APJTB.4.201414B27
25. Swoboda-Kopeć E, Sikora M, Golas M, Piskorska K, Gozdowski D, Netsvyetayeva I. Candida nivariensis in comparison to different phenotypes of Candida glabrata. Mycoses. 2014;57(12):747–53. doi:10.1111/myc.12264
26. Angoulvant A, Guitard J, Hennequin C. Old and new pathogenic Nakaseomyces species: epidemiology, biology, identification, pathogenicity and antifungal resistance. FEMS Yeast Research. 16(2). doi:10.1093/femsyr/fov114
27. Bishop JA, Chase N, Lee R, Kurtzman CP, Merz WG. Production of white colonies on CHROMagar Candida medium by members of the *Candida glabrata* clade and other species with overlapping phenotypic traits. J Clin Microbiol. 2008;46:3498–500.
28. Pinto A, Halliday C, Zahra M, van Hal S, Olma T, Maszewska K, Iredell JR, Meyer W, Chen SC. A Matrix-assisted

laser desorption ionization-time of flight mass spectrometry identification of yeasts is contingent on robust reference spectra. PLoS ONE. 2011;6(10):e25712. doi:10.1371/journal.pone.0025712.
29. Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. J Clin Microbiol. 2011;49:1614–6.
30. Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. Mechanisms of azole resistance in clinical isolates of Candida glabrata collected during a hospital survey of antifungal resistance. Antimicrob Agents Chemother. 2005;49(2):668–79.
31. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arikan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Flörl C, Petrikkos G, Richardson MD, Roilides E, Verweij PE, Viscoli C, Ullmann AJ. ESCMID Fungal Infection Study Group. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect. 2012; 18(Suppl 7):19–37.
32. Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, Edwards JE Jr, Filler SG, Fisher JF, Kullberg BJ, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD. Infectious Diseases Society of America. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(5):503–35.