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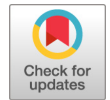

Phenotypic and Molecular Evaluation of Echinocandin Susceptibility of *Candida glabrata*, *Candida bracarensis*, and *Candida nivariensis* Strains Isolated during 30 Years in Argentina

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ABSTRACT The echinocandin susceptibilities of 122 *Candida glabrata* complex strains (including 5 *Candida nivariensis* and 3 *Candida bracarensis* strains) were evaluated by microdilution and compared with the results from a molecular tool able to detect FKS mutations. No echinocandin resistance was detected. The PCR results coincide with the MIC data in 99.25% of the cases (1 *C. glabrata* strain was misidentified as resistant) but were 20 h faster. *C. nivariensis FKS* genes were sequenced and showed differences with *C. glabrata FKS* genes.

KEYWORDS *Candida glabrata* species complex, *Candida nivariensis*, *Candida bracarensis*, echinocandin resistance, hot spot

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The epidemiology of *Candida* infections has undergone recent changes due to the description of cryptic species. *Candida glabrata* species complex includes three human-pathogenic species: *C. glabrata sensu stricto*, *C. nivariensis*, and *C. bracarensis* (1, 2). *Candida glabrata sensu stricto* accounts for 15 to 20% of all cases of *Candida* infections worldwide, and it is the second most common cause of candidemia in the United States (3, 4). In Latin American countries, such as Argentina, *C. glabrata* ranked fourth, representing 4% of the candidemia cases (5).

C. glabrata infection treatment is often difficult due to the increasing prevalence of azole resistance. Thus, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the Infectious Diseases Society of America (IDSA) have proposed echinocandins as the treatment of choice for these infections (6–8). In recent years, the prevalence of echinocandin resistance in *C. glabrata sensu stricto* has increased (9, 10). On the other hand, there are little data about echinocandin susceptibilities for the other two species of the complex, and no treatment recommendations have been released. However, the few available data support them being susceptible to echinocandins (11–14). Clinical resistance to echinocandins has been associated with hot spot *FKS* mutations (15, 16). Recently, our group published a set of classical PCRs able to detect these mutations (17). The aims of this study were to evaluate the *in vitro* activities of echinocandins against Argentinian *C. glabrata sensu lato* strains and to compare the obtained results with those obtained with the described molecular tool.

We analyzed a collection of 122 *C. glabrata* complex clinical strains, including: (i) 114 *C. glabrata sensu stricto* strains (40 isolated from blood, 17 from other normally sterile sites, 20 from the vagina, 14 from urine, 7 from the oral cavity, 2 from a catheter, 1 from stool, and 13 with no origin data), (ii) five *C. nivariensis* strains (throat, renal catheter,

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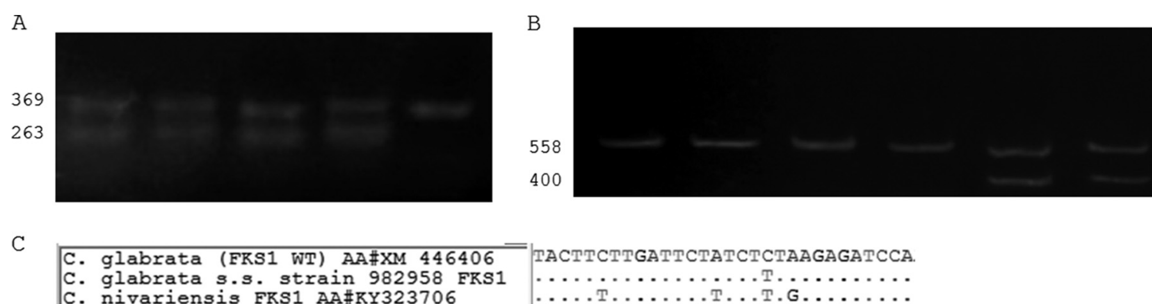

FIG 1 (A) Electrophoresis gel (1.5% agarose) using 1-1670F, 1-S629R, and 1-2225R primers from Dudiuk et al. (16). Lanes 1 to 4, four different *C. glabrata sensu stricto* clinical strains showing wild-type *FKS1* hot spot 1 (TTC TTG ATT CTA TCT CTA AGA GAT CCA); lane 5, *C. glabrata sensu stricto* strain 982958 showing false-resistant genotype due to a silent mutation (underlined) (TTC TTG ATT CTA TCT TTA AGA GAT CCA). (B) Electrophoresis gel (1.5% agarose) using 2-1619F, 2-S663R, and 2-2177R primers from Dudiuk et al. (16). Lanes 1 to 4, *C. nivariensis* strains; lanes 5 and 6, *C. glabrata sensu stricto*. *C. nivariensis* strains harbor silent *FKS2* hot spot 1 mutations compared with *C. glabrata sensu stricto* (TTT TTG ATT CTT TCT TTG AGA GAT CCA versus TTC TTG ATT TTG TCT CTA AGA GAC CCT, respectively). Underlined sequences are the silent mutations. (C) Sequence alignments of the *FKS1* hot spot 1 regions of *C. glabrata sensu stricto* (wild-type and silent mutant strains) and *C. nivariensis*. WT, wild type; s.s. *sensu stricto*. The numbers on the left of each panel are the size of the PCR bands (in bp).

urine, continuous peritoneal dialysis bag, and 1 isolate with no isolation data available), and (iii) three *C. bracarensis* strains (urine, throat, and 1 with no data). All strains were randomly referred from several medical centers from 1984 to 2014 to the Argentinian National Culture Collection of Instituto Nacional de Microbiología Dr. Carlos G. Malbrán. The isolates were identified by classical, molecular (internal transcribed spacer [ITS] sequencing and multiplex PCR), and proteomic (matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS]) methods (18–20). Also, 17 characterized echinocandin-resistant *C. glabrata sensu stricto* strains harboring different *FKS* mutations were used as PCR control (17), together with *Candida parapsilosis sensu stricto* ATCC 22019 and *Candida krusei* ATCC 6258, which were used as MIC controls (21, 22). Anidulafungin (ANF) and caspofungin (CSF) MICs were determined by broth microdilution in accordance with the CLSI M27-A3 and M27-S4 documents (21, 22). Three of the isolates (*C. nivariensis* DMic 144820 and *C. bracarensis* DMic 144819 and DMic 144835) did not grow in RPMI 1640 broth, and susceptibility testing was performed by agar diffusion using Etest strips in RPMI 1640 agar. Echinocandin resistance molecular mechanisms were evaluated by using a recently published set of classical PCRs able to identify hot spot mutations at the *FKS1* and *FKS2* genes. *FKS* gene sequencing was used to confirm the obtained results (17).

Echinocandins showed good *in vitro* activity against all the studied isolates (for *C. nivariensis*, ANF, 0.015 to 0.03 mg/liter, and CSF, 0.06 to 0.13 mg/liter; for *C. bracarensis*, ANF, 0.03 to 0.06 mg/liter, and CSF, 0.015 to 0.06 mg/liter; and for *C. glabrata sensu stricto*, ANF, 0.015 to 0.06 mg/liter, and CSF, 0.015 to 0.25 mg/liter). Turning to the molecular detection of *FKS* mutants, all but one *C. glabrata sensu stricto* strain showed a wild-type band pattern for the *FKS1* and *FKS2* hot spot regions (17). Strain DMic 982958 showed a molecular profile consistent with a substitution at Fks1p (S629). The *FKS* genes of this strain were sequenced. *FKS1* showed a silent mutation (T1888C and no amino acid substitution) that was detected but represents a false-positive result (minor error) (Fig. 1A).

All *C. nivariensis* strains showed a molecular profile consistent with a *C. glabrata sensu stricto FKS* mutant (a single band, using the Dudiuk et al. method [17]) (Fig. 1B). These *C. nivariensis FKS* genes were sequenced by using *FKS* universal primers (23) and showed several nucleotide differences compared with *C. glabrata sensu stricto FKS* genes. However, all the described naturally occurring polymorphisms yielded no amino acid changes. For *C. bracarensis*, it was not possible to obtain PCR bands by using the same primers.

C. glabrata strains with *FKS* mutations and reduced echinocandin susceptibilities have been described worldwide (10, 11, 15). The prevalences of such mutations ranged

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from 2.9% to 18% in different reports from different U.S. centers (9, 24, 25). Neither elevated MIC values nor *FKS* hot spot mutations were detected in our strains. These results are in accordance with other reports from Latin America (26). These geographical differences might be due to the higher use of echinocandin drugs in the United States. Beyda et al. suggested that the unique predictor for echinocandin resistance related to *FKS* mutations is the use of echinocandin drugs in clinical practice (24). In Argentina, the use of these drugs is very scarce and could greatly contribute to the inexistence of such resistance in our collection.

All *C. bracarensis* and *C. nivariensis* isolates showed low echinocandin MIC values and were consistent with previous reports (11, 12, 14).

Shields et al. considered that *FKS* mutation detection is the most significant risk factor for therapy failure for *C. glabrata* infections (16). The PCR method used in this work showed 99.25% accordance with susceptibility testing for *C. glabrata sensu stricto*, and it is at least 20 h faster (17) (95.1% accordance if cryptic species were included). The unique false results (minor error) obtained for the described *C. glabrata sensu stricto* strain were due to a silent mutation at the hot spot 1 region of *FKS1*. In addition, this PCR method would be a suitable tool to circumvent the technical problems reported for caspofungin susceptibility testing (27).

Turning to the cryptic species of the *C. glabrata* complex, the PCR set was unable to correctly classify them regarding echinocandin susceptibility. All *C. nivariensis* strains were incorrectly considered echinocandin resistant, owing to naturally occurring silent substitutions, while no PCR bands were obtained for *C. bracarensis*. Thus, we suggest identifying the cryptic species using PCR (18) or MALDI-TOF MS in order to improve the specificity and sensitivity of the molecular detection of echinocandin resistance.

Accession number(s). The nucleotide sequences for *C. nivariensis FKS1* and *FKS2* genes were deposited in GenBank under the accession numbers KY323706 and KY494841, respectively (Fig. 1C).

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