Title: Comparative study for 147 *Candida* spp. identification and echinocandins susceptibility in isolates obtained from blood cultures in 15 hospitals, Medellin, Colombia

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Highlights

- Overall agreement between MALDI-TOF MS and molecular identification was 99.3%.
- Anidulafungin and caspofungin susceptibility by broth microdilution method were 98% and 88.4%
- Susceptibility to anidulafungin and caspofungin by Etest was 93.9% and 98.6%.
- Agreement between broth microdilution and Etest was 91.8% and 89.8% for anidulafungin and caspofungin.
- No mutations leading to amino acid changes were found in the *FKS* gene(s) in *Candida* spp. isolates.

Abstract

Introduction

Invasive candidiasis has high impact on morbidity and mortality in hospitalized patients. Accurate and timely methods for identification of *Candida* species and determination of equinocandins susceptibility become a priority for laboratories of clinical microbiology.

Methods

A study was performed to compare MALDI-TOF MS identification to sequencing of D1/D2 region of rRNA gene complex 28 subunit, in 147 isolates obtained from patients with candidemia. Susceptibility testing was performed by broth microdilution method and Etest®. DNA sequencing of FKS1 and FKS2 genes was performed.

Results

In this study, the most common species were *C. albicans* (40.8%), followed by *C. parapsilosis* (23.1%) and *C. tropicalis* (17.0%). Overall agreement between the results of identification by MALDI-TOF MS and molecular identification was 99.3%. Anidulafungin and caspofungin susceptibility by broth microdilution method were 98% and 88.4%, respectively. Susceptibility to anidulafungin and caspofungin by Etest was 93.9% and 98.6%. Categorical agreement between Etest and broth microdilution method was 91.8% for anidulafungin and 89.8% for caspofungin; with lower agreements in *C. parapsilosis* for anidulafungin (76.5%) and *C. glabrata* for caspofungin (40.0%). No mutations related to resistance were found in *FKS* genes, although 54 isolates presented synonymous polymorphisms in the hot spots sequenced.

Conclusions

MALDI TOF MS is a good alternative for routine identification of *Candida* isolates. DNA sequencing of *FKS* genes suggests that isolates analyzed are susceptible to echinocandins; alternatively, unknown resistance mechanisms or limitations related to antifungal susceptibility tests may explain the resistance found in few isolates.

Abbreviations

ANI: Anidulafungin

BMD: broth microdilution method

CAS: caspofungin,

CBS-KNAW: Fungal Biodiversity Centre, Utrecht, the Netherlands

CLSI: Clinical and Laboratory Standards Institute

D1/D2 region: large subunit 28S ribosomal RNA.

EUCAST: European Committee on Antimicrobial Susceptibility Testing

HS: hot spot

IC: Invasive candidiasis

IDSA: Infectious Diseases Society of America

IVD database: *In vitro* diagnostics database

MALDI-TOF MS: matrix assisted laser desorption ionization-time of flight-mass spectrometry

ME: major error

MIC: minimum inhibitory concentration

MIE: minor error

NCBI (BLASTn): National Center for Biotechnology Information, Washington, DC

VME: very major error.

Keywords: Candidemia; Candida; echinocandins susceptibility; molecular identification; MALDI-TOF MS Identification; FKS silent mutations.

1. Introduction

Invasive candidiasis (IC) represents over 70% of fungal infections in hospitalized patients (1). Ninety percent of IC is caused by five species: *Candida albicans*, *C. parapsilosis, C. glabrata, C. tropicalis* and *C. krusei* (2, 3). However, there are significant differences in the distribution of *Candida* species and their antifungal susceptibility according to geographic region (4, 5).

Conventional methods for identification of yeasts to the species level, based on macroscopic and microscopic characteristic, biochemical profile by manual or automated system as well as chromogenic agar media are widely used for identification of yeasts and offer efficiency and

accuracy to facilitate the diagnosis of fungal infections (6). Molecular identification of *Candida* species is considered the gold standard (7), however, this technology has high cost, is time-consuming and technically demanding.

Recently, matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used in routine diagnosis for the identification of microorganisms by analyzing their proteins spectra (8). This technology allows a rapid identification of different types of microorganisms, including common bacteria, mycobacteria, anaerobes, yeasts and molds (9). This identification technique has been evaluated compared to conventional methods and it has been found more accurate for *Candida* species identification (10, 11).

Mutations in *Candida FKS* genes, which encode the enzyme targeted by echinocandins, result in elevated minimum inhibitory concentration (MIC) and have been linked to therapeutic failures (12). Patients infected with *C. glabrata* isolates harboring *FKS* mutations are more likely to fail to echinocandins therapy than those infected with isolates without *FKS* mutations (13). In addition, patients with prior echinocandins exposure, treatment failure with echinocandins was frequent, as well as *FKS* mutation rates, in particular caspofungin MICs were significantly higher and caspofungin resistance was more common, compared with patients without prior exposure to echinocandins (14).

Echinocandin resistance is an emerging phenomenon in severely ill patients receiving longer-term therapy. Results of a population-based laboratory surveillance for candidemia in four metropolitan areas in the United States, conducted by Centers for Disease Control and Prevention, demonstrated that the proportion of non-susceptible (NS) *C. glabrata* rose from

4.2% in 2008 to 7.8% in 2014 and the proportion of NS isolates at each hospital ranged from 0% to 25.8% (15,16).

Several studies indicate that resistance to echinocandins in Latin America could be higher than that reported in other populations. A study performed from 2008 to 2010 in 21 tertiary care hospitals in Latin America, showed resistance to anidulafungin in two (4.8%) *C. glabrata* and in one (0.4%) *C. albicans* isolates. Intermediate susceptibility was found in two (1.7%) *C. tropicalis* isolates (17). More recently, Grupo GERMEN reported 7.3% and 7.7% resistance to anidulafungin and caspofungin, respectively; and found higher MICs for these echinocandins in almost all species (18) compared with that reported in previous studies involving isolates from North America, Asia - Pacific, Latin America and Europe (5).

Grupo GERMEN, a local network addressing antimicrobial resistance surveillance in Colombia, in a recent study conducted in 15 hospitals in Medellín, analyzed 300 isolates causing IC and found a variable resistance to fluconazole according to the species: 20% for *C. parapsilosis,* 7.6% for *C. albicans* and 7.4% for *C. tropicalis* (18). The azole-resistant *Candida* infections represent therapeutic challenges because the options for treatment are limited (19). Therefore, it is essential to make a rapid and accurate identification of isolates and determine their susceptibility to other antifungals, particularly echinocandins, which are the first-line therapy in patients with severe sepsis caused by azole-resistant *Candida* species (*C. glabrata*) or for those with prior exposure to azoles (20).

Due to the importance of echinocandins as first line antifungals in severe *Candida* infections it becomes crucial to know the epidemiology of resistant isolates but also the accuracy of laboratory microbiological methods available to identify *Candida* species and their susceptibility

to these antifungals. In the present study, using a collection of isolates from patients with candidemia, obtained and typed in a previous report (18), we compared MICs for echinocandins by Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (BMD) and Etest. In addition, the presence of *FKS* gene mutations were seek in all isolates. Species identification were performed comparatively using MALDI-TOF MS and sequencing of the D1/D2 region of the rRNA gene complex 28 subunit.

2. Material and methods

2.1. Sample

The sample consisted of 147 isolates of *Candida* spp. obtained from candidemia that were collected as part of a previous study that included hospitalized patients recruited from 15 hospitals located within Metropolitan Area of Medellín, Colombia, during a period of 15 months, between August 2010 and November 2011 (18). Preserved isolates were recovered using different methodologies according to their storage conditions and subsequently cultured on Sabouraud dextrose agar (Becton Dickinson and Company ®). All yeasts were incubated 48 to 72 hours, at room temperature (25°C) and their growth and purity were confirmed.

2.2. Molecular identification

The D1/D2 region of the rRNA gene complex 28 subunit was amplified following international guidelines for the molecular identification of fungi for *Candida* identification. Genomic DNA was extracted from isolated colonies grown in Sabouraud dextrose agar (Becton Dickinson and Co.) using a QIAamp DNA mini kit (QIAGEN, Germantown, MD), following the manufacturer**'**s recommendations. The molecular markers were amplified using the primers and protocols previously described for the D1/D2 region (7, 21). The amplified products from the D1/D2 region (~600 bp) were sent to Macrogen (Maryland, USA) for Sanger bidirectional sequencing. Sequencher 5.0 software (Gene Code Corporation) was used for editing and aligning the sequences. A search was then made in the following databases for each sequence to establish similarity with known strains: the NCBI databases (BLAST) (National Center for Biotechnology Information, Washington, DC), CBS-KNAW (Fungal Biodiversity Centre) and Mycobank database (International Mycological Association).

2.3. FKS sequencing

Genomic DNA was extracted from yeast cells grown overnight in YPD broth medium with QIAamp DNA mini kit (QIAGEN, Germantown, MD). PCR and sequencing primers were designed to amplify a ~3 Kb region of the echinocandin drug target genes *FKS1* and *FKS2* as previously described (22-24). PCR amplifications were carried out in a BIORAD iCycler (Biorad) in a 50 µl reaction volume containing 50 ng of genomic DNA, 0.2 µM of each primer and 25 µl of EmeraldAmp Master MIX (Takara Bio Inc). PCR products were purified by QIAquick PCR Purification Kit (Qiagen). Automated fluorescent sequencing was performed in both 5' and 3' by MacroGen USA (Macrogen Corp.). Sequences were assembled and edited using the SeqMan II and EditSeq software packages (Lasergene 12.0; DNAStar, Inc., Madison, WI).

2.4. MALDI-TOF MS Identification

Using a 1 µL plastic loop, a small quantity of a single colony was deposited on a spot of the target slide (disposable 48 wells target slide, bioMérieux) and processed according to manufacturer recommendations in a MALDI-TOF mass spectrometer (bioMérieux, Durham, NC). Resulting spectra were analyzed using the IVD database version 2.0 included in the Myla software. A quantitative value, the percent probability, was calculated and relates to how well the observed spectrum compares to the typical spectrum of each organism, so that values closer to 99.9% indicate a closer match; in this study, a cut-off of 90.0% was used. The calibration was performed using the reference strain *Escherichia coli* ATCC® 8739 and uninoculated matrix was included in each run as a negative control. All isolates were analyzed in duplicate.

2.5. Susceptibility testing

The broth microdilution method (BMD) for echinocandins was determinate in duplicate and performed in accordance with the Clinical and Laboratory Standards Institute (25). Caspofungin

and anidulafungin powders were obtained from Sigma (cat. SML0425-25MG) and Pfizer (Peapack, NJ, USA), respectively. The Etests® (bioMèrieux, Marcy l`Etoile, France) were performed in accordance with the manufacturer's recommendations. Minimum inhibitory concentrations (MICs) were interpreted by applying the clinical interpretive breakpoints defined by the CLSI in 2012 (26), the isolates were classified as susceptible (S), intermediate (I), or resistant (R) to both echinocandins, according to the following breakpoints: for *C. albicans/C. dubliniensis*, *C. tropicalis*, and *C. krusei* isolates with MIC ≤0.25 µg/mL were considered susceptible, and those with MIC ≥1 µg/mL were considered resistant; *C. parapsilosis* complex and *Candida guilliermondii* were considered susceptible with an MIC ≤2 µg/mL and resistant with an MIC ≥8 µg/ml. For *C. glabrata*, an MIC ≤0.12 µg/mL was susceptible, and MIC ≥0.5 µg/mL was considered resistant. Quality control was performed by testing *C. krusei* ATCC 6258 and *C. parapsilosis* 22019.

2.6. Agreement analysis

For each species, percent of agreement was calculated from the results of identification to species level obtained by MALDI -TOF MS and molecular identification of the D1/D2 region of the rRNA gene complex 28 subunit. Likewise, results of the MICs by both methods were compared and overall categorical agreement was calculated. Categorical agreement was defined as susceptible, intermediate, and resistant results that matched between the two methods. The results were considered to be in categorical agreement when the Etest results and reference MICs were within the same interpretive category. The percent of categorical agreement was calculated by dividing the number of tests with no category discrepancy by the number of organisms tested.

The percent of errors was ranked in three categories: very major error, consisted in false-susceptible result by the Etest method; major error, false-resistant result produced by the Etest; and minor error, intermediate result by Etest method and a resistant or susceptible category for the reference method (broth dilution test). According to CLSI, the acceptable levels recommended are <1.5% for VME, <3% for major errors and 10% for minor errors (25).

2.7. Ethics statement

This study, based on national and international ethical considerations, was a retrospective study limited to laboratory analysis of *Candida* spp. isolates from hospitalized patients, obtained as part of their clinical care. No additional procedures were performed and there were no risks associated to this research. The original investigation had the approval of the Ethics Committee of the Corporación para Investigaciones Biológicas and included the review and approval of hospitals participating in the selection of patients.

3. Results

3.1. Identification of *Candida* species

In this study, the most common species were *C. albicans* (40.8%), followed by *C. parapsilosis* (23.1%) and *C. tropicalis* (17.0%). Overall agreement between the results of identification by MALDI-TOF MS and molecular identification by sequencing the D1/D2 region of the rRNA gene complex 28 subunit was 99.3%. For most of species, the agreement between both identification methods was 100%, except for *C. auris*, for which none identification result was obtain by MALDI TOF method (Table 1).

3.2. Susceptibility results to anidulafungin and caspofungin

Overall susceptibility to anidulafungin and caspofungin found by using CLSI BMD method were 98% and 88.4% respectively, while the susceptibility to both echinocandins by Etest was 93.9% and 98.6%.

Differences in anidulafungin susceptibility between the two methods were observed in *C. parapsilosis*, 97.1% by BMD and 79.4% by Etest method. In contrast, susceptibility to caspofungin in *C. glabrata* was higher by Etest compared to the reference method, 100% and 20% respectively (Table 2).

Overall categorical agreement between BMD and Etest methods for anidulafungin and caspofungin was 91.8% and 89.8% respectively. However, differences in agreement were observed according to species and antifungal used; *C. parapsilosis* showed 76.5% and *M. guilliermondii* complex 80.0% for anidulafungin, for caspofungin *C. krusei* showed 33.3% and *C. glabrata* 40.0%. Etest results had higher MICs than the BMD method for anidulafungin, in

particular against *C. parapsilosis* and *M. guilliermondii* complex (MIC_{50} 1 µg/ml for Etest vs 0.5 µg/ml for BMD) (Table 3).

Using Etest, one very major error (VME), two (1.36%) major errors (ME) and nine (6.1%) minor errors (MIE) were found for anidulafungin. Two (1.4%) VME and 16 (10.8%) MIE were observed for caspofungin. A false susceptible result to anidulafungin was obtained by Etest in one isolate of *C. albicans*, and in two isolates of *C. glabrata* for caspofungin (VME).

Etest false resistant result to anidulafungin occurred in two isolates of *C. parapsilosis*. Six of the nine MIE that occurred in anidulafungin were in *C. parapsilosis*, of which five had intermediate susceptibility by Etest, being susceptible by the BMD method. Fourteen of the 16 MIE that occurred in caspofungin correspond to false susceptibility results by Etest, corresponding to an intermediate susceptibility result by the BMD method, especially in *C. glabrata*.

3.3. FKS sequencing

DNA sequencing of the two hot spot (HS) regions of the drug target gene *FKS1* was performed in all isolates of *C. albicans, C. tropicalis, C. krusei, C. lusitaniae and M. guilliermondii* complex. For *C. glabrata and C. parapsilosis*, the DNA sequencing of the two HS regions of the drug target genes *FKS1* and *FKS2* was performed.

No mutations leading to amino acid changes were found in the *FKS* gene(s) in the 147 *Candida* spp. isolates analyzed. However, 54 out of 147 isolates, presented polymorphisms (SNPs) in the hot spots sequenced. Forty-six of them (85%) had silent mutations mainly in the *FKS1* HS1 and 8 of them (15%) presented silent mutations in *FKS1* HS2.

The majority of the *FKS1* HS1 synonymous mutations were found in *C. albicans* (82%). Mutations in the *FKS1* HS2 were found only in *C. tropicalis* isolates. The following strains: *C. haemulonii* (n=1), *C. auris* (n=1) and *C. intermedia* (n=1) were not evaluated due to no reference sequence is available for these specific species.

4. Discussion

Identification of *Candida* species in clinical isolates is important, due to the differences in the antifungal susceptibility profiles that depends on the species with direct consequences in the election of antifungal treatment for these infections. Thus, the rapid identification of species will narrow the spectrum of therapeutic options, reduce costs and improve the outcome (1,2,5,27).

Agreement between MALDI TOF identification for 147 isolates of *Candida* compared to the molecular identification by DNA sequencing obtained in this study was 99.3%. This agreement was higher than the one reported by Galán et al. (96.7%) (8) and Lacroix et al. (98.3%) (28). According to this, MALDI TOF could be an alternative for a routine identification of *Candida* clinical isolates in diagnostic microbiology laboratories, taken advantage of the limited hands on of the method and the rapid results.

The only species that could not be identified by MALDI TOF method was *Candida auris* because this species was not included in the IVD database version 2.0 coupled to the Myla program (bioMérieux, Durham, NC). Recently, the company has released a new version that include *C. auris* in his RUO module. However, it is unknown the experience about the performance of the system identifying *C. auris* with this database addition. This species may be misidentified as *Candida haemulonii* by the Vitek 2 (29). Proper identification of *C. auris* has become particularly important since it is an emerging nosocomial pathogen that often exhibits resistance to fluconazole as it has been described recently in isolates from India and Venezuela (30,31).

No discrepancies in identification of other species were found; however, identification using sequencing showed three yeast isolates belonging to the *Meyerozyma guilliermondii* complex in this study. The genus *Meyerozyma* includes *Candida guilliermondii (Meyerozyma guilliermondii)*

and *Candida fermentati (Meyerozyma caribbica)* (32). These results confirm those reported by Romi et al. (33) who showed that species that are closely related in the *Meyerozima guilliermondii* complex are difficult to differentiate *in vitro* and it may be necessary the use of ITS-RFLP method for this purpose. Difficult identification using phenotype-based methods is also found in related species such as *Candida famata* and *C. haemulonii*, for this reason Brandt and Lockart recommend identification in these cases by using either molecular or MALDI-based methods (32).

In one isolate both identification methods used showed a lack of discrimination between species belonging to the Group *duobushaemuloniil/pseudohaemulonii*. In a study by Cendejas-Bueno et al. (34), it was concluded that *C. haemulonii*, *C. haemulonii* var*. vulnerabilidad*, *C. duobushaemulonii*, *C. pseudohaemulonii* and *C. auris* are part of a group of yeasts that presents difficult identification by different methods, suggesting the use of molecular methods based in non ITS targets.

Overall categorical agreement found between susceptibility results by Etest method and CLSI BMD for anidulafungin and caspofungin was 91.8% and 89.8% respectively. However, lower percentages of categorical agreement were observed in *C. parapsilosis* (76.5%) and *M. guilliermondii* complex (80%) for anidulafungin, and to caspofungin in *C. glabrata* (40%) and *C. krusei* (33.3%). Several studies have pointed out lower percentages of agreement between both methods for caspofungin in *C. glabrata* and *C. krusei* (27,35).

Some isolates of *C. parapsilosis* presented a decreased susceptibility to anidulafungin by Etest, but this phenomenon was not observed for caspofungin. This situation could have an important

clinical impact if Etest is used as routine susceptibility method, since it could lead to consider the no utilization of anidulafungin as a therapeutic option.

Higher MICs for anidulafungin in *C. parapsilosis* and *M. guilliermondii* complex were obtained by Etest as compared to those obtained by BMD method. This confirms the report by Espinel-Ingroff et al (36), in which they concluded that Etest is suitable for determination of susceptibility to anidulafungin in *C. albicans*, *C. tropicalis*, and *C. glabrata*, but not for *C. parapsilosis* and *C. guilliermondii*. These findings suggest that isolates showing decreased susceptibility to this antifungal by Etest in these two species must be tested by an alternative method.

The percentage of agreement obtained for caspofungin in *C. glabrata* with Etest was 40% and 33% for *C. krusei*, showing more isolates that had intermediate susceptibility and resistance than with BMD method. This is consistent with others reports that showed a significant lower percentage of concordance for caspofungin (47.8%) in *C. glabrata* (37).

Susceptibility to caspofungin also has been questioned when BMD is used, since several factors introduce variability in the results according to a recent multicenter study (38). Therefore, susceptibility results to caspofungin against *C. glabrata* with either method should be interpreted with caution in clinical practice when this echinocandin is used for severe infections.

Despite the discrepancies found when Etest is compared to the standard microdilution method, the overall discrepancies found in the present study were lower than the acceptable percentage recommended by CLSI (≤1.5%, and ≤3 % for VME and major errors, respectively) (25). This may support the use of Etest for the routine susceptibility testing of *Candida* isolates for echinocandins due to its easier inclusion in laboratory workflow; however, caution for interpreting

susceptibility results must be applied when *Candida* isolates different from *C. albicans* are predominant.

Sequencing of the two HS regions of the drug target gene(s) *FKS* known to confer echinocandin resistance did not show non-synonymous mutations in *Candida* spp. that were analyzed, even though some isolates turned out to be resistant to caspofungin but susceptible to anidulafungin (see *C. glabrata* Table 2). This may suggest that isolates analyzed in this study are in fact susceptible and the resistant and intermediate results could be due to the to intrinsic limitations of susceptibility phenotypic methods. Conversely, these results could suggest other echinocandins resistance mechanisms yet to be described.

In conclusion, MALDI TOF MS (Vitek MS) is an excellent alternative for a routine identification of *Candida* isolates in diagnostic microbiology laboratories. Due to the lack of agreement found for Etest in species different to *C. albicans*, this method should not be considered as a susceptibility method in these cases. Prospective studies are necessary to know the evolving resistance of echinocandins in *Candida* isolates since its increasing use should have an impact in resistance.

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Declarations

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References

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007 Jan;20(1):133-63.

2. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Meis JF, Gould IM, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance study, 1997 to 2005: an 8.5 years analysis ofsusceptibilities of Candida species and other yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol. 2007 Jun;45(6):1735-45. Epub 2007 Apr 18.

3. Quindós G. Epidemiology of candidaemia and invasive candidiasis. A changing face. Rev Iberoam Micol. 2014 Jan-Mar;31(1):42-8. doi: 10.1016/j.riam.2013.10.001. Epub 2013 Nov 19.

4. Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. Geographic variations in species distribution and echinocandin and azole antifungal resistance rates amongCandida bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to2009). J Clin Microbiol. 2011 Jan;49(1):396-9. doi: 10.1128/JCM.01398-10. Epub 2010 Nov 10.

5. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ng KP, Colombo A, et al. Geographic and temporal trends in isolation and antifungal susceptibility of Candida parapsilopsis: a globalassessment from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J Clin Microbiol. 2008b Mar;46(3):842-9. doi: 10.1128/JCM.02122-07. Epub 2008 Jan 16.

6 Stefaniuk E, Baraniak A, Fortuna M, Hryniewicz W. Usefulness of CHROMagar Candida Medium, Biochemical Methods--API ID32C and VITEK 2 Compact and Two MALDI-TOF MS Systems for Candida spp. Identification. Pol J Microbiol. 2016;65(1):111-4.

7. Clinical and Laboratory Standards Institute. 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing: approved guideline. MM18-A. Clinical and Laboratory Standards Institute. Wayne, PA.

8. Galán F, García-Agudo L, Guerrero I, Marín P, García-Tapia A, García-Martos P, et al. Evaluación de la espectrometría de masas en la identificación de levaduras de interés clínico. Enferm Infecc Microbiol Clin. 2015 Jun-Jul;33(6):372-8. doi: 10.1016/j.eimc.2014.10.003. Epub 2014 Nov 29.

9. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev. 2012 Mar;36(2):380-407. doi: 10.1111/j.1574-6976.2011.00298.x. Epub 2011 Aug 22

10. Keçeli SA, Dündar D, Tamer GS. Comparison of Vitek Matrix-assisted Laser Desorption /Ionization Time-of-Flight Mass Spectrometry Versus Conventional Methods in Candida Identification. Mycopathologia. 2016 Feb;181(1-2):67-73. doi: 10.1007/s11046-015-9944-8. Epub 2015 Sep 23.

11. Sow D, Fall B, Ndiaye M, Ba BS, Sylla K, Tine R, et al. Usefulness of MALDI-TOF Mass Spectrometry for Routine Identification of Candida Species in a Resource-Poor Setting.

Mycopathologia. 2015 Oct;180(3-4):173-9. doi: 10.1007/s11046-015-9905-2. Epub 2015 May 28.

12. Perlin DS. Mechanisms of echinocandin antifungal drug resistance. Ann N Y Acad Sci. 2015 Sep;1354:1-11. doi: 10.1111/nyas.12831. Epub 2015 Jul 17.

13. Shields RK, Nguyen MH, Press EG, Kwa AL, Cheng S, Du C, et al. The presence of an FKS mutation rather than MIC is an independent risk factor for failure of echinocandintherapy among patients with invasive candidiasis due to Candida glabrataAntimicrob Agents Chemother. 2012 Sep;56(9):4862-9. doi: 10.1128/AAC.00027-12. Epub 2012 Jul 2.

14. Shields RK, Nguyen MH, Press EG, Updike CL, Clancy CJ. Caspofungin MICs correlate with treatment outcomes among patients with Candida glabrata invasive candidiasis and prior echinocandin exposure. Antimicrob Agents Chemother. 2013 Aug;57(8):3528-35. doi: 10.1128/AAC.00136-13. Epub 2013 May 13.

15. Vallabhaneni S, Cleveland AA, Farley MM, Harrison LH, Schaffner W, Beldavs ZG, et al. Epidemiology and Risk Factors for Echinocandin Nonsusceptible Candida glabrata Bloodstream Infections:Data From a Large Multisite Population-Based Candidemia Surveillance Program, 2008-2014. Open Forum Infect Dis. 2015 Dec 14;2(4):ofv163. doi: 10.1093/ofid/ofv163. eCollection 2015.

16. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, et al. Increasing echinocandins resistance in Candida glabrata: clinical failure correlates with presence

of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis. 2013 Jun;56(12):1724-32. doi: 10.1093/cid/cit136. Epub 2013 Mar 13.

17. Nucci M, Queiroz-Telles F, Alvarado-Matute T, Tiraboschi IN, Cortes J, Zurita J, et al. Epidemiology of candidemia in Latin America: a laboratory-based survey. PLoS One. 2013;8(3):e59373. doi: 10.1371/journal.pone.0059373. Epub 2013 Mar 19.

18. Maldonado NA, Cano LE, De Bedout C, Arbeláez CA, Roncancio G, Tabares AM, et al. Association of clinical and demographic factors in invasive candidiasis caused by fluconazole-resistantCandida species: a study in 15 hospitals, Medellín, Colombia 2010-2011. Diagn Microbiol Infect Dis. 2014 Jun;79(2):280-6. doi: 10.1016/j.diagmicrobio.2014.02.003. Epub 2014 Feb 22.

19. Bassetti M, Righi E, Costa A, Fasce R, Molinari MP, Rosso R, et al. Epidemiological trends in nosocomial candidemia in intensive care. BMC Infect Dis. 2006 Feb 10;6:21.

20. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016 Feb 15;62(4):e1-50. doi: 10.1093/cid/civ933. Epub 2015 Dec 16.

21. White TJ, Bruns T, Lee S, and Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, eds. Innis MA, Gelfand DH, Sninsky JJ, White TJ. Academic Press, Inc., New York. 1990. 315-322.

22. Douglas CM, D'Ippolito JA, Shei GJ, Meinz M, Onishi J, Marrinan JA, et al. Identification of the FKS1 gene of Candida albicans as the essential target of 1,3-beta-D-glucan synthase inhibitors. Antimicrob Agents Chemother. 1997 Nov;41(11):2471-9.

23. Douglas CM, Marrinan JA, Li W, Kurtz MB. A Saccharomyces cerevisiae mutant with echinocandin-resistant 1,3-beta-D-glucan synthase. J Bacteriol. 1994 Sep;176(18):5686-96.

24. Kurtz MB, Abruzzo G, Flattery A, Bartizal K, Marrinan JA, Li W, et al. Characterization of echinocandin-resistant mutants of Candida albicans: genetic, biochemical, and virulence studies. Infect Immun. 1996 Aug;64(8):3244-51.

25. Clinical and Laboratory Standards Institute (CLSI). 2016. Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters. 4th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute, Wayne, PA.

26. Clinical and Laboratory Standards Institute (CLSI) 2012. Reference method for broth dilution antifungal susceptibility testing of yeasts; 4th informational supplement. CLSI document M27-S4. Clinical and Laboratory Standards Institute, Wayne, PA.

27. Arendrup MC, Dzajic E, Jensen RH, Johansen HK, Kjaeldgaard P, Knudsen JD, et al. Epidemiological changes with potential implication for antifungal prescription recommendations for fungaemia: data from a nationwide fungaemia surveillance programme. Clin Microbiol Infect. 2013 Aug;19(8):E343-53. doi: 10.1111/1469-0691.12212. Epub 2013 Apr 22.

28. Lacroix C, Gicquel A, Sendid B, Meyer J, Accoceberry I, François N, et al. Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of Candida species. Clin Microbiol Infect. 2014 Feb;20(2):153-8. doi: 10.1111/1469-0691.12210. Epub 2013 Apr 17.

29. Lee WG, Shin JH, Uh Y, Kang MG, Kim SH, Park KH, Jang HC. First three reported cases of nosocomial fungemia caused by Candida auris. J Clin Microbiol. 2011 Sep;49(9):3139-42. doi: 10.1128/JCM.00319-11. Epub 2011 Jun 29.

30. Prakash A, Sharma C, Singh A, Kumar Singh P, Kumar A, Hagen F, el al. Evidence of genotypic diversity among Candida auris isolates by multilocus sequence typing, matrix-assisted laser desorption ionization time-of-flight mass spectrometry and amplified fragment length polymorphism. Clin Microbiol Infect. 2016 Mar;22(3):277.e1-9. doi: 10.1016/j.cmi.2015.10.022. Epub 2015 Nov 5.

31. Calvo B, Melo AS, Perozo-Mena A, Hernandez M, Francisco EC, Hagen F et al. First report of Candida auris in America: Clinical and microbiological aspects of 18 episodes of candidemia. J Infect. 2016 Jul 21. pii: S0163-4453(16)30172-4. doi: 10.1016/j.jinf.2016.07.008. [Epub ahead of print]

32. Brandt ME and Lockart SR. Recent taxonomic Developments with Candida and Other Opportunistic Yeast. Curr Fungal Infect Rep 2012 Sep;6(3):170-177. DOI:10.1007/s12281-012-0094-x.

33. Romi W, Keisam S, Ahmed G, Jeyaram K1. Reliable differentiation of Meyerozyma guilliermondii from Meyerozyma caribbica by internal transcribed spacerrestriction fingerprinting. BMC Microbiol. 2014 Feb 28;14:52. doi: 10.1186/1471-2180-14-52.

34. Cendejas-Bueno E, Kolecka A, Alastruey-Izquierdo A, Theelen B, Groenewald M, Kostrzewa M, et al. Reclassification of the Candida haemulonii complex as Candida haemulonii (C. haemulonii group I), C. duobushaemulonii sp. nov. (C. haemulonii group II), and C. haemulonii var. vulnera var. nov.: three multiresistant human pathogenic yeasts. J Clin Microbiol. 2012 Nov;50(11):3641-51. doi: 10.1128/JCM.02248-12. Epub 2012 Sep 5.

35. Bourgeois N, Laurens C, Bertout S, Balard Y, Krasteva D, Rispail P, et al. Assessment of caspofungin susceptibility of Candida glabrata by the Etest®, CLSI, and EUCAST methods, and detection of FKS1 and FKS2 mutations. Eur J Clin Microbiol Infect Dis. 2014 Jul;33(7):1247-52. doi: 10.1007/s10096-014-2069-z. Epub 2014 Feb 13.

36. Espinel-Ingroff A, Canton E, Peman J, Martín-Mazuelo E. Comparison of anidulafungin MICs determined by the clinical and laboratory standards institute broth microdilution method (M27-A3 document) and Etest for Candida species isolates. Antimicrob Agents Chemother. 2010 Mar;54(3):1347-50. doi: 10.1128/AAC.01324-09. Epub 2009 Dec 22.

37. Ben-Ami R, Hilerowicz Y, Novikov A, Giladi M. The impact of new epidemiological cutoff values on Candida glabrata resistance rates and concordance between testing methods. Diagn Microbiol Infect Dis. 2014 Jun;79(2):209-13. doi: 10.1016/j.diagmicrobio.2014.02.008. Epub 2014 Feb 24.

38. Espinel-Ingroff A, Arendrup MC, Pfaller MA, Bonfietti LX, Bustamante B, Canton E, et al. Interlaboratory variability of Caspofungin MICs for Candida spp. Using CLSI and EUCAST methods: should the clinical laboratory be testing this agent? Antimicrob Agents Chemother. 2013 Dec;57(12):5836-42. doi: 10.1128/AAC.01519-13. Epub 2013 Sep 9.

Table 1. Performance of MALDI TOF MS vs. molecular identification by ribosomal RNA sequencing (region D1/D2) of *Candida* spp.

***Candida* spp. identified by sequencing**	**No. of isolates**	**(%)**	**MALDI TOF identification**	**No. of isolates**	**Agreement %**
C.albicans	60	40.8	*C. albicans*	60	100
C.parapsilosis	34	23.1	*C. parapsilosis*	34	100
C. tropicalis	25	17.0	*C. tropicalis*	25	100
C. glabrata	10	6.8	*C. glabrata*	10	100
C. guilliermondii	7	4.7	*C. guilliermondii*	7	100
C. krusei	3	2.04	*C. krusei*	3	100
Meyerozyma guilliermondii complex (Meyerozyma guilliermondii / M. caribbica)	3	2.04	*C. guilliermondii*	3	100
C. lusitaniae	2	1.3	*C. lusitaniae*	2	100
C. intermedia	1	0.68	*C. intermedia*	1	100
C. auris	1	0.68	Without identification	1	0.00
Candida duobushaemulonii / pseudohaemulonii group	1	0.68	*C. haemulonii*	1	100
Overall agreement	147			147	99.3

Table 2. Comparative susceptibility results to anidulafungin and caspofungin by BMD method and Etest

Candida species	Interpretative category*	n (%) susceptibility			
		Anidulafungin		Caspofungin	
		BMD	Etest	BMD	Etest
C. albicans (n=60)	S	59 (98.3)	60 (100)	56 (93.3)	60 (100)
	I	0 (0.0)	0 (0.0)	4 (6.7)	0 (0.0)
	R	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)
C. parapsilosis (n=34)	S	33 (97.1)	27 (79.4)	34 (100)	34 (100)
	I	1 (2.9)	4 (11.7)	0 (0.0)	0 (0.0)
	R	0 (0.0)	3 (8.8)	0 (0.0)	0 (0.0)
C. tropicalis (n=25)	S	25 (100)	25 (100)	23 (92.0)	25 (100)
	I	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)
	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. glabrata (n=10)	S	9 (90)	10 (100)	2 (20.0)	10 (100)
	I	1 (10)	0 (0.0)	6 (60.0)	0 (0.0)
	R	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)
Meyerozyma guilliermondii complex (Meyerozyma guilliermondii / M. caribbica) ** (n=10)	S	10 (100)	8 (80.0)	10 (100)	10 (100)
	I	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)
	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. krusei (n=3)	S	3 (100)	3 (100)	2 (66.7)	2 (66.7)
	I	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)
	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other *Candida* spp. *** (n=5)	S	5 (100)	5 (100)	3 (60.0)	4 (80.0)
	I	0 (0.0)	0 (0.0)	2 (40.0)	1 (20.0)
	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total Candida (n=147)	S	144 (97.9)	138 (93.9)	130 (88.4)	145 (98.6)
	I	2 (1.36)	6 (4.1)	15 (10.2)	2 (1.36)
	R	1 (0.68)	3 (2.0)	2 (1.36)	0 (0.0)

* Susceptible (S), Intermediate (I), Resistant (R).

** Includes: 7 *C. guilliermondii* and 3 *Meyerozyma guilliermondii complex (Meyerozyma guilliermondii / M. caribbica)* isolates.

*** Other *Candida* species includes: *C. lusitaniae, C. intermedia, C. auris*, *C. duobushaemulonii/ pseudohaemulonii group.*

Table 3. MIC_{50}, MIC_{90} and percent of categorical agreement between the susceptibility results by Etest and CLSI BMD method for anidulafungin and caspofungin

	Anidulafungin			Caspofungin		
	MIC_{50}	MIC_{90}	% Categorical agreement	MIC_{50}	MIC_{90}	% Categorical agreement
C. albicans (n=60)						
BMD	0.03	0.064	98.3	0.125	0.25	93.3
Etest	0.002	0.004		0.012	0.064	
C. parapsilosis (n=34)						
BMD	0.5	2	76.5	0.5	1	100
Etest	1	3		0.5	0.5	
C. tropicalis (n=25)						
BMD	0.03	0.03	100	0.125	0.25	92.0
Etest	0.012	0.016		0.023	0.094	
C. glabrata (n=10)						
BMD	0.03	0.03	90.0	0.25	0.5	40.0
Etest	0.004	0.008		0.023	0.094	
Meyerozyma guilliermondii complex (Meyerozyma guilliermondii / M. caribbica) * (n=10)						
BMD	0.5	1	80.0	0.5	0.5	100
Etest	1	3		0.38	1	
C. krusei (n=3)						
BMD	0.064	0.064	100	0.25	0.5	33.3
Etest	0.012	0.023		0.25	0.5	
Other *Candida* species** *(n=5)*						
BMD	0.032	0.032	100	0.25	0.5	80.0
Etest	0.012	0.023		0.25	0.38	
Total						
BMD	0.03	1	**91.8**	0.25	1	**89.8**
Etest	0.008	2		0.064	0.5	

MIC: Minimum Inhibitory Concentration
BMD: broth microdilution method
* Includes: 7 *C. guilliermondii* and 3 *Meyerozyma guilliermondii complex (Meyerozyma guilliermondii / M. caribbica)* isolates
**Other *Candida* species includes: *C. lusitaniae, C. intermedia, C. auris*, *C. duobushaemulonii/ pseudohaemulonii* group