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Candida lusitaniae MICs to the echinocandins are elevated but *FKS*-mediated resistance is rare

Shawn R. Lockhart[*], **Cau D. Pham**, **Randall J. Kuykendall**, **Carol B. Bolden**, and **Angela A. Cleveland**

Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA

Abstract

MIC values were generated for caspofungin, micafungin and anidulafungin against 106 isolates of *C. lusitaniae* and these values were compared to established epidemiologic cutoff values. The majority of isolates were wild type both by MIC value as well as by *FKS1* hotspot sequencing. Although *C. lusitaniae* isolates have MIC values to the echinocandins that are elevated compared to other common species, with regards to known mechanisms of resistance to the echinocandins, isolates with MIC values at or below the ECVs of 0.5 and 1 μg/mL for micafungin and anidulafungin, respectively, should be considered wild type.

Keywords

Candida lusitaniae; echinocandin; FKS; epidemiological cutoff value

Yeast of the genus *Candida* are one of the most frequent causes of bloodstream infections in the US (1, 2). The five most common species of *Candida*, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* comprise up to 95% of *Candida* infections (3–6). Other species, such as *C. dubliniensis*, *C. lusitaniae* and *C. guilliermondii*, comprise 1–2% each of isolates, and a few isolates each of 10–15 other *Candida* species make up the rest of the isolates in any given surveillance (3–5, 7). In recent population-based surveillance in two US cities, *C. lusitaniae* was the sixth most common species overall, surpassing *C. krusei* and falling just below *C. dubliniensis* (5, 8). In a recent report from passive US surveillance, *C. lusitaniae* accounted for 1.6% of 2,496 isolates (9). Globally, *C. lusitaniae* tends to be the 6th or 7th most common species in most surveillance and ranges roughly from 1–2% of the isolates (10–14).

The Clinical and Laboratory Standards Institute (CLSI) recently established species-specific breakpoint minimum inhibitory concentration (MIC) values for caspofungin, micafungin and anidulafungin against six different *Candida* species (15). For species/antifungals with no breakpoints, epidemiological cutoff values (ECVs) are being established, but data on ECVs for *C. lusitaniae* have not been well described (16–20). The ECV is the MIC value that defines the upper limit of the wild type population of MIC values and is meant to distinguish

[*]Send all correspondence to: Dr. Shawn R. Lockhart, Mycotic Diseases Branch, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop G-11, Atlanta, GA 30333, Phone: (404)639-2569, FAX: (404)639-3546, gyi2@cdc.gov.

between a wild type isolate and a non-wild type isolate that may have acquired resistance or reduced susceptibility to the given antifungal agent.

Isolates of *C. lusitaniae* were collected from 2008–2014 as part of a population-based surveillance system in metropolitan Atlanta, GA and Baltimore City and County, MD as described (5) and from 2011–2014 in Knox County, TN and metropolitan Portland area, OR. All isolates were identified as *C. lusitaniae* by either DNA-based Luminex assay or D1D2 sequencing (5). From 5806 bloodstream isolates of *Candida*, 106 (1.8%) were identified as *C. lusitaniae*. The proportion of *C. lusitaniae* varied by site with TN and GA having the highest proportion at 2.1% of isolates, and the lowest proportion in OR at 1.3%.

The role of susceptibility testing is to detect isolates with elevated MIC values that may not respond to antifungal therapy and is generally only useful when there is a breakpoint MIC value for comparison. MIC values were generated according to the methods of the CLSI guideline M27-A3 (21) using frozen RPMI microbroth trays without indicator dye custom manufactured by TREK Diagnostics (Cleveland, OH). Results were read visually after 24 hours of incubation, and quality control isolates *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included on each day of testing. The results for the 106 *C. lusitaniae* isolates are shown in Table 1.

ECV values for *C. lusitaniae* have been previously published in both single laboratory and multi-laboratory studies (17, 22–24). For the echinocandins, the *C. lusitaniae* ECV fall between the lower values seen for *C. albicans* and *C. glabrata* and the higher values seen for *C. parapsilosis* and *C. guilliermondii*. For anidulafungin, the ECV is 1.0 μg/mL and for micafungin it is 0.5 μg/mL. These values are much higher than those for *C. glabrata* and *C. albicans* (0.12 μg/mL and 0.03 μg/mL for anidulafungin and micafungin, respectively), but much lower than the values of 8 μg/mL and 2 μg/mL for *C. guilliermondii* and 8 μg/mL and 4 μg/mL for *C. parapsilosis*. We used the statistical program of Turnidge et al. (25) to calculate the ECVs for our cohort. Our ECVs at a 97.5% cutoff were 1.0 μg/mL, 0.5 μg/mL, and 2.0 μg/mL for caspofungin, micafungin, and anidulafungin, respectively. Our ECVs for micafungin and anidulafungin were equal to and one dilution higher, respectively, than those established by a large multicenter study (22). That study did not evaluate caspofungin due to technical issues that have been noted with the testing of that antifungal (26). According to the ECVs generated here, one, five, and zero isolates would be considered non-wild type by the MICs generated for caspofungin, micafungin, and anidulafungin, respectively. Using the anidulafungin ECV of 1 μg/mL, an additional isolate would be considered non-wild type.

Given that there were five isolates that had non-wild type MIC values to the echinocandins, the *FKS1* hotspot1 and hotspot2 regions from all 106 *C. lusitaniae* isolates were sequenced to look for hotspot mutations. *FKS* sequencing was performed essentially as described by Zimbeck and coworkers (27) with the exception that the primers used were specific for *C. lusitaniae* as described by Desnos-Ollivier and coworkers (28). There was only a single isolate with an amino acid change in hotspot 1, an F640L substitution. That isolate had anidulafungin and caspofungin MICs of ≥ 1 μg/mL and a micafungin MIC of ≥ 2 μg/mL. The single isolate with all three echinocandin MIC values above the ECV (caspofungin, 8 μg/mL; micafungin 4 μg/mL; anidulafungin 2 μg/mL) had a wild type sequence (no amino

acid changes) at *FKS1* hotspot1 and hotspot2. Despite having no mutations in the *FKS1* hotspots, 5.7% of the isolates would be considered non-wild type using the ECVs of Pfaller and coworkers (22).

Information on prior echinocandin exposure was available for 85 of the *C. lusitaniae* isolates. Of these, 10 (12%) had prior exposure to micafungin including the isolates with the *FKS1* mutation. Only one of the isolates with prior echinocandin exposure had elevated MIC values so there was no clear correlation between MIC value and prior echinocandin exposure.

Although the MIC values for *C. lusitaniae* against the echinocandins are elevated, the vast majority of isolates in this population-based cohort had MIC values which were below the statistically calculated echinocandin ECV values. For the few isolates that did have MIC values above the ECV, there was no evidence that *FKS1* mutations were responsible for this elevation. This would indicate that *C. lusitaniae* should be considered more along the lines of *C. parapsilosis* and *C. guilliermondii*, with elevated echinocandin MIC values as the norm rather than the exception. It is suggested here that isolates of *C. lusitaniae* that have an MIC value less than or equal to the ECV of 1.0 μg/mL for anidulafungin and 0.5 μg/mL for micafungin are truly wild type in terms of the *FKS1* gene and can be treated accordingly.

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References

1. Wisplinghoff H, Ebbers J, Geurtz L, Stefanik D, Major Y, Edmond MB, Wenzel RP, Seifert H. Nosocomial bloodstream infections due to *Candida* spp. in the USA: species distribution, clinical features and antifungal susceptibilities. International J. Antimicrob. Agents. 2014; 43:78–81.
2. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect. Contrl. Hosp. Epidemiol. 2008; 29:996–1011.
3. Pfaller MA, Messer SA, Moet GJ, Jones RN, Castanheira M. *Candida* bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in Intensive Care Unit (ICU) and non-ICU settings in the SENTRY Antimicrobial Surveillance Program (2008–2009). Intnational. J antimicrobial agents. 2011; 38:65–69.
4. Lyon GM, Karatela S, Sunay S, Adiri Y. Antifungal susceptibility testing of *Candida* isolates from the *Candida* surveillance study. Journal of clinical microbiology. 2010; 48:1270–1275. [PubMed: 20129963]
5. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, Baughman W, Stein B, Hollick R, Park BJ, Chiller T. Species identification and antifungal susceptibility testing of *Candida*

bloodstream isolates from population-based surveillance studies in two U.S. cities from 2008 to 2011. Journal of clinical microbiology. 2012; 50:3435–3442. [PubMed: 22875889]

6. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, Horn D. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance(R)) registry, 2004–2008. Diagnostic microbiology and infectious disease. 2012; 74:323–331. [PubMed: 23102556]
7. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clinical microbiology reviews. 2007; 20:133–163. [PubMed: 17223626]
8. Cleveland AA, Farley MM, Harrison LH, Stein B, Hollick R, Lockhart SR, Magill SS, Derado G, Park BJ, Chiller TM. Changes in incidence and antifungal drug resistance in candidemia: results from population-based laboratory surveillance in Atlanta and Baltimore, 2008–2011. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012; 55:1352–1361. [PubMed: 22893576]
9. Pfaller MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, Franks B, Azie NE. Epidemiology and outcomes of invasive candidiasis due to non- *albicans* species of *Candida* in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004–2008. PloS one. 2014; 9:e101510. [PubMed: 24991967]
10. Faria-Ramos I, Neves-Maia J, Ricardo E, Santos-Antunes J, Silva AT, Costa-de-Oliveira S, Canton E, Rodrigues AG, Pina-Vaz C. Species distribution and in vitro antifungal susceptibility profiles of yeast isolates from invasive infections during a Portuguese multicenter survey. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2014; 33:2241–2247.
11. Montagna MT, Lovero G, Borghi E, Amato G, Andreoni S, Campion L, Lo Cascio G, Lombardi G, Luzzaro F, Manso E, Mussap M, Pecile P, Perin S, Tangorra E, Tronci M, Iatta R, Morace G. Candidemia in intensive care unit: a nationwide prospective observational survey (GISIA-3 study) and review of the European literature from 2000 through 2013. European review for medical and pharmacological sciences. 2014; 18:661–674. [PubMed: 24668706]
12. Orasch C, Marchetti O, Garbino J, Schrenzel J, Zimmerli S, Muhlethaler K, Pfyffer G, Ruef C, Fehr J, Zbinden R, Calandra T, Bille J. *Candida* species distribution and antifungal susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing and new vs. old Clinical and Laboratory Standards Institute clinical breakpoints: a 6-year prospective candidaemia survey from the fungal infection network of Switzerland. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014; 20:698–705.
13. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-*albicans Candida* spp among candidemia isolates from inpatients in various parts of the world: a systematic review. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2010; 14:e954–966. [PubMed: 20797887]
14. Diekema DJ, Messer SA, Boyken LB, Hollis RJ, Kroeger J, Tendolkar S, Pfaller MA. In vitro activity of seven systemically active antifungal agents against a large global collection of rare *Candida* species as determined by CLSI broth microdilution methods. Journal of clinical microbiology. 2009; 47:3170–3177. [PubMed: 19710283]
15. Clinical and Laboratory Standards Institute. M27-S4 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. Clinical and Laboratory Standards Institute; Wayne, PA: 2012.
16. Espinel-Ingroff A, Aller AI, Canton E, Castanon-Olivares LR, Chowdhary A, Cordoba S, Cuenca-Estrella M, Fothergill A, Fuller J, Govender N, Hagen F, Illnait-Zaragozi MT, Johnson E, Kidd S, Lass-Florl C, Lockhart SR, Martins MA, Meis JF, Melhem MS, Ostrosky-Zeichner L, Pelaez T, Pfaller MA, Schell WA, St-Germain G, Trilles L, Turnidge J. *Cryptococcus neoformans-Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for fluconazole, itraconazole, posaconazole, and voriconazole. Antimicrobial agents and chemotherapy. 2012; 56:5898–5906. [PubMed: 22948877]
17. Espinel-Ingroff A, Pfaller MA, Bustamante B, Canton E, Fothergill A, Fuller J, Gonzalez GM, Lass-Florl C, Lockhart SR, Martin-Mazuelos E, Meis JF, Melhem MS, Ostrosky-Zeichner L, Pelaez T, Szeszs MW, St-Germain G, Bonfietti LX, Guarro J, Turnidge J. Multilaboratory study of

epidemiological cutoff values for detection of resistance in eight *Candida* species to fluconazole, posaconazole, and voriconazole. Antimicrobial agents and chemotherapy. 2014; 58:2006–2012. [PubMed: 24419346]

18. Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida* : time for harmonization of CLSI and EUCAST broth microdilution methods. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy. 2010; 13:180–195. [PubMed: 21050800]
19. Pfaller MA, Espinel-Ingroff A, Canton E, Castanheira M, Cuenca-Estrella M, Diekema DJ, Fothergill A, Fuller J, Ghannoum M, Jones RN, Lockhart SR, Martin-Mazuelos E, Melhem MS, Ostrosky-Zeichner L, Pappas P, Pelaez T, Peman J, Rex J, Szeszs MW. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole and *Candida* spp. as determined by CLSI broth microdilution. Journal of clinical microbiology. 2012; 50:2040–2046. [PubMed: 22461672]
20. Espinel-Ingroff A, Chakrabarti A, Chowdhary A, Cordoba S, Dannaoui E, Dufresne P, Fothergill A, Ghannoum M, Gonzalez GM, Guarro J, Kidd S, Lass-Florl C, Meis JF, Pelaez T, Tortorano AM, Turnidge J. Multicenter evaluation of MIC distributions for epidemiologic cutoff value definition to detect amphotericin B, posaconazole, and itraconazole resistance among the most clinically relevant species of Mucorales. Antimicrobial agents and chemotherapy. 2015; 59:1745–1750. [PubMed: 25583714]
21. Clinical and Laboratory Standards Institute. CLSI Document M27-A3. Clinical and Laboratory Standards Institute; Wayne, PA: 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Third Edition.
22. Pfaller MA, Espinel-Ingroff A, Bustamante B, Canton E, Diekema DJ, Fothergill A, Fuller J, Gonzalez GM, Guarro J, Lass-Florl C, Lockhart SR, Martin-Mazuelos E, Meis JF, Ostrosky-Zeichner L, Pelaez T, St-Germain G, Turnidge J. Multicenter Study of Anidulafungin and Micafungin MIC Distributions and Epidemiological Cutoff Values for Eight *Candida* Species and the CLSI M27-A3 Broth Microdilution Method. Antimicrobial agents and chemotherapy. 2014; 58:916–922. [PubMed: 24277027]
23. Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, Jones RN, Turnidge J, Diekema DJ. Wild-type MIC distributions and epidemiological cutoff values for the echinocandins and *Candida* spp. Journal of clinical microbiology. 2010; 48:52–56. [PubMed: 19923478]
24. Pfaller MA, Castanheira M, Diekema DJ, Messer SA, Jones RN. Triazole and echinocandin MIC distributions with epidemiological cutoff values for differentiation of wild-type strains from non-wild-type strains of six uncommon species of *Candida*. Journal of clinical microbiology. 2011; 49:3800–3804. [PubMed: 21900519]
25. Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2006; 12:418–425.
26. Espinel-Ingroff A, Arendrup MC, Pfaller MA, Bonfietti LX, Bustamante B, Canton E, Chryssanthou E, Cuenca-Estrella M, Dannaoui E, Fothergill A, Fuller J, Gaustad P, Gonzalez GM, Guarro J, Lass-Florl C, Lockhart SR, Meis JF, Moore CB, Ostrosky-Zeichner L, Pelaez T, Pukinskas SR, St-Germain G, Szeszs MW, Turnidge J. Interlaboratory variability of Caspofungin MICs for *Candida* spp. Using CLSI and EUCAST methods: should the clinical laboratory be testing this agent? Antimicrobial agents and chemotherapy. 2013; 57:5836–5842. [PubMed: 24018263]
27. Zimbeck AJ, Iqbal N, Ahlquist AM, Farley MM, Harrison LH, Chiller T, Lockhart SR. *FKS* mutations and elevated echinocandin MIC values among *Candida glabrata* isolates from U.S. population-based surveillance. Antimicrobial agents and chemotherapy. 2010; 54:5042–5047. [PubMed: 20837754]
28. Desnos-Ollivier M, Moquet O, Chouaki T, Guerin AM, Dromer F. Development of echinocandin resistance in *Clavispora lusitaniae* during caspofungin treatment. Journal of clinical microbiology. 2011; 49:2304–2306. [PubMed: 21490186]

Table 1

MIC distributions of 106 *C. lusitaniae* isolates against caspofungin, micafungin and anidulafungin.

	MIC value in μg/ml										
	≤0.008	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**≥8**
caspofungin	0	0	5	9	8	46	32	5	0	0	1
micafungin	1	6	1	5	23	61	4	1	3	1	0
anidulafungin	1	2	3	8	12	25	42	12	1	0	0

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