Journal of Global Antimicrobial Resistance 21 (2020) 331–334

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

In vitro synergy of echinocandins with triazoles against fluconazole-resistant *Candida parapsilosis* complex isolates

Ali Ahmadi[a], Shahram Mahmoudi[b], Sassan Rezaie[a], Sayed Jamal Hashemi[a], Eric Dannaoui[c], Hamid Badali[d,e], Mansoureh Ghaffari[f], Farzad Aala[g], Alireza Izadi[a], Aida Maleki[a], Jacques F. Meis[h,i], Sadegh Khodavaisy[a,*]

[a] *Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[b] *Department of Medical Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
[c] *Université Paris Descartes, Faculté de Médecine, AP-HP, Hôpital Européen Georges Pompidou, Unité de Parasitologie-Mycologie, F-75015, Paris, France*
[d] *Invasive Fungi Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[e] *Fungus Testing Laboratory, Department of Pathology and Laboratory Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States*
[f] *Department of Microbiology, Faculty of Science, Islamic Azad University, Varamin-Pishva, Iran*
[g] *Department of Parasitology and Mycology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*
[h] *Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands*
[i] *Centre of Expertise in Mycology, Radboud University Medical Centre, Canisius-Wilhelmina Hospital Nijmegen, the Netherlands*

ARTICLE INFO

Article history:
Received 6 July 2019
Accepted 1 November 2019
Available online 9 November 2019

Keywords:
Drug synergism
Triazoles
Echinocandins
Candida parapsilosis species complex

ABSTRACT

Introduction: Candida parapsilosis (*C. parapsilosis*) is a common non-*albicans Candida* species ranked as the second common cause of bloodstream infections. Azole resistance and elevated echinocandin MICs have been reported for these fungi. This study was conducted to determine the interactions between azoles and echinocandins against *C. parapsilosis* species complex.

Materials and methods: Fifteen fluconazole-resistant clinical isolates of *C. parapsilosis* complex were included: *C. parapsilosis* sensu stricto (n = 7), *C. orthopsilosis* (n = 5) and *C. metapsilosis* (n = 3). The activity of azoles (fluconazole, itraconazole) and echinocandins (anidulafungin, micafungin) alone and in combination was determined using checkerboard broth microdilution. The results were determined based on the fractional inhibitory concentration index (FICI).

Results: In vitro combination of fluconazole with anidulafungin was found to be synergistic (FICI 0.07–0.37) and decreased the MIC range from 4–64 μg/mL to 0.5–16 μg/mL for fluconazole and from 2–8 μg/mL to 0.125–1 μg/mL for anidulafungin. Similarly, interactions of fluconazole with micafungin (FICI 0.25–0.5), itraconazole with anidulafungin (FICI 0.15–0.37) and itraconazole with micafungin (FICI 0.09–0.37) were synergistic.

Conclusion: The combination of fluconazole and itraconazole with either anidulafungin or micafungin demonstrated synergistic interactions against *C. parapsilosis* species complex, especially against isolates with elevated MIC values. However, the use of these combinations in clinical practice and the clinical relevance of *in vitro* combination results remain unclear.

1. Introduction

In recent decades, a global shift from *Candida albicans* (*C. albicans*) to non-*albicans Candida* species has been reported [1]. *Candida parapsilosis* (*C. parapsilosis*) is a non-*albicans* species serving as the second most common cause of bloodstream infections, preceded by *C. albicans* [2]. It has also been found to be as common as *C. albicans* among cases of invasive candidiasis in Serbia [3] and as the leading cause of candidaemia before 2010 in Europe [4], Asia [5], South America [6], and after 2010 in Iran [7], Venezuela and Colombia [8]. Using molecular analysis, *C. parapsilosis* was found to be a complex of three cryptic species: *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* [9]. *C. parapsilosis* is able to produce biofilm on catheters and other medical devices, leading to increased resistance to azole antifungal drugs [2,10]. It also tends to persist in the hospital environment and has frequently been isolated from hospital surfaces and the hands of healthcare workers [11,12], which may

* Corresponding author at: Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
E-mail address: skhodavaisy@sina.tums.ac.ir (S. Khodavaisy).

http://dx.doi.org/10.1016/j.jgar.2019.11.003

lead to nosocomial transmission of this pathogen. There are reports of *C. parapsilosis* outbreaks in intensive care units, which is indicative of exogenous transmission of this pathogen through direct and indirect contact by healthcare workers' hands [13–15]. Although members of *C. parapsilosis* complex are usually susceptible to azole antifungals [16], resistance has also been reported [17]. In a recent study, 27.6% (55/199) and 4.5% (9/199) of *C. parapsilosis* sensu stricto isolates were resistant and susceptible dose-dependent to fluconazole, respectively [18]. Echinocandins are recommend by the Infectious Diseases Society of America for treatment of candidiasis, while elevated MICs to this class of antifungals have consistently been reported in the *C. parapsilosis* species complex [19,20]. A promising way to deal with drug resistance in fungi is to use combinations of antifungal drugs, particularly those with different mechanisms of action to reduce the toxicity and side effects by shortening the duration of treatment [21]. Combination of echinocandins with azoles could be attractive as they have different targets and mechanisms of action. Therefore, this study investigated the combination of echinocandins with triazoles against fluconazole-resistant *C. parapsilosis* complex clinical isolates.

2. Materials and methods

2.1. Fungal isolates

Fifteen fluconazole-resistant clinical isolates of *C. parapsilosis* complex – including *C. parapsilosis* sensu stricto (n = 7), *C. orthopsilosis* (n = 5) and *C. metapsilosis* (n = 3) – were included in this study. All the isolates were previously identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) and confirmed by molecular methods (i.e. sequencing of internal transcribed spacer ribosomal DNA and D1/D2 regions).

2.2. Antifungal drugs

Stock solutions of antifungal drugs were made by dissolving fluconazole (Pfizer Central Research, Sandwich, UK) in sterile double-distilled water, and itraconazole (Janssen Research Foundation, Beerse, Belgium), anidulafungin (Pfizer Central Research, Sandwich, United Kingdom) and micafungin (Merck Sharp & Dohme, Haarlem, Netherlands) in dimethyl sulfoxide (DMSO). Working solutions at 4X the final concentrations were prepared in RPMI 1640 with glutamine, but without $NaHCO_3$ as recommended by the Clinical Laboratory Standards Institute (CLSI) M27-A3 [22]. The final concentrations ranged from 0.125– 64 μg/mL for fluconazole, 0.03–16 μg/mL for itraconazole and 0.125– 8 μg/mL for echinocandins.

2.3. In vitro *combination testing*

In vitro interaction of azoles with echinocandins (i.e. fluconazole with anidulafungin, fluconazole with micafungin, itraconazole with anidulafungin, and itraconazole with micafungin) were studied using a microdilution checkerboard technique [23]. For preparation of test microplates, 50 μL of each concentration of azoles (fluconazole or itraconazole) were added to columns 1–10, and then 50 μL of echinocandins (anidulafungin or micafungin) were added to rows A–G. Row H and column 11 contained the azoles and echinocandins alone, respectively. Column 12 was the drug-free well that served as the growth control. Fungal inoculum was prepared following the CLSI M27-A3 [24] and 100 μL of it was dispensed to all the wells of test microplates. After 24 h of incubation at 35 °C, the results were visually read. To assess the interaction outcomes, the fractional inhibitory concentration index (FICI) value was calculated as follows: (MIC of Drug A in combination/MIC of Drug A alone) + (MIC of Drug B in combination/MIC of Drug B alone). The interaction was considered to be a synergistic effect when the FICI was $\leq$0.5, indifferent at >0.5 to <4.0, and antagonistic at $\geq$4.

3. Results

The results of *in vitro* combination testing of fluconazole and itraconazole with echinocandins are summarised in Table 1. The MIC ranges of fluconazole when tested alone against *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* isolates were 32–64 μg/mL, 8–16 μg/mL and 4 μg/mL, respectively. The combination of fluconazole with anidulafungin resulted in a synergistic interaction (FICI range 0.07–0.37) and decreased the MIC range of fluconazole to 4–16 μg/mL against *C. parapsilosis* sensu stricto, 0.5–4 μg/mL against *C. orthopsilosis* and 0.5–1 μg/mL against *C. metapsilosis* (Table 1). Similarly, fluconazole combined with micafungin interacted synergistically (FICI range 0.25–0.5) and reduced the MIC range of fluconazole to 2–16 μg/mL against *C. parapsilosis*, 1–4 μg/mL against *C. orthopsilosis* and 0.5–1 μg/mL

Table 1
The MICs of azoles and echinocandins alone and in combination against *Candida parapsilosis* species complex.

Strain No.	MICs alone (μg/mL)				MICs in combination (μg/mL)				FICI/interpretation			
	FLC	ITC	ANF	MCF	FLC/ANF	FLC/MCF	ITC/ANF	ITC/MCF	FLC/ANF	FLC/MCF	ITC/ANF	ITC/MCF
Candida parapsilosis sensu stricto												
TMML 1296	32	4	8	1	4/0.5	8/0.125	0.5/1	0.25/0.125	0.18/SYN	0.37/SYN	0.25/SYN	0.18/SYN
TMML 1297	32	4	8	2	8/0.5	4/0.25	1/1	1/0.25	0.31/SYN	0.25/SYN	0.37/SYN	0.37/SYN
TMML 1298	64	2	2	2	4/0.25	16/0.125	0.5/0.25	0.25/0.25	0.18/SYN	0.31/SYN	0.37/SYN	0.25/SYN
TMML 1299	32	4	4	1	8/0.5	2/0.25	1/0.5	0.5/0.25	0.37/SYN	0.31/SYN	0.37/SYN	0.37/SYN
TMML 1300	64	2	8	2	16/1	16/0.25	0.5/0.5	0.5/0.25	0.37/SYN	0.37/SYN	0.31/SYN	0.37/SYN
TMML 1301	64	4	8	1	4/0.125	8/0.125	0.5/1	1/0.125	0.07/SYN	0.25/SYN	0.25/SYN	0.37/SYN
TMML 1302	32	4	2	2	8/0.25	8/0.25	1/0.25	0.25/0.5	0.37/SYN	0.37/SYN	0.37/SYN	0.31/SYN
Candida orthopsilosis												
TMML 399	8	1	4	4	0.5/0.25	1/0.25	0.06/0.5	0.25/0.25	0.12/SYN	0.18/SYN	0.18/SYN	0.31/SYN
TMML 406	16	1	8	4	4/0.5	4/0.5	0.25/0.25	0.06/0.25	0.31/SYN	0.37/SYN	0.28/SYN	0.12/SYN
TMML 407	16	2	8	2	4/0.25	4/0.25	0.5/1	0.5/0.125	0.28/SYN	0.37/SYN	0.37/SYN	0.31/SYN
TMML 414	8	2	4	4	1/0.25	2/1	0.5/0.125	0.5/0.25	0.18/SYN	0.5/SYN	0.28/SYN	0.31/SYN
TMML 443	16	1	4	4	4/0.5	4/0.125	0.125/0.25	0.03/0.25	0.37/SYN	0.28/SYN	0.18/SYN	0.09/SYN
Candida metapsilosis												
TMML 469	4	1	2	1	1/0.125	1/0.125	0.03/0.25	0.06/0.125	0.31/SYN	0.37/SYN	0.15/SYN	0.18/SYN
TMML 470	4	1	4	1	1/0.5	0.5/0.125	0.06/0.5	0.25/0.125	0.37/SYN	0.25/SYN	0.18/SYN	0.37/SYN
TMML 471	4	1	4	2	0.5/1	1/0.125	0.125/0.25	0.25/0.25	0.37/SYN	0.31/SYN	0.18/SYN	0.37/SYN

Abbreviations: FLC, fluconazole; ITC, itraconazole; ANF, anidulafungin; MCF, micafungin; FICI, fractional inhibitory concentration index; SYN, synergism.

against *C. metapsilosis* (Table 1). The MIC range of itraconazole when tested alone was found to be 2–4 μg/mL against *C. parapsilosis* sensu stricto, 1–2 μg/mL against *C. orthopsilosis* and 1 μg/mL against *C. metapsilosis*. In combination with anidulafungin or micafungin, the MIC range of itraconazole decreased to 0.5–1 μg/mL and 0.25–1 μg/mL against *C. parapsilosis* sensu stricto, 0.06–0.5 μg/mL and 0.03–0.5 μg/mL against *C. orthopsilosis* and 0.03–0.125 μg/mL and 0.06–0.25 μg/mL against *C. metapsilosis*, respectively.

3.1. Discussion

The prophylactic use of azole antifungal drugs, particularly fluconazole, since the late 1990s, significantly reduced the incidence and mortality of systemic *Candida* infections. However, as a consequence, it led to a shift from fluconazole-susceptible to fluconazole-resistant infections, especially due to non-*albicans Candida* species [24]. *C. parapsilosis* is one of the common non-*albicans Candida* species able to form biofilms on catheters and other medical devices and can be transmitted horizontally in healthcare facilities. It is a big threat for patients in intensive care units and a known cause of candidaemia in susceptible paediatric patients [25–28]. Nosocomial outbreaks due to this species have been reported in different geographical regions [13,29–31]. Azole-resistance has been reported in *C. parapsilosis*, especially among cases of invasive infections and those previously treated with fluconazole [18,32,33]. This resistance can also be acquired during systemic antifungal therapy [34,35]. Although echinocandins have been recommended as first-line treatment for invasive candidiasis, reports have shown that *C. parapsilosis* has higher MICs than other *Candida* species to echinocandins [20,36]. An alternative method for treatment of resistant cases is the use of a combination of two drugs with different mechanisms of action [21], which needs *in vitro* and animal model studies prior to being used in a clinical setting.

In the present study, the antifungal activity of triazoles (itraconazole, fluconazole) combined with echinocandins (anidulafungin, micafungin) was determined on fluconazole-resistant *C. parapsilosis* complex isolates. The results showed synergism for all combinations. Antifungal interactions may be different depending on the antifungal classes. In a recent study Chassot et al. investigated the combinations of amphotericin B, fluconazole, voriconazole and flucytosine against echinocandin-susceptible and echinocandin-resistant *C. parapsilosis* sensu stricto strains and, unlike the current results, most of the interactions were indifferent [37]. Variations of in vitro interactions can also occur and members within an antifungal class do not necessarily interact in the same way. Combinations of posaconazole with either caspofungin or anidulafungin and voriconazole with anidulafungin were found to be indifferent, while voriconazole with caspofungin resulted in an antagonistic interaction against *C. parapsilosis* sensu stricto isolates [38]. Moreover, voriconazole in combination with micafungin exhibited a synergistic interaction against the emerging pathogen *C. auris*, while the interaction of voriconazole with caspofungin and fluconazole with either caspofungin or anidulafungin was indifferent [39]. Although inter-species variations have been reported for the results of antifungal combinations [40], no difference was noted in the current results obtained for *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis*. This might be due to the close relatedness of these species, which belong to the same phylogenetic complex. However, this finding is based on a limited number of isolates and because there are no other data on the combination of antifungal drugs against *C. orthopsilosis* and *C. metapsilosis*, future studies in this area may change our understanding.

In conclusion, the combination of fluconazole or itraconazole with either anidulafungin or micafungin showed a synergistic interaction against the *C. parapsilosis* species complex, especially against isolates with elevated MIC values. The use of these combinations in clinical practice and the clinical relevancy of these *in vitro* results should be further explored.

Funding

This study was part of a M.S. dissertation supported by Tehran University of Medical Sciences (Grant no. 9511352001). This study has been funded and supported by Tehran University of Medical Sciences (TUMS); Grant no. 97-02-211-38592.

Competing interests

The authors declare no conflicts of interest regarding this study.

Ethical approval

Ethical Approval Number: IR.TUMS.IKHC.REC.1397.213.

Authors' contributions

SK: designed and supervised the study; AA, AM, AI, MG and SM: performed the experiments and drafted the manuscript, SR, SJH, HB and FA: performed data analysis; ED and JFM: critical review of the manuscript. All authors approved the final version of the manuscript.

References

[1] Papadimitriou-Olivgeris M, Spiliopoulou A, Kolonitsiou F, Bartzavali C, Lambropoulou A, Xaplanteri P, et al. Increasing incidence of candidaemia and shifting epidemiology in favor of *Candida* non-*albicans* in a 9-year period (2009–2017) in a university Greek hospital. Infection 2018;47(2):209–16.
[2] van Asbeck EC, Clemons KV, Stevens DA. *Candida parapsilosis*: a review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility. Crit Rev Microbiol 2009;35(4):283–309.
[3] Arsić Arsenijević V, Otasević S, Janić D, Minic P, Matijasevic J, Medic D, et al. *Candida* bloodstream infections in Serbia: first multicentre report of a national prospective observational survey in intensive care units. Mycoses 2018;61(2):70–8.
[4] Pfaller MA, Diekema DJ, Jones RN, Sader HS, Fluit AC, Hollis RJ, et al. International Surveillance of Bloodstream Infections Due toCandida Species: Frequency of Occurrence and *In Vitro* Susceptibilities to Fluconazole, Ravuconazole, and Voriconazole of Isolates Collected from 1997 through 1999 in the SENTRY Antimicrobial Surveillance Program. J Clin Microbiol 2001;39(9):3254–9.
[5] Nakamura T, Takahashi H. Epidemiological study of Candida infections in blood: susceptibilities of *Candida* spp. to antifungal agents, and clinical features associated with the candidemia. J Infect Chemother 2006;12(3):132–8.
[6] Medrano DJA, Brilhante RSN, Cordeiro RDA, Rocha MFG, Rabenhorst SHB, Sidrim JJC. Candidemia in a Brazilian hospital: the importance of *Candida parapsilosis*. Rev Inst Med Trop Sao Paulo 2006;48(1):17–20.
[7] Vaezi A, Fakhim H, Khodavaisy S, Alizadeh A, Nazeri M, Soleimani A, et al. Epidemiological and mycological characteristics of candidemia in Iran: a systematic review and meta-analysis. J Mycol Med 2017;27(2):146–52.
[8] 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. Andes DR, editor. PLoS One 2013;8(3)e59373.
[9] Tavanti A, Davidson AD, Gow NAR, Maiden MCJ, Odds FC. *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. J Clin Microbiol 2005;43(1):284–92.
[10] Tumbarello M, Posteraro B, Trecarichi EM, Fiori B, Rossi M, Porta R, et al. Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. J Clin Microbiol 2007;45(6):1843–50.
[11] Wang H, Zhang L, Kudinha T, Kong F, Ma X-J, Chu Y-Z, et al. Investigation of an unrecognized large-scale outbreak of *Candida parapsilosis* sensu stricto fungaemia in a tertiary-care hospital in China. Sci Rep 2016;6(1):27099.
[12] Barbedo LS, Vaz C, Pais C, Figueiredo-Carvalho MHG, Muniz MM, Zancope-Oliveira RM, et al. Different scenarios for *Candida parapsilosis* fungaemia reveal high numbers of mixed *C. parapsilosis* and *Candida orthopsilosis* infections. J Med Microbiol 2015;64(Pt_1):7–17.

[13] Vaz C, Sampaio P, Clemons KV, Huang Y-C, Stevens DA, Pais C. Microsatellite multilocus genotyping clarifies the relationship of *Candida parapsilosis* strains involved in a neonatal intensive care unit outbreak. Diagn Microbiol Infect Dis 2011;71(2):159–62.
[14] Asadzadeh M, Ahmad S, Al-Sweih N, Hagen F, Meis JF, Khan Z. High-resolution fingerprinting of *Candida parapsilosis* isolates suggests persistence and transmission of infections among neonatal intensive care unit patients in Kuwait. Sci Rep 2019;9(1):1340.
[15] Diab-Elschahawi M, Forstner C, Hagen F, Meis JF, Lassnig AM, Presterl E, et al. Microsatellite Genotyping Clarified Conspicuous Accumulation of *Candida parapsilosis* at a Cardiothoracic Surgery Intensive Care Unit. J Clin Microbiol 2012;50(11):3422–6.
[16] Neji S, Hadrich I, Trabelsi H, Abbes S, Cheikhrouhou F, Sellami H, et al. Virulence factors, antifungal susceptibility and molecular mechanisms of azole resistance among *Candida parapsilosis* complex isolates recovered from clinical specimens. J Biomed Sci 2017;24(1):1–16.
[17] Souza ACR, Fuchs BB, Pinhati HMS, Siqueira RA, Hagen F, Meis JF, et al. *Candida parapsilosis* resistance to fluconazole: molecular mechanisms and *in vivo* impact in infected *Galleria mellonella* larvae. Antimicrob Agents Chemother 2015;59(10):6581–7.
[18] Singh A, Singh PK, de Groot T, Kumar A, Mathur P, Tarai B, et al. Emergence of clonal fluconazole-resistant *Candida parapsilosis* clinical isolates in a multicentre laboratory-based surveillance study in India. J Antimicrob Chemother 2019;1260–8, doi:http://dx.doi.org/10.1093/jac/dkz029.
[19] Garcia-Effron G, Park S, Perlin DS. Correlating echinocandin MIC and kinetic inhibition of fks1 mutant glucan synthases for *Candida albicans*: implications for interpretive breakpoints. Antimicrob Agents Chemother 2009;53(1):112–22.
[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 2015;62(4):e1–50.
[21] Ernst EJ, Rogers PD. Antifungal agents: methods and protocols. Totowa, NJ: Humana Press; 2005. p. 209.
[22] CLSI. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard - third edition, Vol. 28. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. p. 1–25.
[23] Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother 2003;52(1) 1–1.
[24] Maertens JA. Invasive fungal infections. The EBMT Handbook. Cham: Springer International Publishing; 2019. p. 273–80.
[25] Harrington R, Kindermann SL, Hou Q, Taylor RJ, Azie N, Horn DL. Candidemia and invasive candidiasis among hospitalized neonates and pediatric patients. Curr Med Res Opin 2017;33(10):1803–12.
[26] Paramythiotou E, Frantzeskaki F, Flevari A, Armaganidis A, Dimopoulos G, Paramythiotou E, et al. Invasive fungal infections in the ICU: how to approach, how to treat. Molecules 2014;19(1):1085–119.
[27] Arastehfar A, Khodavaisy S, Daneshnia F, Najafzadeh MJ, Mahmoudi S, Charsizadeh A, et al. Molecular Identification, Genotypic Diversity, Antifungal Susceptibility, and Clinical Outcomes of Infections Caused by Clinically Underrated Yeasts, *Candida orthopsilosis*, and *Candida metapsilosis*: An Iranian Multicenter Study (2014–2019). Front Cell Infect Microbiol 2019;9:264, doi: http://dx.doi.org/10.3389/fcimb.2019.00264.
[28] Lupetti A, Tavanti A, Davini P, Ghelardi E, Corsini V, Merusi I, et al. Horizontal transmission of *Candida parapsilosis* Candidemia in a neonatal intensive care unit. J Clin Microbiol 2002;40(7):2363–9.
[29] Badali H, Rezaie S, Jacques FM, Afshari SAK, Modiri M, Hagen F, et al. Microsatellite genotyping of clinical *Candida parapsilosis* isolates. Curr Med Mycol 2017;3(4):15–20.
[30] Qi L, Fan W, Xia X, Yao L, Liu L, Zhao H, et al. Nosocomial outbreak of *Candida parapsilosis* sensu stricto fungaemia in a neonatal intensive care unit in China. J Hosp Infect 2018;100(4):e246–52.
[31] Pinhati HMS, Casulari LA, Souza ACR, Siqueira RA, Damasceno CMG, Colombo AL. Outbreak of candidemia caused by fluconazole resistant *Candida parapsilosis* strains in an intensive care unit. BMC Infect Dis 2016;16(1):433.
[32] Zhang L, Xiao M, Watts MR, Wang H, Fan X, Kong F, et al. Development of fluconazole resistance in a series of *Candida parapsilosis* isolates from a persistent candidemia patient with prolonged antifungal therapy. BMC Infect Dis 2015;15(1):340.
[33] Sarvikivi E, Lyytikäinen O, Soll DR, Pfaller MA, Richardson M, Luukkainen P, et al. Emergence of fluconazole resistance in a *Candida parapsilosis* strain that caused infections in a neonatal intensive care unit. J Clin Microbiol 2005;43(6):2729–35.
[34] Ben-Ami R, Olshtain-Pops K, Krieger M, Oren I, Bishara J, Dan M, et al. Antibiotic exposure as a risk factor for fluconazole-resistant *Candida* bloodstream infection. Antimicrob Agents Chemother 2012;56(5):2518–23.
[35] Hebert C, Villaran R, Tolentino J, Best L, Boonlayangoor S, Pitrak D, et al. Prior antimicrobial exposure and the risk for bloodstream infection with fluconazole-non-susceptible *Candida* strains. Scand J Infect Dis 2010;42(6–7):506–9.
[36] Tóth R, Nosek J, Mora-Montes HM, Gabaldon T, Bliss JM, Nosanchuk JD, et al. *Candida parapsilosis*: from Genes to the Bedside. Clin Microbiol Rev 2019;32(2):e00111–118, doi:http://dx.doi.org/10.1128/CMR.00111-18.
[37] Chassot F, Venturini TP, Piasentin FB, Santurio JM, Svidzinski TIE, Alves SH. Activity of antifungal agents alone and in combination against echinocandin-susceptible and -resistant *Candida parapsilosis* strains. Rev Iberoam Micol 2019;36(1):44–7.
[38] Chatzimoschou A, Katragkou A, Simitsopoulou M, Antachopoulos C, Georgiadou E, Walsh TJ, et al. Activities of triazole-echinocandin combinations against *Candida* species in biofilms and as planktonic cells. Antimicrob Agents Chemother 2011;55(5):1968–74.
[39] Fakhim H, Chowdhary A, Prakash A, Vaezi A, Dannaoui E, Meis JF, et al. *In vitro* interactions of echinocandins with Triazoles against multidrug-resistant *Candida auris*. Antimicrob Agents Chemother 2017;61(11):e01056–17, doi: http://dx.doi.org/10.1128/AAC.01056-17.
[40] Katragkou A, McCarthy M, Meletiadis J, Hussain K, Moradi PW, Strauss GE, et al. In vitro combination therapy with isavuconazole against *Candida* spp. Med Mycol 2017;55(8):859–68.