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First multicentre report of in vitro resistance rates in candidaemia isolates in Turkey

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ABSTRACT

Objectives: This study investigated the antifungal resistance rates of isolates from candidaemia patients in 12 tertiary-care centres in Turkey.
Methods: A total of 1991 *Candida* spp. isolates from 12 centres isolated from 1997–2017 were included in the study. Species/species complex (SC) identification was performed using conventional methods in all centres, occasionally accompanied by MALDI-TOF/MS. Antifungal susceptibility testing was performed for amphotericin B, fluconazole, itraconazole, posaconazole, voriconazole and micafungin (as echinocandin class representative) using the CLSI microdilution method. Resistance rates were determined according to CLSI clinical breakpoints (CBPs). For drugs and species with undetermined CBPs, epidemiological cut-off values were used for wild-type (WT)/non-WT categorisation.
Results: No or low rates of resistance were detected in general for tested *Candida* spp. isolates. Specifically, overall resistance to fluconazole in isolates of *Candida parapsilosis* SC and *Candida glabrata* SC were 7.7% and 0.9%, respectively. Resistance rates for *C. parapsilosis* SC varied extensively from one center to other (0–47.1%). Importantly, no echinocandin resistance was detected. Rates of non-WT isolates were also generally low: fluconazole against *Candida lusitaniae*, 4.3%; posaconazole against *C. parapsilosis* SC, 3.5%; posaconazole against *Candida krusei*, 1.9%; and voriconazole against *C. glabrata* SC, 0.5%.
Conclusion: This is the first multicentre report of antifungal resistance rates among candidaemia isolates in Turkey, suggesting low resistance rates in general. Due to varying rates of fluconazole resistance in *C. parapsilosis* SC isolates that was detected at remarkably high levels in some centres, further studies are warranted to explore the source, clonal relatedness and resistance mechanisms of the isolates.

1. Introduction

Antifungal resistance is gaining increasing importance due not only to awareness and increasing rates but also to developments in the detection of resistance mechanisms. International surveillance programmes as well as multicentre national studies have contributed

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to increasing knowledge regarding the extent of antifungal resistance [1–20]. Focusing on *Candida* spp., secondary echinocandin resistance has been one of the hot topics drawing attention in recent years [21]. Whilst rates of resistance in *Candida* strains have been investigated in studies in Turkey, studies using Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) reference methods and the currently accepted clinical breakpoints (CBPs) or epidemiological cut-off values (ECVs) for interpretation of the results are limited. Additional limitations are the availability of only single-centre data in each of these reports, exploring resistance rates only for one species of *Candida* and/or testing *Candida* isolated from non-sterile clinical samples [22–30]. We aimed to conduct the first multicenter study to document the antifungal resistance rates in candidaemia isolates in Turkey using the CLSI reference antifungal susceptibility testing method.

2. Materials and methods

A total of 1991 *Candida* strains isolated between 1997 and 2017 from patients with candidaemia at 12 centres in Turkey were included in the study. All centres were tertiary-care university hospitals with the number of beds varying from 700 to 1850. The candidaemia isolates included from these centres were from mixed populations including haematology/oncology patients and those hospitalised in intensive care units. Species identification was performed in each centre using one or more of the following methods: germ tube test followed by ID32C or API20C AUX (bioMérieux, France) assimilation profile; morphology on cornmeal agar with Tween 80; and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) proteomic analysis (Bruker) [31–34]. MALDI-TOF/MS was used for species identification in three of the centres (Nos. 3, 8 and 9), accompanying the biochemical profiles using commercial strips and/or morphological characteristics on cornmeal agar with Tween 80. The isolates included 851 *Candida albicans*, 575 *Candida parapsilosis* species complex (SC), 216 *Candida glabrata* SC, 203 *Candida tropicalis*, 52 *Candida krusei*, 33 *Candida kefyr*, 23 *Candida lusitaniae*, 16 *Candida guilliermondii* SC and 22 isolates belonging to other *Candida* spp. [*Candida inconspicua*/*norvegensis* (7), *Candida dubliniensis* (6), *Candida pelliculosa* (3), *Candida rugosa* (2), *Candida utilis* (2), *Candida lipolytica* (1) and *Candida sake* (1)]. All antifungal susceptibility tests were performed in the Mycology Laboratory of the Department of Medical Microbiology, Hacettepe University Medical School (Ankara, Turkey) using the CLSI microdilution method [35]. In vitro activities of amphotericin B (Sigma Aldrich, USA), fluconazole (Pfizer, Ireland), itraconazole (Janssen-Cilag, USA), posaconazole (Merck Sharp & Dohme, USA), voriconazole (Pfizer, USA) and micafungin (Astellas, Japan) (as a representative of the echinocandins) [36] were determined against the studied isolates. Minimum inhibitory concentrations (MICs) were evaluated visually following incubation for 24 h; the incubation period was extended to 48 h in case of insufficient growth. MICs were interpreted using the established species-specific CLSI CBPs to categorise the isolates as susceptible, susceptible dose-dependent (S-DD), intermediate or resistant [37,38]. In the case of lack of determined CBPs for a species–drug combination, CLSI ECVs were used to classify the isolate as wild-type (WT) or non-WT [38]. If neither CBPs nor ECVs are available for a particular species–drug combination, only the generated MICs were presented. Based on the well-known species-based intrinsic resistance pattern, strains of *C. krusei* were categorised as fluconazole-resistant. The analysis was focused on the rates of resistance (or non-WT isolates) for each particular species and antifungal drug and inter-centre variations in rates of resistance.

3. Results

MICs and percentages of susceptibility, resistance and non-WT strains for all 1991 *Candida* spp. isolates included in the study are shown in Table 1. According to the available CBPs, the overall resistance rate for fluconazole against *C. parapsilosis* SC was noteworthy (7.7%). On the other hand, the overall fluconazole resistance rate against *C. glabrata* SC was as low as 0.9%. According to the available ECVs, the rates of non-WT isolates were also low in general, the highest values being detected for fluconazole against *C. lusitaniae* (4.3%), posaconazole against *C. parapsilosis* SC (3.5%) and posaconazole against *C. krusei* (1.9%). These results suggest very low rates of antifungal resistance in candidaemia isolates in general and emphasise the relatively high rates of fluconazole resistance in *C. parapsilosis* SC isolates in Turkey. In relation to this significant finding, the fluconazole resistance rate for *C. parapsilosis* SC varied extensively between centres (range, 0–47.1%) (Table 2). Fluconazole-resistant *C. parapsilosis* SC isolates were rather scattered with no remarkable strict cumulation in any of the time period or years. Importantly, no echinocandin resistance was detected in any of the isolates (Table 1). Except for fluconazole against *C. glabrata* SC where the category of 'susceptible' is lacking (S-DD: 99.1%), the number of strains included in the S-DD category was also very low to 0 for all species–drug combinations (Table 1).

4. Discussion

This is the first multicentre evaluation of antifungal resistance rates in candidaemia isolates in Turkey using one of the reference microdilution susceptibility testing methods. In many previous reports, methods other than CLSI or EUCAST reference microdilution were used. Also, previous studies from Turkey mostly consisted of an evaluation for a single centre or for isolates belonging only to a specific species and thus provided a rather limited perspective on the current extent of antifungal resistance in the country [22–30,39]. Given the high number of isolates included in this study ($n = 1991$) isolated in 12 tertiary-care university hospitals, the attained conclusions may optimally represent the current status and reveal that antifungal resistance rates are very low in general in *Candida* isolates in Turkey. Remarkable variations in antifungal resistance rates in *Candida* isolates have been observed in published reports of multicentre studies from various countries, including low and high resistance rates [2–6,8,9,11–18,20]. Centre-based variations and specific factors, including previous exposure to antifungal drugs, are known to influence resistance rates, as questioned and explored in previously published reports [10,11,15,17,40].

In the current study, three of the conclusions that we would like to focus on are particularly noteworthy. First is the remarkable rate of fluconazole resistance in *C. parapsilosis* SC. High fluconazole resistance rates in *C. parapsilosis* SC have also been reported in multicentre studies from other countries. A laboratory-based surveillance in South Africa showed that only 37% of *C. parapsilosis* SC isolates were susceptible to fluconazole [40]. On the other hand, fluconazole resistance rate of 19.3% was reported from China [8] among *C. parapsilosis* isolates. Other multicentre studies have revealed low rates or absence of resistance, including the Korean (<1%) [17], Asia-Pacific (2.2%) [15], Portuguese (4%) [5], Belgian (5.6%, using the EUCAST reference method) [16], Romanian (0% fluconazole resistance in bloodstream isolates of *C. parapsilosis* using the EUCAST reference method) [9] and Spanish (0–0.6%) [11,13] multicentre surveys as well as a report from Peru (5%) [14]. In the current study, inter-centre variations in fluconazole resistance rates among *C. parapsilosis* SC isolates were remarkable. Based on the conclusions obtained in this study, further studies to explore any possible clonal spread of fluconazole-resistant strains,

Table 1
Minimum inhibitory concentrations (MICs) following 24 h of incubation and resistance/non-wild-type (non-WT) rates obtained for all *Candida* spp. isolates included in the study (n = 1991).

Candida spp. (n)	Antifungal drug	MIC (μg/mL)				Rate of resistance/non–WT (%)			
		MIC_{50}	MIC_{90}	GM	Range	S	S-DD/I	R	Non-WT[a]
C. albicans (851)	AMB	1	2	0.95	0.125–2				0
	FLU	0.25	0.5	0.26	<0.125–4	99.8	0.2	0	
	ITR	0.06	0.125	0.04	≤0.015–0.5	99.4	0.6	0	
	MFG	≤0.03	≤0.03	0.03	≤0.03–0.06	100	0	0	
	POS	≤0.03	0.06	0.03	≤0.03–1				0
	VRC	≤0.015	≤0.015	0.02	≤0.015–0.06	100	0	0	
C. parapsilosis SC (575)	AMB	1	2	0.89	0.125–2				0
	FLU	1	4	0.95	≤0.125 to >64	89	3.3	7.7	
	ITR	0.06	0.25	0.07	≤0.015–1				0.2
	MFG	1	1	0.83	0.125–4	99.8	0.2	0	
	POS	≤0.03	0.25	0.05	≤0.03–0.5				3.5
	VRC	≤0.015	0.03	0.02	≤0.015–0.5	97.9	2.1	0	
C. glabrata SC (216)	AMB	1	2	1.13	0.125–2				0
	FLU	4	16	4.17	0.5–64	–	99.1	0.9	
	ITR	0.25	0.5	0.23	≤0.015–2				0
	MFG	≤0.03	≤0.03	0.03	≤0.03–0.06	100	0	0	
	POS	0.25	1	0.23	<0.03–2				0
	VRC	0.03	0.125	0.03	≤0.015–1				0.5
C. tropicalis (203)	AMB	1	2	1.17	0.25–2				0
	FLU	0.5	1	0.45	≤0.125–2	100	0	0	
	ITR	0.6	0.25	0.08	≤0.015–0.5				0
	MFG	≤0.03	≤0.03	0.03	≤0.03–0.06	100	0	0	
	POS	≤0.03	0.125	0.04	≤0.03–0.125				0
	VRC	≤0.015	≤0.015	0.02	≤0.015–0.06	100	0	0	
C. krusei (52)	AMB	2	2	1.32	0.5–2				0
	FLU	32	64	27.64	8 to >64	–	–	100[b]	
	ITR	0.25	0.5	0.17	≤0.015–0.5				0
	MFG	0.06	0.25	0.08	≤0.03–0.25	100	0	0	
	POS	0.25	0.5	0.14	≤0.03–1				1.9
	VRC	0.06	0.125	0.07	0.03–0.125	100	0	0	
C. kefyr (33)	AMB	2	2	1.37	0.5–2			[c]	[c]
	FLU	0.25	0.5	0.28	≤0.125–1				0
	ITR	0.06	0.125	0.07	≤0.015–0.5			[c]	[c]
	MFG	≤0.03	≤0.03	0.03	≤0.03–0.06				0
	POS	≤0.03	0.125	0.05	≤0.03–0.25				0
	VRC	≤0.015	≤0.015	0.02	≤0.015				0
C. lusitaniae (23)	AMB	1	1	1	0.5–2				0
	FLU	0.25	1	0.3	≤0.125–4				4.3
	ITR	0.06	0.125	0.06	0.03–0.125				0
	MFG	≤0.03	0.125	0.04	≤0.03–0.25				0
	POS	≤0.03	0.06	0.04	≤0.03–0.125				0
	VRC	≤0.015	≤0.015	0.02	≤0.015				0
C. guilliermondii SC (16)	AMB	1	1	0.81	0.25–1				0
	FLU	2	8	2.18	0.5–8				0
	ITR	0.125	0.25	0.14	0.03–0.5				0
	MFG	0.125	1	0.15	≤0.03–1	100	0	0	
	POS	0.125	0.5	0.13	≤0.03–0.5				0
	VRC	≤0.015	0.03	0.02	≤0.015–0.03				0
Other *Candida* spp. (22)[d]	AMB	–	–	–	0.25–2				
	FLU	–	–	–	≤0.125–32				
	ITR	–	–	–	0.03–0.125				
	MFG	–	–	–	≤0.03–0.25				
	POS	–	–	–	≤0.03–0.25				
	VRC	–	–	–	≤0.015–0.125				

$MIC_{50/90}$, MIC for 50% and 90% of the isolates, respectively; GM, geometric mean; S, susceptible; S-DD, susceptible dose-dependent (for FLU and ITR); I, intermediate (for echinocandins, i.e. MFG); R, resistant; AMB, amphotericin B; FLU, fluconazole; ITR, itraconazole; MFG, micafungin; POS, posaconazole; VRC, voriconazole; SC, species complex; ECV, epidemiological cut-off value.

[a] Rate of resistance could not be determined for these drug–species combinations owing to lack of determined clinical breakpoints.

[b] Intrinsic resistance to FLU.

[c] Not determined owing to lack of established clinical breakpoints and ECVs.

[d] Includes *C. inconspicua*/*norvegensis* (7), *C. dubliniensis* (6), *C. pelliculosa* (3), *C. rugosa* (2), *C. utilis* (2), *C. lipolytica* (1) and *C. sake* (1). Among these, ECVs are available for FLU, VRC and POS against *C. dubliniensis* and *C. pelliculosa* and for AMB, ITR and MFG only against *C. dubliniensis*. All isolates of these species with available ECVs are wild-type for the denoted antifungal drugs.

particularly in centre No. 12, and the identification of cryptic species within *C. parapsilosis* SC are among the planned future projects to be conducted.

Second, a very low resistance rate of fluconazole against *C. glabrata* SC (0.9%) was detected (Table 1). Fluconazole susceptibility in *C. glabrata* SC isolates includes only categories of S-DD and resistant [37], and resistance to fluconazole has been a growing concern in a remarkable number of reports [41,42]. Fluconazole resistance rates of 2.8% from South Korea [6], 4% from China (China-SCAN study) [8], 5.2% from the Asia-Pacific region [using Sensititre® YeastOne® (Thermo Fisher) and CLSI breakpoints] [15]. 8% from South Africa [40], 9% from Portugal [5], 10.3%

Table 2
Inter-centre variation in rates of resistance/percentages of non-wild-type (non-WT) isolates for all species–antifungal combinations detected within the categories of resistance or non-WT.

Centre no.	Rate of resistance (%)/percentage of non-WT isolates/total *n* in each centre										
	C. parapsilosis SC				*C. glabrata* SC			*C. krusei*		*C. lusitaniae*	
	FLU [a]	ITR[b]	POS[b]	Total *n*[c]	FLU[a]	VRC[b]	Total *n*[c]	POS[b]	Total *n*[c]	FLU[b]	Total *n*[c]
1	6.3	0	0.8	126	1.2	0	82	0	11	0	11
2	3.4	0	3.4	29	4.0	4.0	25	0	6	[d]	1
3	5.1	0	0	39	0	0	3	–	0	–	0
4	7.1	0	0	14	0	0	15	0	5	–	0
5	3.1	0	0	64	0	0	20	0	13	0	3
6	0	0	2.7	37	0	0	6	–	0	–	0
7	0	0	0	11	0	0	2	–	0	0	1
8	0	0	0	84	0	0	19	0	5	0	2
9	7.9	0	0	63	0	0	11	0	5	0	4
10	1.9	0	0	52	0	0	22	0	1	0	1
11	0	0	0	5	0	0	2	–	0	–	0
12	47.1	2.0	33.3	51	0	0	9	16.7	6	–	0
Overall	7.7	0.2	3.5	575	0.9	0.5	216	1.9	52	4.3	23
Range	0–47.1	0–2.0	0–33.3	–	0–4.0	0–4.0	–	0–16.7	–	[d]	–

SC, species complex; FLU, fluconazole; ITR, itraconazole; POS, posaconazole; VRC, voriconazole.
[a] Resistance rate (%).
[b] Percentage of non-WT isolates (%).
[c] Total number of isolates per denoted species identified in each centre.
[d] Only one resistant isolate.

from South Korea [17], 10.7–11.3% from Belgium [16,42], 16% from Romania (using the EUCAST reference method) [9] and 36.4% from Brazil [2] are noteworthy for fluconazole and *C. glabrata* SC. On the other hand and similar to the current findings, (very) low resistance rates or no resistance have been reported from multicentre studies conducted in Spain (0–1.1%) [11,13], Peru (0%) [14] and South Korea (0%) [6]. These reports clearly show the variability of resistance rates for fluconazole against *C. glabrata* SC isolates and the results of the current study emphasise the very low rates of resistance observed in Turkish centres for this particular antifungal–SC combination.

Third, no echinocandin-resistant isolates were detected among the tested candidaemia isolates. Micafungin was included in this study as one of the two echinocandins (anidulafungin and micafungin) recommended for detection of in vitro resistance to this class of antifungal drugs [36]. Echinocandins are of particular significance as being one of the two first-line therapeutic choices in the treatment of invasive candidiasis, and echinocandin resistance is being increasingly encountered in *Candida* isolates [21]. The emergence of stepwise multidrug resistance, including to echinocandins, has also been reported in some isolates [43]. Echinocandin resistance rates of 1% from Belgium (tested using the EUCAST reference method, anidulafungin and micafungin) [16], 1.7% from the Asia-Pacific region (tested using caspofungin in the Sensititre YeastOne panel) [15], 1.9% in *C. glabrata* in a surveillance study from South Africa [40] and 3.8% in a retrospective fungaemia survey in Sweden (tested with anidulafungin) [7] are among the relatively low rates reported so far. On the other hand, rates varying from 0–10% and 0–8.3% have been reported depending on species for micafungin in the Portuguese [5] and Spanish [13] multicentre surveys, respectively. Unlike these findings and similar to the current results, no echinocandin resistance was detected for *Candida* spp. in the multicentre studies from Spain (tested with caspofungin and anidulafungin) [11], China (tested with caspofungin) [8], South Korea (tested with caspofungin and micafungin) [17] and Peru (tested with anidulafungin) [14]. The echinocandin resistance rate among *Candida* isolates, which is currently nil in this study, awaits periodic surveillance to determine any possible change.

To conclude, this study remains significant as being the first multicentre antifungal resistance report for candidaemia isolates in Turkey. The results suggest surveillance and detailed analysis for fluconazole resistance in *C. parapsilosis* SC isolates in particular and continuation of surveillance studies to determine any possible change in antifungal resistance rates.

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Competing interests

SA-A has received lecture honoraria or travel grants from Astellas, Gilead, Merck and Pfizer outside of the submitted work. All other authors declare no competing interests.

Ethical approval

Not required.

References

[1] Pfaller MA, Messer SA, Rhomberg PR, Castanheira M. CD101, a long-acting echinocandin, and comparator antifungal agents tested against a global collection of invasive fungal isolates in the SENTRY 2015 Antifungal Surveillance Program. Int J Antimicrob Agents 2017;50:352–8, doi:http://dx.doi.org/10.1016/j.ijantimicag.2017.03.028.
[2] Doi AM, Pignatari AC, Edmond MB, Marra AR, Camargo LF, Siqueira RA, et al. Epidemiology and microbiologic characterization of nosocomial candidemia from a Brazilian national surveillance program. PLoS One 2016;11:e0146909, doi:http://dx.doi.org/10.1371/journal.pone.0146909.
[3] Badiee P, Badali H, Boekhout T, Diba K, Moghadam AG, Hossaini Nasab A, et al. Antifungal susceptibility testing of *Candida* species isolated from the immunocompromised patients admitted to ten university hospitals in Iran: comparison of colonizing and infecting isolates. BMC Infect Dis 2017;17:727, doi:http://dx.doi.org/10.1186/s12879-017-2825-7.
[4] Bassetti M, Merelli M, Righi E, Diaz-Martin A, Rosello EM, Luzzati R, et al. Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol 2013;51:4167–72, doi:http://dx.doi.org/10.1128/JCM.01998-13.
[5] Faria-Ramos I, Neves-Maia J, Ricardo E, Santos-Antunes J, Silva AT, Costa-de-Oliveira S, et al. Species distribution and in vitro antifungal susceptibility profiles of yeast isolates from invasive infections during a Portuguese

multicenter survey. Eur J Clin Microbiol Infect Dis 2014;33:2241–7, doi:http://dx.doi.org/10.1007/s10096-014-2194-8.
[6] Jung SI, Shin JH, Song JH, Peck KR, Lee K, Kim MN, et al. Multicenter surveillance of species distribution and antifungal susceptibilities of *Candida* bloodstream isolates in South Korea. Med Mycol 2010;48:669–74, doi:http://dx.doi.org/10.3109/13693780903410386.
[7] Klingspor L, Ullberg M, Rydberg J, Kondori N, Serrander L, Swanberg J, et al. Epidemiology of fungaemia in Sweden: a nationwide retrospective observational survey. Mycoses 2018;61:777–85, doi:http://dx.doi.org/10.1111/myc.12816.
[8] Liu W, Tan J, Sun J, Xu Z, Li M, Yang Q, et al. Invasive candidiasis in intensive care units in China: in vitro antifungal susceptibility in the China-SCAN study. J Antimicrob Chemother 2014;69:162–7, doi:http://dx.doi.org/10.1093/jac/dkt330.
[9] Minea B, Nastasa V, Moraru RF, Kolecka A, Flonta MM, Marincu I, et al. Species distribution and susceptibility profile to fluconazole, voriconazole and MXP-4509 of 551 clinical yeast isolates from a Romanian multi-centre study. Eur J Clin Microbiol Infect Dis 2015;34:367–83, doi:http://dx.doi.org/10.1007/s10096-014-2240-6.
[10] Montagna MT, Caggiano G, Lovero G, De Giglio O, Coretti C, Cuna T, et al. Epidemiology of invasive fungal infections in the intensive care unit: results of a multicenter Italian survey (AURORA Project). Infection 2013;41:645–53, doi:http://dx.doi.org/10.1007/s15010-013-0432-0.
[11] Nieto MC, Telleria O, Cisterna R. Sentinel surveillance of invasive candidiasis in Spain: epidemiology and antifungal susceptibility. Diagn Microbiol Infect Dis 2015;81:34–40, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2014.05.021.
[12] Pemán J, Cantón E, Quindós G, Eraso E, Alcoba J, Guinea J, et al. Epidemiology, species distribution and in vitro antifungal susceptibility of fungaemia in a Spanish multicentre prospective survey. J Antimicrob Chemother 2012;67:1181–7, doi:http://dx.doi.org/10.1093/jac/dks019.
[13] Pemán J, Cantón E, Linares-Sicilia MJ, Roselló EM, Borrell N, Ruiz-Pérez-de-Pipaon MT, et al. Epidemiology and antifungal susceptibility of bloodstream fungal isolates in pediatric patients: a Spanish multicenter prospective survey. J Clin Microbiol 2011;49:4158–63, doi:http://dx.doi.org/10.1128/JCM.05474-11.
[14] Rodriguez L, Bustamante B, Huaroto L, Agurto C, Illescas R, Ramirez R, et al. A multi-centric study of *Candida* bloodstream infection in Lima-Callao, Peru: species distribution, antifungal resistance and clinical outcomes. PLoS One 2017;12:e0175172, doi:http://dx.doi.org/10.1371/journal.pone.0175172.
[15] Tan TY, Hsu LY, Alejandria MM, Chaiwarith R, Chinniah T, Chayakulkeeree M, et al. Antifungal susceptibility of invasive *Candida* bloodstream isolates from the Asia-Pacific region. Med Mycol 2016;54:471–7, doi:http://dx.doi.org/10.1093/mmy/myv114.
[16] Trouve C, Blot S, Hayette MP, Jonckheere S, Patteet S, Rodriguez-Villalobos H, et al. Epidemiology and reporting of candidaemia in Belgium: a multi-centre study. Eur J Clin Microbiol Infect Dis 2017;36:649–55, doi:http://dx.doi.org/10.1007/s10096-016-2841-3.
[17] Won EJ, Shin JH, Choi MJ, Lee WG, Park YJ, Uh Y, et al. Antifungal susceptibilities of bloodstream isolates of *Candida* species from nine hospitals in Korea: application of new antifungal breakpoints and relationship to antifungal usage. PLoS One 2015;10:e0118770, doi:http://dx.doi.org/10.1371/journal.pone.0118770.
[18] Xiao M, Fan X, Chen SC, Wang H, Sun ZY, Liao K, et al. Antifungal susceptibilities of *Candida glabrata* species complex, *Candida krusei*, *Candida parapsilosis* species complex and *Candida tropicalis* causing invasive candidiasis in China: 3 year national surveillance. J Antimicrob Chemother 2015;70:802–10, doi:http://dx.doi.org/10.1093/jac/dku460.
[19] Yang YL, Chen HT, Lin CC, Chu WL, Lo HJ, TSARY Hospitals. Species distribution and drug susceptibilities of *Candida* isolates in TSARY 2010. Diagn Microbiol Infect Dis 2013;76:182–6, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2013.03.003.
[20] Zhou ZL, Lin CC, Chu WL, Yang YL, Lo HJ, TSARY Hospitals. The distribution and drug susceptibilities of clinical *Candida* species in TSARY 2014. Diagn Microbiol Infect Dis 2016;86:399–404, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2016.09.009.
[21] Arendrup MC, Perlin DS. Echinocandin resistance: an emerging clinical problem? Curr Opin Infect Dis 2014;27:484–92, doi:http://dx.doi.org/10.1097/QCO.0000000000000111.
[22] Ozhak-Baysan B, Ogunc D, Colak D, Ongut G, Donmez L, Vural T, et al. Distribution and antifungal susceptibility of *Candida* species causing nosocomial candiduria. Med Mycol 2012;50:529–32, doi:http://dx.doi.org/10.3109/13693786.2011.618996.
[23] Eksi F, Gayyurhan ED, Balci I. In vitro susceptibility of *Candida* species to four antifungal agents assessed by the reference broth microdilution method. ScientificWorldJournal 2013;2013:236903, doi:http://dx.doi.org/10.1155/2013/236903.
[24] Dalyan Cilo B, Topaç T, Ağca H, Sağlam S, Efe K, Ener B. Comparison of Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution methods for determining the susceptibilities of *Candida* isolates [in Turkish]. Mikrobiyol Bul 2018;52:35–48, doi:http://dx.doi.org/10.5578/mb.63991.
[25] Hilmioglu-Polat S, Sharifynia S, Oz Y, Aslan M, Gundogdu N, Serin A, et al. Genetic diversity and antifungal susceptibility of *Candida parapsilosis* sensu stricto isolated from bloodstream infections in Turkish patients. Mycopathologia 2018;183:701–8, doi:http://dx.doi.org/10.1007/s11046-018-0261-x.
[26] Karabıçak N, Alem N. Antifungal susceptibility profiles of *Candida* species to triazole: application of new CLSI species-specific clinical breakpoints and epidemiological cutoff values for characterization of antifungal resistance [in Turkish]. Mikrobiyol Bul 2016;50:122–32, doi:http://dx.doi.org/10.5578/mb.10682.
[27] Hazırolan G, Sarıbaş Z, Arıkan Akdağlı S. Comparison of microdilution and disk diffusion methods for the detection of fluconazole and voriconazole susceptibility against clinical *Candida glabrata* isolates and determination of changing susceptibility with new CLSI breakpoints [in Turkish]. Mikrobiyol Bul 2016;50:428–37, doi:http://dx.doi.org/10.5578/mb.26544.
[28] Toka Özer T, Durmaz S, Yula E. Antifungal susceptibilities of *Candida* species isolated from urine culture. J Infect Chemother 2016;22:629–32, doi:http://dx.doi.org/10.1016/j.jiac.2016.06.012.
[29] Kazak E, Akin H, Ener B, Sigirli D, Ozkan O, Gurcuoglu E, et al. An investigation of *Candida* species isolated from blood cultures during 17 years in a university hospital. Mycoses 2014;57:623–9, doi:http://dx.doi.org/10.1111/myc.12209.
[30] Metin DY, Hilmioglu-Polat S, Samlioglu P, Doganay-Oflazoglu B, Inci R, Tumbay E. Evaluation of antifungal susceptibility testing with microdilution and Etest methods of *Candida* blood isolates. Mycopathologia 2011;172:187–99, doi:http://dx.doi.org/10.1007/s11046-011-9413-y.
[31] Larone DH. Medically important fungi: a guide to identification. Washington, DC: ASM Press; 2011.
[32] Normand AC, Becker P, Gabriel F, Cassagne C, Accoceberry I, Gari-Toussaint M, et al. Validation of a new web application for identification of fungi by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 2017;55:2661–70, doi:http://dx.doi.org/10.1128/JCM.00263-17.
[33] Criseo G, Scordino F, Romeo O. Current methods for identifying clinically important cryptic *Candida* species. J Microbiol Methods 2015;111:50–6, doi:http://dx.doi.org/10.1016/j.mimet.2015.02.004.
[34] Chalupova J, Raus M, Sedlarova M, Sebela M. Identification of fungal microorganisms by MALDI-TOF mass spectrometry. Biotechnol Adv 2014;32:230–41, doi:http://dx.doi.org/10.1016/j.biotechadv.2013.11.002.
[35] Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard—third edition CLSI document M27-A3. Wayne, PA: CLSI; 2008.
[36] 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;57:5836–42, doi:http://dx.doi.org/10.1128/AAC.01519-13.
[37] Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. 4th ed. Wayne, PA: CLSI; 2017 CLSI standard M27.
[38] Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of *Candida* spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. J Clin Microbiol 2012;50:2846–56, doi:http://dx.doi.org/10.1128/JCM.00937-12.
[39] Dagi HT, Findik D, Senkeles C, Arslan U. Identification and antifungal susceptibility of *Candida* species isolated from bloodstream infections in Konya, Turkey. Ann Clin Microbiol Antimicrob 2016;15:36, doi:http://dx.doi.org/10.1186/s12941-016-0153-1.
[40] Govender NP, Patel J, Magobo RE, Naicker S, Wadula J, Whitelaw A, et al. Emergence of azole-resistant *Candida parapsilosis* causing bloodstream infection: results from laboratory-based sentinel surveillance in South Africa. J Antimicrob Chemother 2016;71:1994–2004, doi:http://dx.doi.org/10.1093/jac/dkw091.
[41] Ko JH, Peck KR, Jung DS, Lee JY, Kim HA, Ryu SY, et al. Impact of high MIC of fluconazole on outcomes of *Candida glabrata* bloodstream infection: a retrospective multicenter cohort study. Diagn Microbiol Infect Dis 2018;92:127–32, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2018.05.001.
[42] Goemaere B, Lagrou K, Spriet I, Hendrickx M, Becker P. Clonal spread of *Candida glabrata* bloodstream isolates and fluconazole resistance affected by prolonged exposure: a 12-year single-center study in Belgium. Antimicrob Agents Chemother 2018;62:, doi:http://dx.doi.org/10.1128/AAC.00591-18 pii: e00591-18.
[43] Jensen RH, Astvad KM, Silva LV, Sanglard D, Jorgensen R, Nielsen KF, et al. Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in *Candida albicans* orchestrated by multiple genetic alterations. J Antimicrob Chemother 2015;70:2551–5, doi:http://dx.doi.org/10.1093/jac/dkv140.