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Species distribution and antifungal susceptibility profile of *Candida* isolates from blood and other normally sterile foci from pediatric ICU patients in Tehran, Iran

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Abstract

As data on pediatric invasive candidiasis (IC) and the antifungal susceptibility pattern of associated isolates are scarce in Iran, this study aimed to determine species distribution and antifungal susceptibility profile of *Candida* species isolated from pediatric patients with suspected or documented IC. A total of 235 yeast strains recovered from normally sterile body fluids of patients admitted at the intensive care units of Children's Medical Centre, Tehran, Iran, were identified using CHROMagar Candida, molecular methods (ITS PCR-RFLP and sequencing), and MALDI-TOF. Susceptibility to amphotericin B, fluconazole, voriconazole, micafungin, and anidulafungin was determined according to the European on Antimicrobial Susceptibility testing reference microdilution method (EUCAST E.Def 7.3.1). *Candida albicans* (53.6%), *C. parapsilosis* (24.7%), and *C. tropicalis* (8.5%) were the most common species, followed by *C. lusitaniae* (4.3%), *C. glabrata* (3.0%), *C. guilliermondii* and *C. orthopsilosis* (each 1.7%), *C. kefyr* (1.3%), *C. dubliniensis* (0.8%), and *C. intermedia* (0.4%). Amphotericin B MICs were $\leq$1 mg/l for all *Candida* isolates. *C. albicans* isolates were susceptible to all five antifungal agents. All *C. parapsilosis* isolates categorised as intermediate to micafungin and anidulafungin, except two isolates that had the MICs >2 mg/l for micafungin. MIC_{50}, MIC_{90}, and MIC range for fluconazole were 0.25 mg/l, 1 mg/l, and 0.125 – $\geq$32 mg/l, respectively. Fluconazole and voriconazole showed 100% activity against the most prevalent *Candida* species. The low resistance rate, favorable safety profile and low cost of fluconazole make it a reasonable choice for treatment of candidemia/invasive candidemia in Iran.

Key words: *Candida,* susceptibility, Paediatric ICU, Iran.

Introduction

The patient population at risk of invasive candidiasis (IC) including candidaemia is increasing due to advances in medical technologies and therapeutic interventions.[1] Diagnosing IC and candidemia is challenging as specific signs and symptoms are often lacking and diagnostic tools insensitive, in part explaining the associated increased crude and attributable mortality rates.[2] Candidemia as the most common invasive fungal infection represents a serious and rising challenge in neonatal and pediatric intensive care units (ICU).[1] ICU admission and use of broad-spectrum antibiotics, central venous lines, mechanical

ventilation, and total parenteral nutrition are the main risk factors.[1–5]

The strategies for prevention and treatment of Candida infections in critically ill children have been evaluated.[4] Prophylactic and empirical therapeutic strategies appear attractive but also associated with increased risk of emerging intrinsic and acquired resistance. For example, *C. glabrata* and *C. parapsilosis*, which are intrinsically nonsusceptible to fluconazole and echinocandins, respectively, have increased proportionally at several institutions following increased use of antifungal prophylaxis.[5–7] Therefore, antifungal susceptibility testing is essential for targeted management of patients with invasive fungal infections, patients who are intolerant or refractory to some antifungal agents, patients previously exposed to antifungal agents or who are involved with rare *Candida* species, and also for local epidemiological studies and resistance surveillance.[2,4]

There are significant geographical differences in the distribution and *in vitro* susceptibility pattern to antifungal agents among the different species of *Candida*.[8,9] In Iran, pediatric candidaemia data and antifungal susceptibility patterns of related strains are scarce. We have previously described the epidemiology of 156 episodes of invasive candidiasis in Iranian paediatric patients.[3] We here extend this study to include more patients and include the antifungal susceptibility data of the *Candida* species isolated from paediatric patients with documented and suspected invasive candidiasis (mainly candidaemia), hospitalized in a reference children hospital in Tehran, Iran.

Methods

Between July 2014 and December 2017, all yeast isolates obtained from blood ($n = 186$) and other normally sterile body fluids including biopsies ($n = 10$), cerebrospinal fluid (CSF) ($n = 8$), dialysis fluid ($n = 4$), synovial fluid ($n = 2$), bronchoalveolar lavage (BAL) ($n = 24$, among which 13 patients had also positive blood culture), and ascites fluid ($n = 1$), of ≤16-year-old paediatric patients admitted at the neonatal and pediatric ICUs of Children's Medical Centre, Tehran, Iran, were collected. The epidemiological aspects (but not the susceptibility data) of a part of the patients (156/235 isolates) have previously been published.[3] All strains were subcultured on CHROMagar *Candida* and species identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) technique (MicroFlex LT system, Bruker Daltonics, Germany),[10] in addition to the molecular methods, ITS-PCR-RFLP and ITS-sequencing.[3]

Susceptibility tests were carried out according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) definitive document E.Def 7.3.1.[11] Briefly, stock solution (5000 mg/ml except fluconazole (10 000 mg/ml)) of amphotericin B and fluconazole (Sigma-Aldrich, Vallensbæk Strand, Denmark), voriconazole and anidulafungin (Pfizer A/S, Ballerup, Denmark) and micafungin (Astellas Pharma Inc, Japan) were prepared in dimethyl sulfoxide (DMSO), (D8779, Sigma-Aldrich). For each isolate, drug-free wells (growth control), and for each run *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as the quality controls, and the run was accepted if it was within the recommended control minimal inhibitory concentration (MIC) ranges.[11] The susceptibility classification was done adopting the EUCAST clinical breakpoints (http://www.eucast.org/clinical_breakpoints/; version 9.0). For *C. tropicalis* for which a micafungin breakpoint has not yet been established, the MICs were interpreted adopting epidemiological cut-off (ECOFF) of 0.06 mg/l (http://www.eucast.org/ast_of_fungi/rationale_documents_for_antifungals/). Moreover, the echinocandin breakpoints *C. glabrata* were adopted for the two phylogenetically related species *C. lusitaniae* and *C. guilliermondii* and, similarly, the *C. parapsilosis* breakpoints for *C. orthopsilosis*.

Results

During the period study, a total of 235 *Candida* isolates were recovered from 224 pediatric patients with suspected or proven IC. Nine patients had two episodes, and one had three episodes of candidemia, whereas all other isolates were from unique patients. Prematurity and related organ disorders (35.4%), genetic disorders (33.1%), trauma and prior surgery (19.6%), and congenital heart diseases (10.9%) were the main underlying diseases. The species distribution was as follows: *C. albicans* 126 (53.6%), *C. parapsilosis* 58 (24.7%), *C. tropicalis* 20 (8.5%), *C. lusitaniae* 10 (4.3%), *C. glabrata* 7 (3.0%), *C. guilliermondii* and *C. orthopsilosis* 4 (1.7%) each, *C. kefyr* 3 (1.3%), *C. dubliniensis* 2 (0.8%), and *C. intermedia* 1 (0.4%).

Amphotericin B displayed uniform activity against all isolates (MIC_{50} 0.125 mg/l/ MIC_{90} 0.25 mg/l) with no MICs above the nonspecies-specific breakpoint of 1 mg/l (Table 1). *C. albicans* and *C. dubliniensis* isolates were the most susceptible species at a mg/l basis and all isolates were susceptible to all five antifungal agents.

The overall MIC ranges for anidulafungin and micafungin were 0.03–4 mg/l, and specifically for *C. parapsilosis* was 0.25–4 mg/l and 0.5–4 mg/l, respectively. Nonsusceptibility was found for *C. parapsilosis* isolates as per definition, as all were intermediate to anidulafungin and two, with micafungin MICs = 4 mg/l, were resistant to micafungin. Three/4 and 4/4 *C. guilliermondii* were classified as anidulafungin and micafungin resistant, respectively. Similarly, 2/10 and 7/10 *C. lusitaniae* isolates were deemed resistant due to anidulafungin MICs >0.06 mg/l and micafungin MICs >0.03 mg/l if adopting the *C. glabrata* breakpoints for this species. Finally, micafungin MICs for 1/3 *C. kefyr* and 1/1 *C. intermedia* were 0.06 mg/l and thus above the micafungin breakpoint.

Table 1. Susceptibility of *Candida* isolates to five antifungal agents at children medical center's ICUs

		MIC (mg/l)														
	No.	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	≥ 32	MIC_{50}	MIC_{90}	S/WT	S/WT (%)
Amphotericin B																
Candida albicans	126		37	76	12	1							0.125	0.25	126	100.0
Candida parapsilosis	58		6	30	20	2							0.125	0.25	58	100.0
Candida tropicalis	20		6	6	7	1							0.125	0.25	20	100.0
Candida lusitaniae	10		7	3									0.06	0.125	7	100.0
Candida glabrata	7			1	6								ND	ND	7	ND
Candida guilliermondii	4			3		1							ND	ND	4	ND
Candida orthopsilosis	4		2	2									ND	ND	4	ND
Candida spp.	6	2			2	1	1						ND	ND	6	ND
In total	235	2	58	121	47	6	1						0.125	0.25	235	100.0
Micafungin																
Candida albicans	126	126											≤0.03	≤0.03	126	100.0
Candida parapsilosis	58					5	19	32	2				2.0	2.0	0	0.0
Candida tropicalis	20	18	2										0.03	0.03	20	100
Candida lusitaniae	10	3	2	4	1								≤0.03	≤0.03	3	ND
Candida glabrata	7	7											ND	ND	7	ND
Candida guilliermondii	4					3	1						ND	ND	0	0.0
Candida orthopsilosis	4					4							ND	ND	0	0.0
Candida spp.	6	4	2										ND	ND	4	ND
In total	235	158	6	4	1	12	20	32	2				≤0.03	2.0	160	68.1
Anidulafungin																
Candida albicans	126	126											≤0.03	≤0.03	126	100.0
Candida parapsilosis	58				1	12	28	16	1				1.0	2.0	0	0.0
Candida tropicalis	20	19	1										≤0.03	≤0.03	20	100
Candida lusitaniae	10	2	6	2									0.06	0.125	8	ND
Candida glabrata	7	7											ND	ND	7	ND
Candida guilliermondii	4			1			2	1					ND	ND	0	0.0
Candida orthopsilosis	4					4							ND	ND	0	0.0
Candida spp.	6	5	1										ND	ND	6	ND
In total	235	159	8	3	1	16	30	17	1				≤0.03	1.0	167	71.1
Fluconazole																
Candida albicans	126			90	35	1							0.125	0.25	126	100.0
Candida parapsilosis	58				8	31	16	3					0.5	1.0	58	100.0
Candida tropicalis	20				13	7							0.25	0.5	20	100.0
Candida lusitaniae	10			1	7	2							0.25	0.5	10	100.0
Candida glabrata	7							3	3			1	ND	ND	0	0.0
Candida guilliermondii	4							1	1	1	1		ND	ND	2	ND
Candida orthopsilosis	4				2	1		1					ND	ND	4	ND
Candida spp.	6			2	2	1		1					ND	ND	6	ND
In total	235			93	67	43	16	9	4	1	1	1	0.25	1.0	226	96.2
Voriconazole																
Candida albicans	126	126											≤0.03	≤0.03	126	100.0
Candida parapsilosis	58	58											≤0.03	≤0.03	58	100.0
Candida tropicalis	20	17	3										≤0.03	0.06	20	100.0
Candida lusitaniae	10	10											≤0.03	≤0.03	10	100.0
Candida glabrata	7		6					1					ND	ND	6	ND
Candida guilliermondii	4			4									ND	ND	4	ND
Candida orthopsilosis	4	4											ND	ND	4	ND
Candida spp.*	6	5	1										ND	ND	6	ND
In total	235	220	10	4				1					≤0.03	≤0.03	234	99.6

**Candida* spp. (no. 6) included the following isolates: *C. kefyr* 3, *C. dubliniensis* 2, *C. intermedia* 1.
ND, non-definable.

Table 2. Distribution of *Candida* species (%) in this study and other pediatric studies

Countries	No. of isolates	*C. albicans*	*C. parapsilosis* complex	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. lusitaniae*	*C. guilliermondii*	Other	Reference
This study	235	54.3	26.6	8.0	2.8	...	4.0	1.6	3.2	
Spain 2011	201	36.5	46.8	5.9	3.9	1.0	2.0	2.5	0.5	13
Germany 2011	35	45.7	17.1	5.7	14.3	...	8.6	...	8.6	17
Kuwait 2013	89	47.2	38.2	...	...	...	...	...	14.6	18
Mexico 2013	342	37.1	37.1	21.1	2.6	...	...	...	...	15
Taiwan 2016	295	45.4	29.1	4.8	3.4	...	...		17.3	21
Brazil 2017	65	37.0	31.0	8.0	3.0	3.0	1.5	5.0	12.0	20
Italy 2017	41	34.1	60.9	...	2.4	...	...	2.4	...	22
Turkey 2017	54	50.0	24.0	11.1	5.6	...	3.7		5.6	23

The fluconazole MIC range was 0.125 to ≥32 mg/l. With the exception of *C. glabrata* and *C. guilliermondii* isolates which due to the intrinsic resistance were not susceptible to fluconazole, all other *Candida* isolates were susceptible to this compound. One *C. glabrata* isolate with MIC ≥32 mg/l and thus potentially resistant to fluconazole, whereas the remaining *C. glabrata* isolates were intermediate to fluconazole. Moreover, the fluconazole MICs for the four *C. guilliermondii* strains were 2, 4, 8, and 16 mg/l, respectively, and thus straddled the non-species-specific clinical breakpoints (S: ≤2 mg/l and R: >4 mg/l).

Voriconazole with overall MIC_{50} and MIC_{90} ≤0.03 mg/l, and MIC range ≤0.03–2 mg/l, showed 100% activity against *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. One *C. glabrata* strain had the MIC = 2 mg/l, while the rest of them had the MIC = 0.06 mg/l. The MICs for the control strains were within the acceptable range for the tested drugs.

Discussion

In the previous study conducted from July 2014 to December 2016, a total of 158 isolates were collected.[3] In the current article the study period was extended with 1 year, providing 77 more isolates and susceptibility profile for all 235 samples were studied. Thus, this is the first and largest study to our knowledge of susceptibility profile of documented and suspected invasive candidiasis performed in a paediatric population in Iran. *C. albicans* and *C. parapsilosis* were responsible for almost half (53.6%) and a quarter (24.7%) of the candidemia cases in children, respectively (Table 2). Our data thus align with the worldwide shift from *C. albicans* to non-*albicans Candida* species.[2] Approximately 13% of all *Candida* isolates belonged to uncommon species outside the five most common ones (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis,* and *C. tropicalis*) confirming these species are also rare in Iran. Compared to other studies,[12–15] *C. glabrata* and *C. krusei* were very rare among our isolates. This may be due to the low implementation of azole prophylaxis in the management of candidemia in Iran but also to geographical and demographic characteristics in Iran.

The rate of acquired resistance was low, in agreement with some studies in other countries[13,15–21] (Table 3). Amphotericin B showed excellent activity against all *Candida* isolates consistent with studies in Turkey, Kuwait, Taiwan, Italy, Germany, Brazil, Argentina, and USA.[8,16,17,19–23] Sutcu et al. found that all except two isolates of *C. lusitaniae,* were susceptible to amphotericin B.[22] Of note, none of 10 *C. lusitaniae* isolates in our study were amphotericin B resistant, in agreement with the findings elsewhere.[12] However, this species can develop secondary resistance to this drug due to a higher mutational rate compared to other *Candida* spp. and therefore is not regarded a good target for amphotericin B therapy regardless the MIC.[9,24]

Resistance to fluconazole is of concern because it is recommended as an alternative to amphotericin B or first line treatment for infantile and neonatal candidaemia.[25] In our setting, the fluconazole susceptibility rate was 100% for the four most common species *C. albicans*, *C. parapsilosis*, *C. tropicalis,* and *C. lusitaniae* (accounting for 91% of the isolates). This is in agreement with the susceptibility profile of *Candida* species causing candidaemia in Switzerland[26] and Taiwan.[20] Similarly, susceptibility to voriconazole was high with only one *C. glabrata* (voriconazole MIC of 2 mg/l, fluconazole MIC ≥ 32 mg/l) displaying acquired resistance in our setting. Farooqi et al. and Motta et al. both reported a single *C. glabrata* isolate with MIC of 4 mg/l as the mono-resistance *Candida* isolate.[27] Lyon et al. found that resistance to fluconazole predict resistance to voriconazole.[28] In this study, we found no correlation between fluconazole and voriconazole susceptibility for *C. guilliermondii,* and overall, voriconazole MICs were low in agreement with the findings of others.[29]

In contrast with some reports,[28,30] we found no indications of acquired echinocandin resistance among the species that are normally susceptible to this drug class. *C. parapsilosis* is intrinsically intermediate to echinocandins due to an intrinsic alteration in the hot spot region of the target genes FKS1.[31,32] A minority of *C. parapsilosis* were classified as resistant to micafungin due to an MIC one dilution above the intermediate breakpoint, suggesting either a rare occurrence of acquired micafungin

Table 3. Epidemiology of antifungal resistance in our study and other pediatric studies

Antifungals	Countries	No. of tested isolates	*C. albicans*	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. lusitaniae*	Others	Total	Method	References
Amphotericin B	Current study	235								0.0	EUCAST	
	Spain 2011	201			8.3					0.5	CLSI	13
	Germany 2011	32			50.0	25.0	…*	50.0		9.4	CLSI	17
	Kuwait 2013	89			…	…	…	…		0.0	CLSI	18
	Mexico 2013	342			1.4	11.1	…	…	…	…	CLSI	15
	Taiwan 2016	295					…	…	5.9	1.0	EUCAST	21
	Brazil 2017	47								0.0	CLSI	20
	Italy 2017	41			…	…	…	…	…	…	CLSI	22
	Turkey 2017	54					…			0.0	CLSI	23
Fluconazole	Current study	235				100.0	…	100.0	12.5	3.8	EUCAST	
	Spain 2011	201	1.4		16.6		100.0		33.3	3.0	CLSI	13
	Germany 2011	32				25.0	…	50.0		6.3	CLSI	17
	Kuwait 2013	89								0.0	CLSI	18
	Mexico 2013	342			1.4	11.11	…	…	…	…	CLSI	15
	Taiwan 2016	295	0.7	All NS**	7.1	…	…	…	11 NS	26 NS	EUCAST	21
	Brazil 2017	47	3.0			3.6	…	…		4.3	CLSI	20
	Italy 2017	41			…	…	…	…	…	0.0	CLSI	22
	Turkey 2017	54	7.4				…	50.0	33.7	15.2	CLSI	23
Voriconazole	Current study	235					14.3	…		0.4	EUCAST	
	Spain 2011	201	1.4		8.3				33.3	1.5	CLSI	13
	Germany 2011	32				25.0	…			3.1	CLSI	17
	Kuwait 2013	89	…	…	…	…	…	…	…	…	CLSI	18
	Mexico 2013	342				11.11	…	…	…	…	CLSI	15
	Taiwan 2016	295	3.0		28.6	90.0	…	…	5.9	6.5	EUCAST	21
	Brazil 2017	47	…	…	…	…	…	…	…	…	CLSI	20
	Italy 2017	41	…	…	…	…	…	…	…	…	CLSI	22
	Turkey 2017	54	9.1			…	…			5.0	CLSI	23
Micafungin	Current study	235		3.5			…	70.0	50.0	66.7	EUCAST	
	Spain 2011	201		1.1	8.3				66.6	2.0	CLSI	13
	Germany 2011	32								0.0	CLSI	17
	Kuwait 2013	89	…	…	…	…	…	…	…	…	CLSI	18
	Mexico 2013	342		3.3			…	…	…	…	CLSI	15
	Taiwan 2016	295	17.2	2.3	10.0		…	…	78.4	22.4	EUCAST	21
	Brazil 2017	47								0.0	CLSI	20
	Italy 2017	41			…	…	…	…	…	0.0	CLSI	22
	Turkey 2017	54	…	…	…	…	…	…	…	…	CLSI	23
Anidulafungin	Current study	235		100.0	5.0		…	20.0	25.0	29.1	EUCAST	
	Spain 2011	201		1.0	8.3				66.6	2.48	CLSI	13
	Germany 2011	32		18.7			…			3.1	CLSI	17
	Kuwait 2013	89	…	…	…	…	…	…	…	…	CLSI	18
	Mexico 2013	342		2.47			…	…	…	…	CLSI	15
	Taiwan 2016	295	53.7		57.0	10.0	…	…	76.5	40.7	EUCAST	21
	Brazil 2017	47								0.0	CLSI	20
	Italy 2017	41			…	…	…	…	…	…	CLSI	22
	Turkey 2017	54				…	…	100.0	…	…	CLSI	23

*Were not done; **nonsusceptible.

resistance in these isolates or more likely that technical errors caused the few elevated MICs. MIC values of echinocandins were high also for *C. guilliermondii* isolates consistent with the findings of others.[33] *C. lusitaniae* strains also showed moderately elevated echinocandins MIC values compared to *C. Albicans*, as has been found previously in the context of a wild-type target gene.[34] However, Asner et al. described resistance of *C. lusitaniae* to all common antifungals, and also they showed that while echinocandins or azole resistance followed monotherapy, multidrug antifungal resistance emerged during combined therapy.[24] On the other hand, it is believed that echinocandin resistance has become increasingly common among isolates of *C. glabrata*, *C. lusitaniae*, and *C. krusei*.[35] Hence, it is important to be aware of possible selection of echinocandin resistance especially during long-term therapy.

In conclusion, knowledge of the local epidemiology is of utmost importance when selecting the primary therapy before the infections species in known.[36] In Iran, *C. albicans* is still the most common cause of paediatrics candidaemia, although a significant number of the yeasts isolated are non-*albicans Candida* species with *C. parapsilosis* accounted for a quarter of all isolates. Acquired resistance was rare. Therefore, as the echinocandins are expensive and not preferable for *C. parapsilosis*, amphotericin B associated with toxicity, and fluconazole which covers more than 90% of our isolates, we suggest fluconazole remain a valid choice for empiric therapy for patients at high risk of invasive

candidiasis in Iranian neonatal and pediatric intensive care unit patients. However, the recovery of some *Candida* species resistant to echinocandins, and the presence of resistance in uncommon *Candida* species, makes the species identification and drug susceptibility testing crucial.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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