

# Impact of antifungal stewardship interventions on the susceptibility of *Candida* species in pediatric patients with malignancy

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## Research

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## Abstract

## Background

There is a worldwide concern with respect to the antimicrobial resistance and the inappropriate use of antifungal agents, which had led to an ever-increasing antifungal resistance. This study aimed to identify the antifungal susceptibility of *Candida* species isolated from the pediatric patients with cancer and also to evaluate the clinical impact of antifungal stewardship (AFS) interventions on antifungal susceptibility of *Candida* species.

## Methods

In this study, *Candida* species colonization were evaluated among hospitalized children with cancer in a tertiary teaching hospital, Shiraz 2017-18. The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) for polyenes (amphotericin B), echinocandins (caspofungin), and azoles (voriconazole, fluconazole, posaconazole, and itraconazole). Antifungal susceptibility of *Candida* species was compared with our previous study results to determine the clinical impact of AFS interventions on the antifungal susceptibility.

## Results

The prevalence of *Candida albicans* in the present study was significantly higher than other *Candida* species. *Candida albicans* species were completely susceptible to the azoles. The sensitivity rate of *C. albicans* to amphotericin B and caspofungin was 93.1% and 97.1%, respectively. The results confirm the positive effect of optimized antifungal usage and bedside intervention on the susceptibility of *Candida* species after the implementation of the AFS program. *C. albicans* and *C. glabrata* exhibited significant increase in sensitivity after the execution of AFS program.

## Conclusions

Improving the antifungal agent usage can improve antifungal susceptibility and reduce resistance. The AFS is recommended to be addressed, applied, and regularly assessed in centers.

## Introduction

The prevalence of *Candida* infections is on the rise as a result of excessive usage of broad-spectrum antibiotics, HIV infection, malignancies, transplants, invasive procedures, and prolonged hospitalization [1–3]. More than 17 different species of *Candida* are recognized that can infect humans, and amongst them, 90% of all invasive infections are related to *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* [4, 5].

Antifungal resistance usually occurs following selective pressure induced by the use or misuse of antifungal agents in high-risk patients, especially those with malignancy [6–8]. *Candida* infections epidemiology changes by the duration of the antifungal therapy and its type during preventive strategies, such as prophylaxis[9].

*C. albicans* susceptibility breakpoint is continuously updated by the Clinical and Laboratory Standards Institute (CLSI), which can be used as an indicator to manage systemic candidiasis [10]. Although *C. albicans* is the most common cause of *Candida* infections, the prevalence of non-*albicans* species is increasing [6]. The emergence of non-*albicans* *Candida* infections have become a global concern; however, as we described previously, change in its epidemiology after adherence to the AFS program was observed [11]. Similar positive effects could be expected by improving the susceptibility pattern of *Candida* species after AFS implementation. Therefore, the aim of this study was to identify the antifungal susceptibility of *Candida* species amongst pediatric patients with *Candida* colonization and also to investigate the ASP intervention effects on antifungal susceptibility in pediatric patients with hematological malignancy.

The incidence of IFDs ranged from 7.7 to 12.5/1000 admissions during 2015-18 in our center. Invasive candidiasis (IC) is the most common form of IFDs (47.2%), and its annual incidence ranges 22.5–55.3%.

## Methods And Materials

# Settings

The university hospital Amir is a tertiary oncology center in Shiraz, Iran, with more than 1.5 million inhabitants. The department of hematology/medical oncology has seven wards (100 active beds), an autologous transplant ward, and annually over 5000 pediatric patients are given admission.

## Population

All children aged <18-year-old between 2017-18 with hematologic malignancy or solid organ tumors were included in this study. Samples were collected from oral/nasal secretions and urine/stool specimens. In children with severe thrombocytopenia or bleeding tendency, only urine and stool samples were collected. Weekly sampling was performed for patients with more than one-week of hospitalization. This cross-sectional study was approved by the Ethics Committee of Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences. After explaining the study objectives, informed consent of children's parents or guardians was obtained.

## Mycological study

Urine, nasal, oral, and stool samples were cultured on Sabouraud Dextrose Agar (Merck, Germany) medium. The cultured samples were transferred to the Mycology Laboratory of Professor Alborzi Clinical Microbiology Research Center for identification and susceptibility testing. The fungi isolates were cultured twice on Potato Dextrose Agar medium at 35°C for seven days to ensure the culture purity, and then the cultures were read at 530 nm by a spectrophotometer. *C. parapsilosis* ATCC-22019 and *C. krusei*-ATCC-6258 were used as standard quality control CLSI-recommended strains.

## Antifungal drugs

The amphotericin B, caspofungin, voriconazole, fluconazole, posaconazole, and itraconazole powders (Sigma-Aldrich, Germany) were used to make serial dilutions. The broth microdilution method was applied to determine the susceptibility. CLSI M51-A, M59, and M60 followed in specifying the susceptibility pattern of isolates [10, 12, 13]. Inoculum suspension was prepared, using the spectrophotometric method at 530 nm. The dilutions of 1:1000 was prepared in RPMI 1640 medium (Sigma-Aldrich, England), supplemented with 1.5% agar and 2% glucose at pH 7.0, using 0.165 M-morpholine propane sulfuric acid (MOPS). The final concentration was adjusted to 0.5 McFarland, with a volume of 100 µl conidium dilution, were added to wells and incubated at 35 °C. Positive and negative wells (wells without drug and yeast, respectively) were considered for each species, and the minimum inhibitory concentration (MIC) was read visually after 24 and 48 hours. For amphotericin B, complete growth inhibition was considered as MIC value. Then, MIC<sub>50</sub> and MIC<sub>90</sub> values of the isolated species were calculated. The susceptibility pattern of each isolate was analyzed based on CLSI breakpoints [10].

## Antifungal Stewardship program principles in Amir medical oncology center

AFS is a "strategic planning" that can be summarized in learning, training, and continuous practice to improve evidence-based skills in the management of IFDs in high-risk patients. By the sustained adherence to the AFS, indiscriminate use of antifungal agents, drug resistance, side effects and costs will be reduced. The AFS has been executed in our center since June 2015. Characteristics of AFS interventions summarized in Table 1. It should be noted that the diagnosis and treatment of the IFDs was significantly improved after the implementation of the AFS. Changing from empiric therapy to pre-emptive antifungal treatment strategies was accomplished by the application of non-culture-based methods, such as galactomannan (GM) antigen test and polymerase chain reaction (PCR). Therapeutic drug monitoring and antifungal susceptibility testing has become the standard of care for monitor serum voriconazole concentrations and targeted therapy since the early 2016. We used the original data from our recent investigation, which was conducted before the implementation of AFS during 2011-2012 in pediatric patients with malignancy (period-1; p1) [14]. This was done to compare the clinical impact of AFS interventions on antifungal susceptibility of different *Candida* species with the present study (period-2; p2).

<b>Table 1. Main components of implemented AFS interventions in AMOC</b>
· <b>Appropriate treatment of the suspected IFDs</b>
· Disposition to targeted therapy (by diagnostic driven approach) instead of empiric treatment
· Adherence to current evidence-based guidelines in the treatment of the IFDs instead of individual decision making
· <b>Appropriate antifungal prescription</b>
· Appropriate antifungal selection
· Appropriate duration
· Appropriate administration route
· Appropriate dosage
· <b>Limited use of azoles for prophylaxis of the IFDs (only for secondary prophylaxis in patients with a previous history of IMDs)</b>
· <b>Regular epidemiologic surveillance to estimate fungal infection incidence and detection of any epidemiologic shift</b>
· <b>Regular surveillance of the sensitivity pattern to antifungal drugs</b>
· <b>Appropriate use of new diagnostic modalities (implementation of routine GM test, twice/week during prolonged and profound neutropenic phase (ANC&lt;500 cells/mm<sup>3</sup>))</b>
· <b>Improving mycological diagnostic approach with judicious use of bronchoalveolar lavage and ultrasound/CT scan guided lung biopsy (or other organs as needed)</b>
· <b>Time-sensitive automatic stop orders for specified antifungal prescriptions</b>
· <b>Switching from intravenous to oral antifungal, when appropriate and confirmed by the infectious disease consultant</b>
· <b>Full-time laboratory services (24-hour, seven days per week coverage) and strategies for reducing lab turnaround time (establishing a "hotline" for contributors to call about the lab test results)</b>
· <b>Non-medical approach to prevent fungal infections</b>
· Applying modalities to reduce the nosocomial infections (for example diminished colonization by the appropriate use of an indwelling catheter)
· Surveillance of the possible environmental roots of infection (for example surveillance of indoor spore load in the hospital's wards)

AFS: antifungal Stewardship; IFDs: invasive fungal diseases; IMDs: invasive mold diseases; GM: galactomannan; ANC: absolute neutrophil count; AMOC: Amir Medical Oncology Center,

### Statistical analysis

Data were analyzed using IBM SPSS Statistics 21 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). All categorical variables are reported in percentages and numbers. P values were calculated, using the Chi-square test and Fisher's exact test. P values <0.05 were considered to be statistically significant. The Pearson correlation test was used to investigate the correlations between quantitative variables.

## Results

From May 2017 to November 2018, 482 oral/nasal samples, and urine and stool specimens were collected from 136 pediatric patients with hematological malignancies or solid organ tumors. Most patients were male (53.3%), and the mean age was 7.57 years (Median: 6.5, Std. Deviation: ± 4.85, range from 4.8 months to 18 years). Eighty-two cases were detected having at least one *Candida* spp. colony, and amongst them 59 were *C. albicans* colonies.

Acute lymphoblastic leukemia (41/136, 30.1%), acute myeloblastic leukemia (18/136, 13.2%), and neuroblastoma (13/136, 9.5%) were the most common underlying diseases, respectively. In total, 51.4% (70) were neutropenic (absolute neutrophil count < 1500 cells/mm<sup>3</sup>).

One hundred thirty-three strains of *Candida* species were detected (2 species not identified), amongst which 102 strains were *C. albicans* followed by *C. krusei* (7), *C. kefyr* (7), *C. parapsilosis* (5), *C. glabrata* (4), *C. tropicalis* (3), and *C. famata* (3). Susceptibility of *Candida* species to different antifungal drugs are summarized in Table 2.

Among the 102 *C. albicans* isolates collected during 2017-18 surveillance program, all were susceptible to the azole agents. The sensitivity rate of *C. albicans* to amphotericin B and caspofungin was 93.1% (95) and 97.1% (99), respectively.

Among the seven *C. krusei* isolates, 28.6% were resistant to caspofungin. All the *C. krusei* isolates were sensitive to amphotericin B, voriconazole. For itraconazole, 85.7% were sensitive, and 14.3% were susceptible dose-dependent.

All the five *C. parapsilosis* isolates were sensitive to amphotericin B, caspofungin. For itraconazole, 80% were sensitive, and 20% were susceptible dose-dependent. In total, 80% of *C. parapsilosis* were found to be susceptible to fluconazole.

Among all the tested strains of *C. glabrata* and *C. tropicalis*, none of them were resistant to any of the antifungal agents.

CLSI breakpoints are not available for *C. kefyr* and *C. famata*. Susceptibility of different antifungals to *C. kefyr* and *C. famata* is provided in Table 3.

Table 2

Susceptibility of 133 *Candida* spp. to antifungal drugs and distributions of MIC ( $\mu\text{g/ml}$ ) by CLSI broth microdilution method <sup>a</sup>

Organism	AF	Breakpoints	S	SDD	I	R	ECV*	WT	N-WT	MIC50*	MIC90*	MIC Range*	
C. albicans	AmpB	$S \leq 1, R \geq 1$	93.1%	-	-	6.9%	4	96%	4%	0.250	0.50	0.032-8	
	CSF	$S \leq 0.25, I = 0.5, R \geq 1$	97.1%	-	1.96%	1%	0.25	97%	3%	0.032	0.064	0.032-1	
	VCZ	$S \leq 0.12, I = 0.25, - 0.5 R \geq 1$	100%	-	-	-	0.032	98%	2%	0.032	0.032	0.032-0.125	
	FCZ	$S \leq 2, SDD = 4, R \geq 8$	100%	-	-	-	0.25	98%	2%	0.032	0.125	0.032-4	
	PSZ	This drug doesn't have any breakpoint						-	-	-	-	-	-
	ITC	$S \leq 0.12, SDD = 0.25, - 0.5 R \geq 1$	100%	-	-	-	0.064	98%	2%	0.032	0.032	0.032-0.064	
C. glabrata	AmpB	$S \leq 1, R \geq 1$	100%	-	-	-	0.25	75%	25%	0.250	0.5	0.25-0.5	
	CSF	$S \leq 0.12, I = 0.25, R \geq 0.5$	75%	-	25%	-	0.125	75%	25%	0.125	0.25	0.064-0.25	
	VCZ	ECV = 0.5, WT: MIC $\leq$ ECV & Non-WT: MIC $>$ ECV						0.032	100%	-	0.032	0.032	0.032
	FCZ	$SDD \leq 32, R \geq 64$	-	100%	-	-	0.25	75%	25%	0.25	1	0.125-1	
	PSZ	This drug doesn't have any breakpoint						-	-	-	-	-	-
	ITC	$S \leq 0.12, SDD = 0.25, - 0.5 R \geq 1$	100%	-	-	-	0.064	75%	25%	0.064	0.125	0.064-0.125	
C. krusei	AmpB	$S \leq 1, R \geq 1$	100%	-	-	-	0.5	85.7%	28.6%	0.5	1	0.25-1	
	CSF	$S \leq 0.25, I = 0.5, R \geq 1$	14.3%	-	57.1%	28.6%	0.5	71.4%	14.3%	0.5	1	0.25-1	
	VCZ	$S \leq 0.5, I = 1, R \geq 2$	100%	-	-	-	0.125	85.7%	28.6%	0.125	0.25	0.064-0.25	
	FCZ	C. krusei is considered resistant to FCZ, irrespective of the MIC						-	-	-	-	-	-
	PSZ	This drug doesn't have any breakpoint						-	-	-	-	-	-
	ITC	$S \leq 0.12, SDD = 0.25, - 0.5 R \geq 1$	85.7%	14.3%	-	-	0.125	85.7%	28.6%	0.125	0.25	0.125-0.25	
C. tropicalis	AmpB	$S \leq 1, R \geq 1$	100%	-	-	-	0.25	66.7%	33.3%	0.25	0.5	0.25-5	
	CSF	$S \leq 2$	100%	-	-	-	0.064	66.7%	33.3%	0.064	1	.032 - 1	
	VCZ	$S \leq 0.12, I = 0.25, - 0.5 R \geq 1$	100%	-	-	-	0.032	100%	-	0.032	0.032	0.032	
	FCZ	$S \leq 8, R \geq 64$	100%	-	-	-	0.032	66.7%	33.3%	0.032	0.125	0.032-0.25	

\* ( $\mu\text{g/ml}$ ). a. Based on recommended CLSI 24-hour minimum inhibitory concentration limits [10].AmpB: Amphotericin B, CSF: Caspofungin, VCZ: Voriconazole, FCZ: Fluconazole, PSZ: Posaconazole, ITC: Itraconazole, AF: antifungal, SDD: susceptible dose-dependent, S: sensitive, I: intermediate, R: resistant, ECV: Epidemiological Cutoff Value; ECVs capture  $\geq 97.5\%$  of the statistically modelled population [12], WT: Wild-type, NWT: non-wild-type, MIC50: Minimum Inhibitory Concentration required to inhibit the growth of 50% of organisms. MIC90: Minimum Inhibitory Concentration required to inhibit the growth of 90% of fungal species.

Organism	AF	Breakpoints	S	SDD	I	R	ECV*	WT	N-WT	MIC50*	MIC90*	MIC Range*	
	PSZ	This drug doesn't have any breakpoint					-	-	-	-	-	-	-
	ITC	S ≤ 0.12, SDD = 0.25, - 0.5 R ≥ 1	100%	-	-	-	0.032	100%	-	0.032	0.032	0.032	
<i>C. parapsilosis</i>	AmpB	S ≤ 1, R ≥ 1	100%	-	-	-	0.25	80%	20%	0.25	0.5	.032-.5	
	CSF	S ≤ 2, I = 4, R ≥ 8	100%	-	-	-	0.5	80%	20%	0.064	0.125	.032-0.125	
	VCZ	S ≤ 0.12, I = 0.25, - 0.5 R ≥ 1	80%	-	20%	-	0.032	80%	20%	0.032	0.5	.032-.5	
	FCZ	S ≤ 2, SDD = 4, R ≥ 8	80%	-	-	20%	0.064	80%	20%	0.064	16	.032 - 16	
	PSZ	This drug doesn't have any breakpoint					-	-	-	-	-	-	
	ITC	S ≤ 0.12, SDD = 0.25, - 0.5 R ≥ 1	80%	20%	-	-	0.032	80%	20%	0.032	0.25	.032-.25	

\* (µg/ml). a. Based on recommended CLSI 24-hour minimum inhibitory concentration limits [10].

AmpB: Amphotericin B, CSF: Caspofungin, VCZ: Voriconazole, FCZ: Fluconazole, PSZ: Posaconazole, ITC: Itraconazole, AF: antifungal, SDD: susceptible dose-dependent, S: sensitive, I: intermediate, R: resistant, ECV: Epidemiological Cutoff Value; ECVs capture ≥ 97.5% of the statistically modelled population [12], WT: Wild-type, NWT: non-wild-type, MIC50: Minimum Inhibitory Concentration required to inhibit the growth of 50% of organisms. MIC90: Minimum Inhibitory Concentration required to inhibit the growth of 90% of fungal spores.

Table 3  
Susceptibilities of different antifungals to *C. kefyr* and *C. famata*

Species (no. tested)	Antifungal agent	MIC (µg/ml)		
		Range	50%	90%
<i>C. kefyr</i> (7)	Fluconazole	0.032–0.25	0.064	0.125
	Voriconazole	0.032	0.032	0.032
	Itraconazole	0.032	0.032	0.032
	Caspofungin	0.032-1	0.064	0.125
	Amphotericin B	0.064-2	0.25	0.5
<i>C. famata</i> (3)	Fluconazole	0.032–0.25	0.032	0.032
	Voriconazole	0.032	0.032	0.032
	Itraconazole	0.032–0.25	0.032	0.032
	Caspofungin	0.032–0.125	0.032	0.032
	Amphotericin B	0.25–0.5	0.25	0.25

The epidemiologic cutoff value (ECV) for *in vitro* susceptibility testing of *Candida* spp. was calculated based on "CLSI epidemiological cutoff values for antifungal susceptibility testing. 2nd ed. CLSI supplement M59" [12].

CLSI does not provide posaconazole minimal inhibitory concentration breakpoint for *C. albicans*. Posaconazole 24-hour and 48-hour MIC statistics were determined for 106 *C. albicans* isolates. Accordingly, mean 24-hour and 48-hour MIC were 0.0361 and 0.0394, respectively (Table 4, Fig. 1).

Table 4  
Posaconazole 24-hour and 48-hour MIC statistics for 102 isolates of *C. albicans*

	24-hour MIC	48-hour MIC
Mean	0.0361	0.0394
Median	0.0320	0.0320
Mode	0.03	0.03
Std. Deviation	0.02470	0.04727
Variance	0.001	0.002
Range	0.22	0.47
Minimum	0.03	0.03
Maximum	0.25	0.50

## Discussion

Amongst the 136 studied cases, 59.9% (82) were colonized with at least one *Candida* species. Most of them were colonized with *C. albicans* 59 (72%). *C. krusei*, *C. kefyr*, *C. glaberata*, *C. parapsilosis*, *C. tropicalis*, and *C. famata* were the least common *Candida* species. Detail information regarding the colonization pattern of the studied cases can be found in our recently published paper [11].

According to the results, all *C. krusei*, *C. glaberata*, *C. parapsilosis*, and *C. tropicalis* isolates were susceptible to amphotericin B (the most active agent for the treatment of non-albicans *Candida* species). Caspofungin exhibited high resistance against *C. krusei* and *C. glaberata* isolates (28.6% and 25%, respectively). Accordingly, amphotericin B can be considered as a more active agent than caspofungin for the treatment of non-albicans *Candida* species, especially, *C. krusei* and *C. glaberata*.

### Frequency of azoles, caspofungin and amphotericin B resistance amongst uncommon *Candida* species

There are limited reports on azole MIC range for *C. kefyr*. However, based on a large epidemiological study in our region [8], the available MIC range in colonizing isolates were 0.064-2 (MIC 90%: 0.5 µg/ml); 0.032–0.25 (MIC 90%: 0.125 µg/ml); 0.032–0.125 (MIC 90%: 0.032 µg/ml) for fluconazole, itraconazole and voriconazole, respectively. The caspofungin MIC range was reported between 0.032-2 (MIC 50%: 0.15 µg/ml and MIC 90%: 0.03 µg/ml) [8, 15]. *C. kefyr* is generally considered susceptible to amphotericin B (MIC < 1 µg/ml); however, wide range of MICs have been reported worldwide (0.5–64) [16–18]. *C. kefyr* is usually considered susceptible to posaconazole (MIC 90%: 0.032 to 0.12 µg/ml) [8, 17].

The reported fluconazole MIC range for *C. famata* is 0.25–16 (MIC 50%: 2–4 µg/ml and MIC 90%: 16 µg/ml) [19]; for itraconazole is 0.06–2 (MIC 50%: 0.25 µg/ml and MIC 90%: 0.5 µg/ml) [20]; and for voriconazole is 0.032-1 (MIC 90%: 0.125 µg/ml) [8]. The caspofungin MIC range is 0.032–0.25 (MIC 50%: 0.5 µg/ml and MIC 90%: 1 µg/ml) [8, 15]. *C. famata* is generally considered susceptible to amphotericin B (MIC < 1 µg/ml) [8, 21]. While higher posaconazole MICs were reported for *C. famata* (0.015–1; MIC 50%: 0.25 µg/ml, and MIC 90%: 1 µg/ml) [22], we found lower MIC range in our study (0.032–0.125, MIC 50%: 0.032 µg/ml). It should be noted that MIC ranges are different between colonized and infected isolates [8].

Accordingly, in this study the colonized isolates of *C. Kefyr* and *C. famata* were susceptible to the tested antifungal agents.

Therapeutic options for fungal infections are limited even before the global rise of antifungal resistance [23, 24]; hence, judicious prescription of available choices, especially non-azole antifungals, should be considered in high-risk settings, such as oncology centers. Our results confirmed that caspofungin and amphotericin B are more active antifungal agents compare to azoles in some isolates, such as *C. parapsilosis*.

The emergence of azole-resistant *C. glabrata* is also of significant concern in the setting that use fluconazole prophylaxis [9].

### Clinical impact of antifungal stewardship interventions on antifungal susceptibility of *Candida* species during the two study periods

Epidemiological changes in the *Candida* colonization pattern was described in our previous report. Briefly, we found a significant reduction in non-*albicans* species colonization after the implementation of AFS. During period 1 (p1), 46.5% (88) of the studied cases (n = 188) were

colonized, while in the 2nd period, the colonization rate reached 59.9% (P value = 0. 0.017) [11]. In total, 25.3% (23) of the cases were receiving antifungal prophylaxis during the 2nd period, mainly with the liposomal formulation of amphotericin B, while 54% were on antifungal prophylaxis during p1, mostly with fluconazole or itraconazole [Difference 21.2%, 95% CI: 9.16–31.77%, P = 0.0007]. This success was achieved by controlling and restricting antifungal usage during p2.

In a study by Hadadi et al. during 2011-12 *C. albicans* was the most common species followed by *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. famata*, *C. parapsilosis*, *C. dubliniensis*, and *C. kefyr* during the p1. *C. glabrata* was the most resistant isolated *Candida* species, showing 70% resistant to fluconazole and 50% to itraconazole, 7.5% to amphotericin B, and 14% to ketoconazole [25].

#### Frequency of azole and caspofungin resistance *C. albicans*

Amongst the 117 tested isolates of *C. albicans*, 52.5% (53) of the isolates were found to be azole-resistant during p1, while only 1.5% (2) were resistant during p2 (P value < 0.001). No fluconazole-resistant (MIC  $\geq$  8  $\mu$ g/ml) *C. albicans* was detected during p2 (P value < 0.001). Multidrug-resistant strains, including azole, caspofungin and amphotericin B resistant isolates were not found within the two study periods (Table 4 and Fig. 2).

#### Frequency of amphotericin B resistance *C. albicans*

Despite the significant reduction in fluconazole and caspofungin-resistant, during p2, some increase in the incidence of amphotericin B-resistant *C. albicans* was detected during p2 (Table 5). A small but significant increase in amphotericin B-resistant *C. albicans* strains can be partially explained by changing antifungal preventive strategy to liposomal amphotericin B, since 2015. However, the frequency of amphotericin B-resistant *C. albicans* was not affected by liposomal amphotericin B prophylaxis between the two periods ( $p = 0.619$ ).

Table 5  
The sensitivity of isolated *C. albicans* against fluconazole, caspofungin, and amphotericin B, during 2011-12 (period 1) and 2017-18 (period 2), No. (%) of isolates

Antifungal agent	Sensitivity	Period 1	Period 2*	p-value
Fluconazole	Sensitive	53 (67.1)	102 (102)	< 0.001**
	Resistant	26 (32.9)	0	
Caspofungin	Sensitive	54 (83.1)	99 (97.1)	< 0.001**
	Resistant	11 (16.9)	1 (1)	
Amphotericin B	Sensitive	83 (100)	95 (93.1)	< 0.001**
	Resistant	0	7 (6.9)	
* No fluconazole-resistant isolates of <i>C. albicans</i> was found during period 2 (2017–2018)				
** Statistically significant by Fisher's exact test				

#### Frequency of azoles, caspofungin, and amphotericin B resistance amongst the non-*albicans* colonized species

We also analyzed the rate of fluconazole, voriconazole, itraconazole, caspofungin and amphotericin B resistance amongst the non-*albicans* colonized species between the two study periods. Significant decrease in fluconazole, itraconazole and caspofungin resistance was found among the *C. glabrata* strains during the second study period compared with 2011-12. Also, a statistically significant reduction in amphotericin B resistance was found during p2 in *C. krusei* isolates (Table 6).

#### Clinical impact of *Candida* colonization in hospitalized patients with hematological malignancies

Most of *Candida* bloodstream infections, including central line-associated candidemia originate from endogenous host flora [26–28]. The clinical impact of *Candida* colonization on the short-term mortality rate of patients with hematological malignancies was reported in a previous study [29]. On the other hand, short-term survival was affected in patients with non-*albicans* species, including *C. glabrata*, *C. kefyr*, and *C. krusei*, compare with *C. albicans* [29, 30]. Azole prophylaxis has a critical role in the development of either unsusceptible strains or even in the selection of yeast with intrinsic azole-resistant, such as *C. krusei* [6, 9, 31, 32].

As discussed earlier, during p1, more than 35% of cases were colonized with non-*albicans* species, mostly *C. glabrata* and *C. krusei*. However, after the implementation of the AFS, non-*albicans* colonization decreased to less than 20%, mostly *C. krusei* and *C. Parapsilosis*, with a significant decrease in *C. glabrata* colonization [11]. *C. glabrata* is considered as the second most common gastrointestinal yeast

flora, after *C. albicans* [33]. In our opinion, the shift toward more *C. albicans* colonization is the result of sustained adherence to the AFS and restricted azole prescription (Table 1).

### **Comparison of the mean minimum inhibitory concentration (MIC) value of the six antifungal drugs between different *Candida* spp. during the two study periods**

In addition to the susceptibility results, we also compared the mean MIC value of each antifungal drug for *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. famata*, and *C. kefyr* during two study periods (Fig. 4). As shown in Fig. 4, a significant reduction in mean FCZ-MIC was found for *C. albicans*, *C. glabrata*, and *C. tropicalis* in p2 compare to p1.

There are lots of data with respect to the positive effect of stewardship program on bacterial resistance [34–37]; however, due to its multifactorial development, antifungal resistance is more challenging to measure. Even in colonized patients, susceptibility patterns might change over time, especially in immunocompromised hosts [8]. Although the AFS has known short-term effects (e.g., reduction in antifungal consumption, costs and outcomes) on the management of IFDs and patient safety [38–41], its long-term effects has been described on resistance patterns [42]. Based on stewardship program metrics, change in resistance patterns, and pathogen-specific resistance is the most difficult target to reach [34]. To the best of our knowledge, there is a shortage of reports on the improvement of antifungal susceptibility of *Candida* species overtimes after the implementation of AFS. Hence, the results of our study highlight the importance of strict adherence to the stewardship programs amongst the cancer patients.

## **Limitations**

This study was limited by the small number of samples. Additionally, further studies using next generation sequencing are needed to detect AFS effects on antifungal resistance genes in *Candida* spices.

## **Conclusion**

*C. albicans* is the most prevalent colony amongst the pediatric patients with malignancy, and azoles remain the most effective choice when they are used wisely. Improving *Candida* species antifungal susceptibility after the implementation of AFS is promising. Knowledge of etiologic agents, along with the regular identification of antifungal susceptibility patterns is necessary in oncology settings.

## **Declarations**

### **Ethics and consent to participate**

The study was approved by medical ethics committee of *Professor Alborzi clinical microbiology research center*. All individuals (or their parents) in the study population were informed about the current study, with written consents obtained before enrolment in the present study.

## **Consent for publication**

Not applicable

### **Availability of data and material**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors do not have any financial or other relationships which could be regarded as a conflict of interest.

## **Funding**

Not applicable

## **Author contributions:**

Study concept and design: AA and HSS; Acquisition of data: AA, JH, HSS and GF; Mycological analysis: BP, JH and GF; Statistical Analysis: AA, HS, and NS, Analysis and interpretation of data: AA and HSS; Drafting of the manuscript: AA, Critical revision of the manuscript for important intellectual content: AA and BP; Study supervision: AA and BP. All individuals listed as (co)-authors have met the authorship criteria, and nobody who qualifies for authorship has been omitted from the list. The final manuscript was corrected and approved by all authors.

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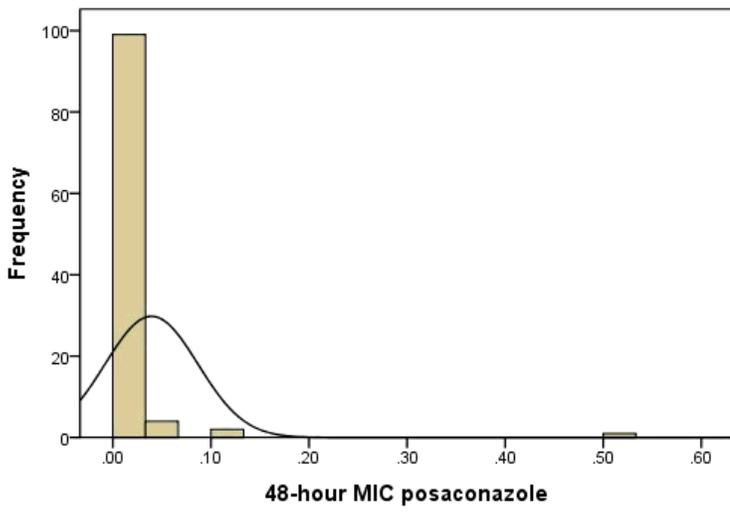
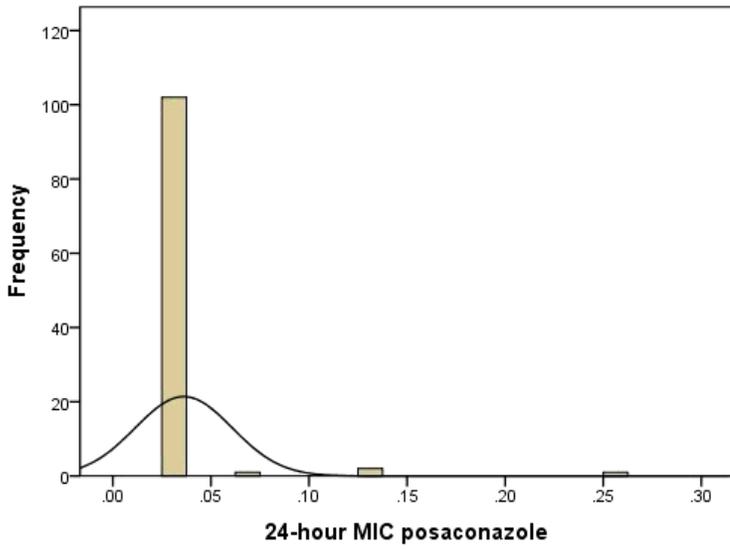
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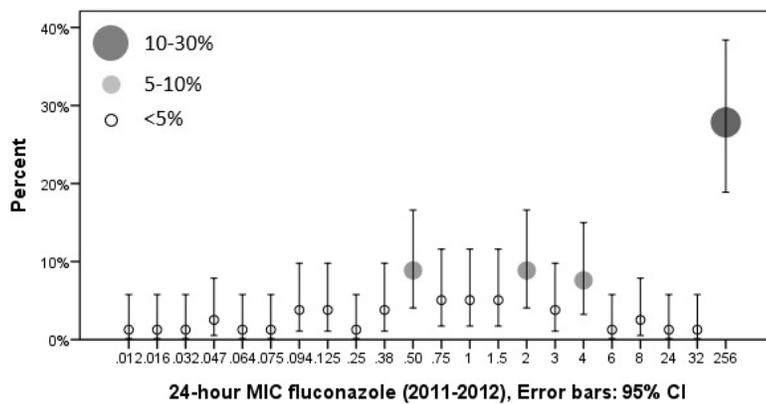
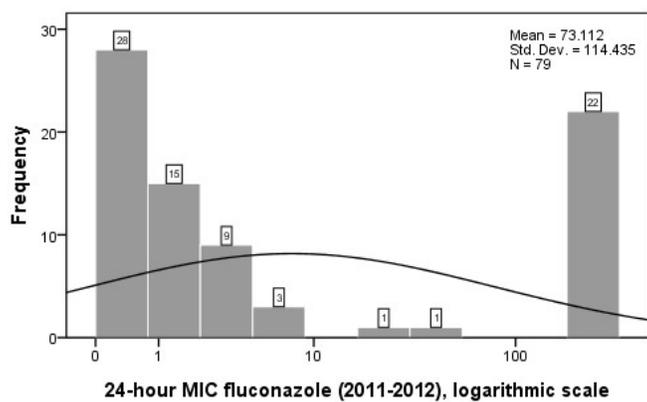
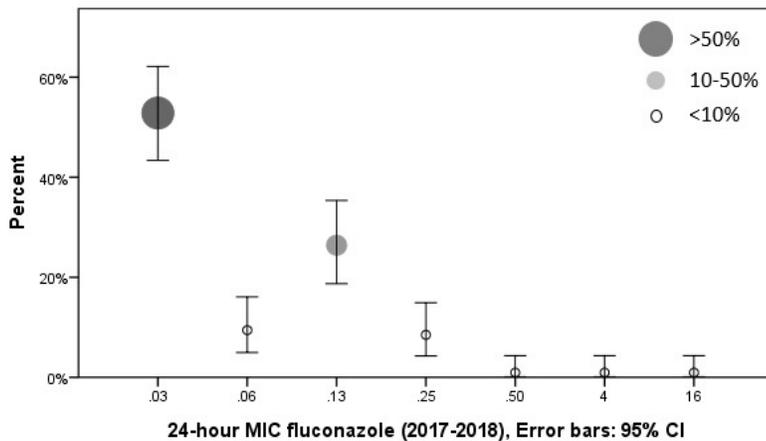
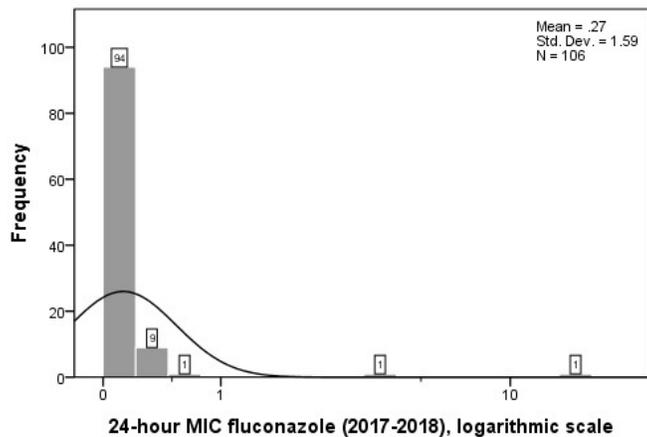
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## Figures



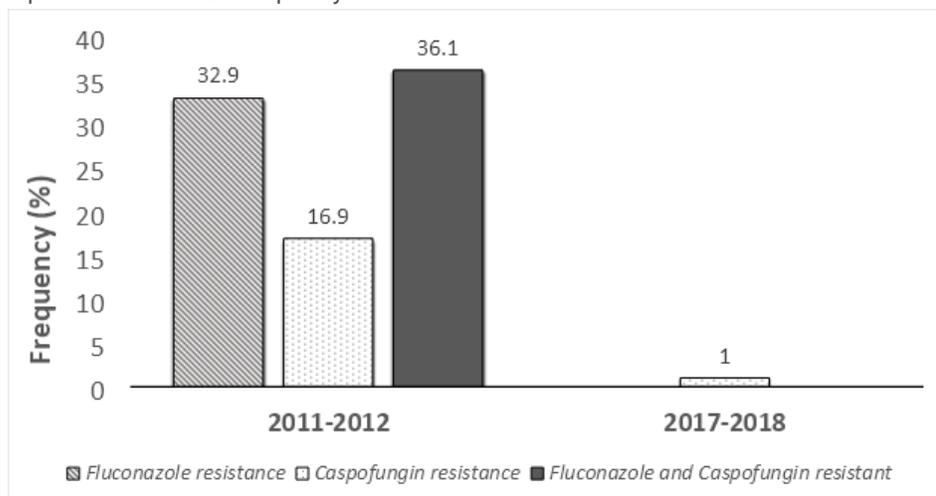
**Figure 1**

24-hour and 48-hour MIC distribution with a histogram of the isolated *C. albicans*



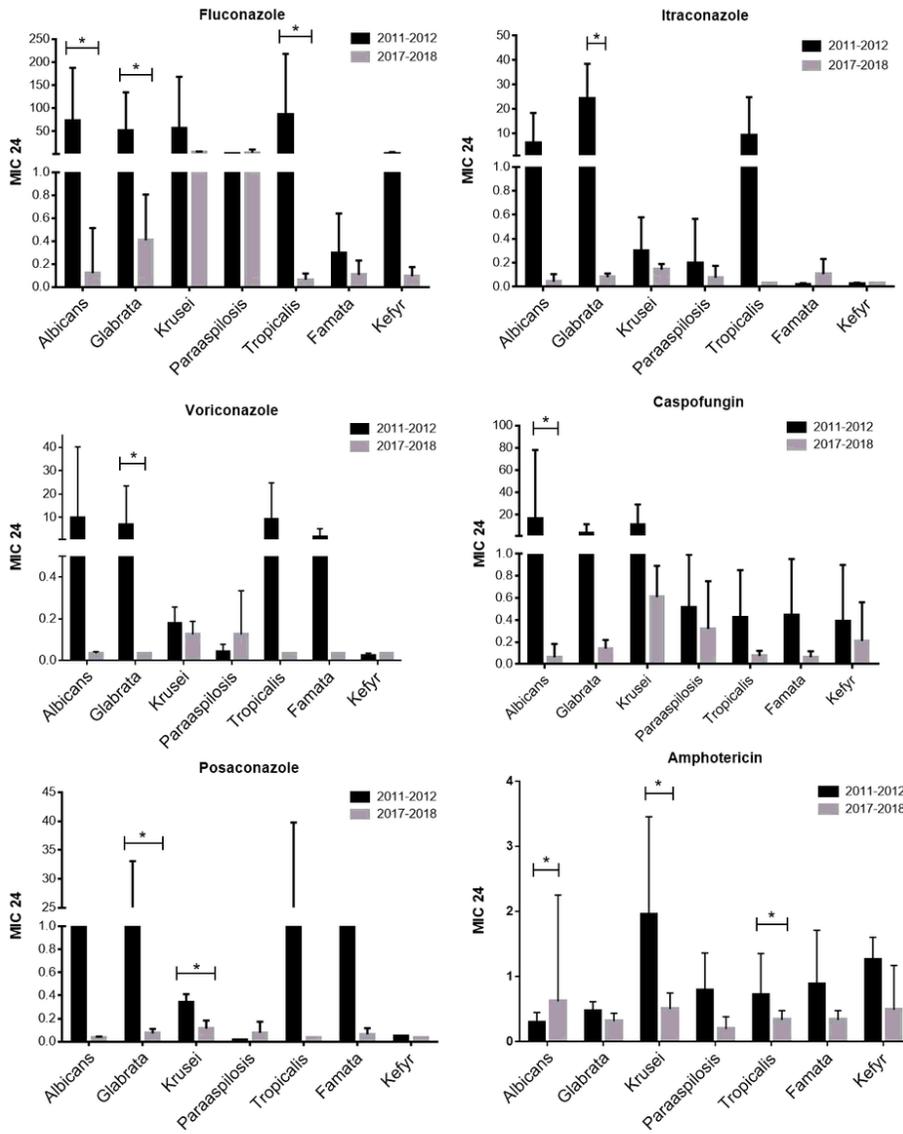
**Figure 2**

24-hour MIC fluconazole of 117 (2011-2012), and 106 (2017-2018) strains of *C. albicans*. Left, chart bar. Logarithmic scales of 24-hour MIC fluconazole, each bar is labelled with the number of isolates. MIC distribution histogram also is provided for better comparison between the two periods. Right, Error bar. Frequency of MIC results is presented in error bars with 95% CI. Each error bar is labelled by circles that are representative of MIC frequency.



**Figure 3**

Frequency of fluconazole-resistant, caspofungin-resistant and fluconazole/caspofungin-resistant strains of *C. albicans* during the two study periods



**Figure 4**

The mean MIC value (24-hour) of *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. famata*, and *C. kefyr* for fluconazole, itraconazole, voriconazole, caspofungin, posaconazole, and amphotericin B, during the two study periods. Error bars represent standard deviations. \*P ≤ 0.05 by the two-way ANOVA test.