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Original Article

Candida profiles and antifungal resistance evolution over a decade in Lebanon

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Abstract

Introduction: Infection with and antifungal resistance of *Candida* species have been on the rise globally. Relevant data on these pathogens are relatively few in our region, including Lebanon, thus warranting this study.

Methodology: This retrospective study of *Candida* spp. profiles and their *in vitro* antifungal susceptibility was based on analysis requests for 186 *Candida* non-*albicans* and 61 *C. albicans* during three periods (2005–2007, 2009–2011, and 2012–2014) over the span of the last 10 years at the American University of Beirut Medical Center (AUBMC), a major tertiary care center in Lebanon. Identification of *Candida* was done using the API 20C AUX system, and the E-test was used to determine the minimum inhibitory concentrations (MICs) of antifungal agents.

Results: Among the 1,300–1,500 *Candida* isolates recovered yearly, *C. albicans* rates decreased from 86% in 2005 to around 60% in 2014. Simultaneously, the non-*albicans* rates increased from 14% in 2005 to around 40% in 2014, revealing 11 species, the most frequent of which were *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. All these demonstrated high resistance (35%–79%) against itraconazole, but remained uniformly susceptible (100%) to amphotericin B. Though C. *albicans* and the other species maintained high susceptibility against fluconazole and voriconazole, their MIC_{90} showed an elevated trend over time, and *C. glabrata* had the highest resistance rates.

Conclusions: The observed rise in resistance among *Candida* spp. in Lebanon mandates the need for close surveillance and monitoring of antifungal drug resistance for both epidemiologic and treatment purposes.

Key words: antifungal resistance; *Candida* spp.; *Candida* non-*albicans*; Lebanon.

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Introduction

Medical advances have been contributing to the increasing number of sustained immunocompromised patients. This has been paralleled by the increasing susceptibility of such patients to opportunistic infections, especially *Candida* infections [1-4].

Though *Candida* spp. are normal commensals in many parts of the body including the skin, mucous membranes, respiratory and gastrointestinal tracts, the rates of *Candida* infections have been on the rise worldwide for the past two decades. This rise ranked *Candida* fourth among the most common bloodstream isolates and nosocomial bloodstream infections, and among the most commonly cultured organisms from all sites in intensive care units (ICUs) in the United States of America (USA) [5-7]. Certain risk factors augment the risk of candidemia, including immunosuppression, prolonged use of antibiotics, prolonged stay in ICUs, and use of central venous catheters [8].

Despite improvement in medical care, infection control measures, and antifungal therapy, significant morbidity and mortality remains very high. For example, high rates of mortality ranging between 40% and 90% in high-risk patients, such as those with hematologic malignancies, are still being encountered [9-10].

Generally, antifungal agents shown to be effective for the treatment of *Candida* infections include amphotericin, the triazoles (fluconazole, itraconazole, voriconazole, posaconazole), the echinocandins, and flucytosine. However, there is emerging evidence from different parts of the world of increasing resistance among *Candida albicans* and non-*albicans* species to these agents [4,11]. Nonetheless, data concerning the profile and antifungal susceptibility of *Candida* spp. are relatively few in some Arab countries [12-19] and other countries in the region [20-24]. In Lebanon, only one study exists, which was published 17 years ago [25]; thus, this subject warrants an update.

This retrospective study aimed to identify the *Candida* species and determine their susceptibility to fluconazole, voriconazole, intraconazole and amphotericin B at the American University of Beirut Medical Center (AUBMC) throughout three different periods of time over the last 10 years.

Methodology

Candida isolates

Candida isolates analyzed and evaluated in this study were those recovered from patient specimens submitted for fungal investigation at the clinical microbiology laboratory (CML) of the AUBMC. These were submitted for speciation and antifungal susceptibility testing prior to commencing patients' therapy. The isolates were non-duplicates, and each was recovered from one patient over three periods of time (2005–207, 2009–2011, and 2012–2014) over a span of 10 years.

Identification and speciation of Candida isolates

The routine identification and speciation of *Candida* isolates was based on microscopic and macroscopic growth morphology, and germ-tube testing. *C. dubliniensis*, being also germ-tube positive, is differentiated from *C. albicans* based on its failure to grow at 45°C.

Speciation of the *Candida* isolates was done using the API 20C AUX system (bio Merieux, Cedex, France). Reading of reactions and interpretation of results were done after 48 hours and 72 hours of incubation at 26°C before the results were finalized.

Antifungal susceptibility testing of Candida species

The E-test (AB Biodisk, Solna, Sweden) was used to determine the minimum inhibitory concentrations (MICs) of fluconazole (range 0.016–256 ug/mL), itraconazole (range 0.002–32 ug/mL), and voriconazole (range 0.002–32 ug/mL), based on the manufacturer's instructions, essentially as reported earlier [25,26]. Briefly, fresh inoculums of isolates from a 0.5 McFarland turbidity suspension were streaked onto RPMI 1640 media (Sigma, St. Louis, MO, USA). The inoculated plates were allowed to dry for around 15 minutes before the antifungal E-strips were placed on top. The plates were incubated in ambient air at 35°C, and the MICs were recorded at 24 hours of incubation. Readings and interpretations of the MICs in the E-test were done according to the manufacturer's instructions, generally determined based on where the border of the elliptical inhibition zone intersected the scale on the strips or where there was a sharp decline in the amount of growth (≈ 80% inhibition). In the case of growth of small colonies inside the inhibition zone, the limit of the inhibition zone was defined as the border where the colonies started to change size and the density of growth decreased (≈80% inhibition).

MIC breakpoints of antifungal agents

The species-specific antifungal MIC breakpoints were used to classify isolates as susceptible (S), susceptible dose dependent (SDD) or resistant (R) were based on the clinical breakpoints (CBPs) as adopted by the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) and reported by Pfaller and Diekem [26-29]. The antifungal breakpoints for different species are as follows. *C. albicans*: fluconazole ($S \leq 2$; SDD: 4; $R \geq 8$), voriconazole ($S \leq 0.12$; SDD: 0.25–0.5; $R \geq 1$), itraconazole ($S \leq 0.12$; SDD: 0.25–0.5; $R \geq 1$), and amphotericin B ($S \leq 2$; $R > 2$). *C. tropicalis*: fluconazole ($S \leq 2$; SDD: 4; $R \geq 8$), voriconazole ($S \leq 0.12$; SDD: 0.25–0.5; $R \geq 1$), itraconazole ($S \leq 0.5$; $R > 0.5$), and amphotericin B ($S \leq 2$; $R > 2$). *C. glabrata*: fluconazole ($S \leq 32$; $R \geq 64$), voriconazole ($S \leq 0.5$; $R > 0.5$), itraconazole ($S \leq 2$; $R > 2$), and amphotericin B ($S \leq 2$; $R > 2$). *C. parapsilosis*: fluconazole ($S \leq 2$; SDD: 4; $R \geq 8$), voriconazole ($S \leq 0.12$; SDD: 0.25–0.5; $R \geq 1$), itraconazole ($S \leq 0.5$; $R > 0.5$), and amphotericin B ($S \leq 2$; $R > 2$). *C. kruseii*: fluconazole ($S \leq 32$; $R \geq 32$), voriconazole ($S \leq 0.5$; SDD: 1; $R \geq 2$), itraconazole ($S \leq 1$; $R > 1$), and amphotericin B ($S \leq 2$; $R > 2$).

Quality control isolates

The quality of test performance was controlled by including the reference strains *C. albicans* (ATCC 10231), C. *parapsilosis* (ATCC 22019), and *C. kruseii* (ATCC 6258) on each day of testing in the E-test.

Results

Yearly recovery of Candida isolates

The yearly total of *Candida* isolates recovered from different clinical specimens at this College of American Pathologists (CAP)-accredited CML during the study periods ranged between 1,300 and 1,500 isolates per year. Among these isolates, the yearly rates of *C. albicans* showed a decrease from 86% in 2005 to 64% in 2007. Since 2008 and until 2014, however, the yearly rate of recovering *C. albicans* was stabilized between 58% and 60%. This situation was paralleled by increasing rates of recovering non-

albicans isolates, 14% in 2005 and reaching 42% in subsequent years. Though it was not an objective of this study, and though it is extremely difficult to retrieve and associate *Candida* isolates for each body site/source from the blood source, an almost steady trend of *Candida* recovery among total pathogens was observed during the study period: 1.9% in 2005–2007, 3% in 2009–2011, and 2.6% in 2012–2014.

Request basis for speciation and susceptibility testing

In this CML, speciation and antifungal susceptibility testing is done on a request basis and not routinely. During the study period, there were 186 *Candida* non-*albicans* requested for speciation and susceptibility testing, and 61 *C. albicans* requested for susceptibility testing.

Types of recovered Candida species

The most common species among the non-*albicans* 186 isolates that had been requested for speciation are shown in Figure 1. Eleven different species were found, and the percentages of the most common among the three different study periods were *C. tropicalis* (34%–45%), *C. glabrata* (25%–36%), and *C. parapsilosis* (9%–22%). *C. krusei* (5%–11%) was also recovered, but at low numbers and in only

Figure 1. Distribution of *Candida* non-*albicans* species during the three study periods

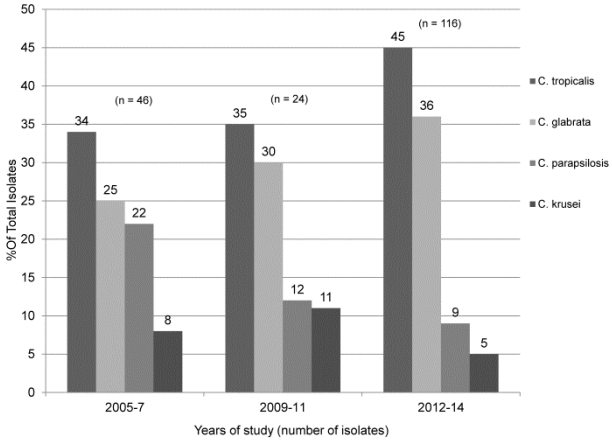

Table 1. Distribution of *Candida* non-*albicans* species during the three study periods

Candida species/year		Aspects related to antifungal												
			Fluconazole			Voriconazole			Itraconazole			Amphotericin		
		No. tested	MIC range (ug/mL)	MIC_{90}	%S	MIC range (ug/ml)	MIC_{90}	%S	MIC range (ug/mL)	MIC_{90}	%S	MIC range (ug/mL)	MIC_{90}	%S
C. albicans	2005–2007	11	0.125–1.5	0.5	100									
	2009–2011	17	0.125–1	0.38	100	0.016–0.19	0.023	94	0.023–2	2	65			
	2012–2014	33	0.125–2	2	94	≤ 0.016–1	0.125	97	0.19–6	2	47	0.047–1	1	100
C. tropicalis	2005–2007	7	0.19–8	0.75	86									
	2009–2011	29	0.125–4	3	79	0.16–0.75	0.19	86	0.124–4	3	62			
	2012–2014	29	0.125– ≥ 256	3	86	0.016–12	0.125	93	0.25–12	8	27	0.25–0.5	0.5	100
C. glabrata	2005–2007	14	0.5– ≥ 256	≥ 256	56									
	2009–2011	16	0.19– ≥ 256	≥ 256	75	0.016– ≥ 32	8	75	0.125– ≥ 32	≥ 32	38			
	2012–2014	24	0.125– ≥ 256	≥ 256	83	0.016–8	8	75	0.125– ≥ 32	≥ 32	21	0.25–1	1	100
C. parapsilosis	2005–2007	6	0.064–0.75	0.5	100									
	2009–2011	3	0.75–1.5	1	100	0.016–0.016	0.016	100	0.023–0.12	0.094	100			
	2012–2014	7	0.75–3	1.5	86	0.016–1.25	0.125	100	0.25–3	1	50	0.047–1	0.047	100
C. krusei	2005–2007													
	2009–2011	8	3–32	32	100	0.016–0.25	0.19	100	0.016–0.19	0.19	100			
	2012–2014													

MIC: minimum inhibitory concentration; %S: percentage susceptible

one of the study periods. Other species recovered in very low rates (1%–4%) included *C. guillermondi*, *C. stellatoide*, *C. kefyr*, *C. norvegiensis*, *C. lusitanae*, and *C. dubliniensis*.

Distribution of clinical material/source

The distribution (%) of the clinical source of the recovered *Candida* species were urine (28%), blood (25%), respiratory tract (19%), fluids (15%), wounds/abscesses (11%), and catheters (2%).

Antifungal susceptibility

The susceptibility of fluconazole, voriconazole, itraconazole and amphotericin B test findings against *Candida albicans* and non-*albicans* isolates requested for testing are presented in Table 1. The MIC (ug/mL) range, the MIC that defines the inhibition of isolates at proportions of 90% (MIC_{90}), and the percentage of susceptible strains are shown for each of the species over the study periods. Susceptibility to itraconazole was lowest among all the species, and over all the study periods. Fluconazole remained highly active (94%–100%) against *C. albicans*, though the trend of higher MIC values and lower susceptibility was observed during the period 2012–2014 compared to the previous periods. *C. tropicalis* showed almost consistent stability in susceptibility (79%–86%) over the three study periods. *C. glabrata* showed lower susceptibility than the other species (56%–83%). However, the latest trend showed higher susceptibility. Though low numbers of *C. parapsilosis* were tested and showed overall high susceptibility (86%–100%) to fluconazole, the trend showed a decrease in susceptibility over time.

Unfortunately, only few *C. krusei* isolates were tested, and in only one period; thus, commenting on *C. krusei* would not be rational. Voriconazole demonstrated high susceptibility (86%–100%) against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, but its activity against *C. glabrata* was lower (74%–75%). Amphotericin B was tested at the latest study period and showed uniform susceptibility to all tested *Candida* species.

Discussion

Surveillance and epidemiology studies of *Candida* infections are very limited or non-existent in Lebanon and the surrounding region, unlike the extensive numbers of studies in North America and Europe [30,31]. The increase in fungal infections has prompted an increase in the use of antifungal agents and in practice resulted in measurable rates of acquired or innate fungal resistance in *Candida* species that necessitates each institution to assess this for the welfare of patients [3]. In this context, this study presents updated information on the frequently recovered *Candida* species and their antifungal susceptibility profile covering a long gap pertaining to this subject since the last relevant publication in 1998 from this country [25].

Although *C. albicans* topped the rank, non-*albicans* showed an increasing trend throughout the ten-year study period, and recently both groups seem to be stabilizing at overall recovery rates of around 60% and 40%, respectively.

Among the ten recovered types of non-*albicans* strains throughout the three study periods, *C. tropicalis* accounted for the highest recovery (34%–45%), followed by *C. glabrata* (25%–36%), *C. parapsilosis* (12%–22%), and *C. krusei* (5%–11%). Such epidemiological distribution can vary among different hospitals and/or geographic locations in the world. For example, the recovery of *C. glabrata* is extremely low in Latin America compared to North America and Europe [32]. In Jordan, *C. glabrata* ranked second after *C. albicans* and showed significant increase in incidence among patients with vulvoalvuovaginitis studied between 1994–1996 (17.9%) and 1999–2001 (32.5%) [19]. On the other hand, a study of candidemia episodes in a Brazilian tertiary hospital found that *C. parapsilosis* and *C. tropicalis* but not *C. albicans* were the most common agents [6].

Similar to other global studies, the recovery of *C. dubliniensis* was extremely low (1 to 5 isolates among the 1,300 and 1,500 *Candida* isolates recovered yearly). Though *C. dubliniensis* is known to be a causative agent of oral mucocutaneous infection, and has been described as a candidemia agent since 1999, our findings are in concordance with the reported low frequency (0.35%–3%) from different parts of the world [33,34].

The association of *Candida* species recovery with hospital units, risk factors, or mortality involved was not an aim of this study. However, published studies have reported that both *C. albicans* and non-*albicans* distribution was more frequently recovered from ICU patients than from those on the wards, mostly ascribed to the use of central venous catheterization; comorbidity conditions such as diabetes mellitus were additional contributors to infection [19,33,34].

Moreover, the mortality rate among *Candida* spp. is not necessarily paralleled or associated with the higher recovered species. For example, Bonfietti *et al*.

[34] reported that *C. glabrata* ranked the lowest in recovery but its mortality in patients was the highest (80%) compared to *C. tropicalis* (77%), *C. albicans* (55%), and *C. parapsilosis* (47%).

Concerning the anti-*Candida* agents, amphotericin B is considered the standard treatment (except for *C. lusitaniae* and *C. guilliermondii*), while the azole group of agents are the most frequently used antifungals systemically and locally.

In our study, all isolates showed uniform susceptibility to amphotericin B. This is in accord with most studies from our region [12-24] and from different parts of the world [31,34,35], where all *Candida* isolates exhibited high susceptibility to amphotericin B, with MICs of up to 1 mg/L. In contrast, the majority of the isolates in our study showed high levels of resistance (35%–79%) to itraconazole. This is different from what was reported in a Brazilian study, where all the isolates exhibited high susceptibility to this drug [34]. However, in the USA [31], high resistance was reported among *C. tropicalis* (22%), *C. glabrata* (59%), and *C. krusei* (23%). In our region, very high resistance was also reported among *C. glabrata* from Israel (43%) and Iran (85%), and among *C. albicans* (38%) and *C. krusei* (87%) from Iran [20,23].

Comparing the findings in the current study with those of an earlier one from our center in 1998 [25] reveals that *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were more susceptible to both fluconazole (94%–100%) and itraconazole (83%–100%) in 1998 than in the current study.

In this study, *C. albicans* maintained high susceptibility to fluconazole (94%–100%) and voriconazole (94%–97%), though their MIC_{90} showed an increasing trend over time. This is also similar to resistant rates reported from Iran (11% for fluconazole, and 6% for itraconazole) [23] and Turkey (6% fully resistant and 11% SDD against fluconazole) [24]. However, rare-to-minimal resistance to these azoles in *C. albicans* isolates were reported by several countries in our region [14-21] and internationally [31,34].

Regarding *C. tropicalis* isolates, our findings showed that they maintained relatively high susceptibility over time against both fluconazole (79%–86%) and voriconazole (86%–93%). Though the voriconazole MIC_{90} remained very low, the increasing trend of fluconazole MIC_{90} from 0.75 ug/mL in 2005–2007 to 3 ug/mL in later study periods is worrying. Variable azole MIC values ranging from 0.06 to 8 mg/L have been reported for *C. tropicalis* bloodstream strains [34].

No rates of resistance against fluconazole were reported from Egypt [15,16], Kuwait [14], and Israel [20,21]. Some resistance (6%) was reported from the USA [31], while moderate to high rates of resistance were reported against fluconazole from Turkey (33%) [24]. Resistance against voriconazole was reported from Qatar (8%) [17], the USA (3%) [31], and most notably from Turkey (33%) [24]. Israel [20] and Turkey [24] also reported resistance rates against itraconazole: 3% and 22%, respectively. It is interesting to observe that high crude mortality (77%) was noted in patients with *C. tropicalis*, although no *in vitro* resistance was observed [34].

C. glabrata in our study, on the other hand, showed relatively low susceptibility against both fluconazole (56%–83%) and voriconazole (74%–75%) with high MIC_{90}: $\geq$ 256 ug/mL and 8 ug/mL, respectively. In our region, studies reported variable resistance rates against fluconazole. For example, fluconazole resistance rates ranging between 6% and 12% were reported from Egypt [15,16] and Kuwait [14], while very high rates ranging between 21% and 100% were reported from Israel [20], Iran [23], Qatar [17], and Turkey [24]. Similarly, the susceptibility data against fluconazole were controversial as contrasts in findings were reported from different parts of the world. For example, low rates of azole resistance were reported from Brazil [36,37] while moderate and high rates were reported from Greece and the USA [24,31]. Although primary intrinsic *in vitro* resistance to fluconazole has been reported, secondary resistance is the most common form of resistance in *C. glabrata* [38]. The ability to acquire resistance to fluconazole and, furthermore, the differences in methodology used in the studies confirm the low reproducibility of tests performed with this species [32].

Though *C. parapsilosis*, in our study, maintained high susceptibility rates to fluconazole, it showed a decreasing trend from 100% to 86% with increasing MIC_{90} from 0.5 ug/mL in the first study period to 1.5 ug/mL in the last. This is in accord with what was reported from several countries in our region [14-20,23] and from the USA [31].

C. krusei recovery in our study was low. This is in agreement with published studies from other parts of the world, where it is also found at low rates, ranging from 0 to 4.5% [3,39,40]. This species is intrinsically resistant to fluconazole, but in our study and others, many strains were susceptible.

Conclusions

Our results revealed elevated incidence of non-*albicans Candida* strains over time. The results also highlighted the importance of determining the incidence of different species and performing antifungal susceptibility tests, as this helps reveal which strains can be resistant to which antifungals. Moreover, this study indicated that although there is a trend towards increasing MICs levels, fluconazole and voriconazole, as well as amphotericin B, maintain adequate activity against the most commonly encountered *Candida* species in our institution, while itraconazole does not. In addition, this study establishes a comparative basis for future studies and surveillance programs to help guide empiric therapeutic approaches in treating *Candida* infections.

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