Changing trends in epidemiology and antifungal susceptibility patterns of six bloodstream *Candida* species isolates over a 12-year period in Kuwait

Ziauddin Khan[1]*, **Suhail Ahmad**[1], **Noura Al-Sweih**[1,2], **Eiman Mokaddas**[1,3], **Khalifa Al-Banwan**[1,4], **Wadha Alfouzan**[1,5], **Inaam Al-Obaid**[6], **Khaled Al-Obaid**[7], **Mohammad Asadzadeh**[1], **Ahlam Jeragh**[8], **Leena Joseph**[1], **Soumya Varghese**[1], **Sandhya Vayalil**[9], **Omar Al-Musallam**[9]

1 Department of Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait, 2 Department of Microbiology, Maternity Hospital, Shuwaikh, Kuwait, 3 Department of Microbiology, Ibn-Sina Hospital, Shuwaikh, Kuwait, 4 Department of Microbiology, Al-Amiri Hospital, Kuwait City, Kuwait, 5 Department of Microbiology, Farwaniya Hospital, Farwaniya, Kuwait, 6 Department of Microbiology, Al-Sabah Hospital, Shuwaikh, Kuwait, 7 Department of Microbiology, Mubarak Al-Kabeer Hospital, Jabriya, Kuwait, 8 Department of Microbiology, Al-Adan Hospital, Hadyia, Kuwait, 9 Mycology Reference Laboratory, Department of Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait

* zkhan@hsc.edu.kw

Abstract

Changing trends in incidence and antifungal susceptibility patterns of six *Candida* species causing candidemia in Kuwait between 2006–2017 are reported. A total of 2075 isolates obtained from 1448 patients were analyzed. Identity of *Candida* species isolates was determined by phenotypic methods and confirmed by PCR amplification/PCR-sequencing of rDNA and/or MALDI-TOF MS. Antifungal susceptibility was determined by Etest. *C. albicans* accounted for 539 (37.22%) cases followed by *C. parapsilosis* (n = 502, 34.67%), *C. tropicalis* (n = 210, 14.5%), *C. glabrata* (n = 148, 10.22%), *C. krusei* (n = 27, 1.81%) and *C. dubliniensis* (n = 22, 1.5%). The comparative percent distribution of *Candida* species causing candidemia between 2006–2011 and 2012–2017 was as follows: *C. albicans* 41.8% and 33.1%, *C. parapsilosis* complex 32.01% and 37.04%, *C. tropicalis* 13.59% and 15.31%, and *C. glabrata* 8.77% and 11.51%, *C. krusei* 2.0% and 1.7%, and *C. dubliniensis* 1.75 and 1.3%, respectively. Three of 371 *C. albicans* isolates during 2006–2011 and five of 363 during 2012–2017 were resistant to fluconazole. Among *C. parapsilosis* isolates, one of 310 during 2006–2011 and 21 of 446 during 2012–2017 were resistant to this drug. Furthermore, at an epidemiologic cutoff value (ECV) of ≤0.5 μg/ml, 70.1% *C. albicans* isolates were wild-type for fluconazole during 2006–2011 as compared to 58.1% during 2012–2017. Likewise, at an ECV of ≤2 μg/ml, 98.0% of *C. parapsilosis* isolates were wild-type during 2006–2011 as compared to 93.4% during 2012–2017. Clonal spread of fluconazole-resistant *C. parapsilosis* in one major hospital was documented. An 8.8% shift in favor of non-*albicans Candida* species with concomitant increase in MICs between the two periods preludes emergence of fluconazole-resistant candidemia cases in Kuwait.

Introduction

Candida species are a major cause of healthcare-associated bloodstream infection (BSI) world-wide [1, 2]. They are associated with considerable infection-related morbidity and mortality, particularly in intensive care unit (ICU), where at least 50% episodes of candidemia occur [3]. While *C. albicans* continues to be the most frequently isolated bloodstream pathogen in most studies, there is a gradual shift towards non-*albicans Candida* species (*C. glabrata*, *C. tropicalis*, and *C. parapsilosis*), which together account for >50% cases of candidemia [1, 2, 4]. Considerable differences exist in the number of cases caused by individual non-*albicans Candida* species depending upon the geographic region, patient population, age and prior exposure to antifungal agents [1, 5]. Regardless of the geographical area, *C. albicans* is more frequent in patients aged up to 18 years, the frequency of *C. parapsilosis* decreases with age and *C. glabrata* is more common among the elderly [1]. Studies from Northern Europe and the USA [4, 6] have reported relatively a higher number of cases due to *C. glabrata* as compared to Spain and Brazil, where a higher number of cases were caused by *C. parapsilosis* [1]. On the contrary, *C. tropicalis* is the most common non-*albicans Candida* species in Asia [7]. Like many other countries, candidemia is also an important cause of BSI both in adult and pediatric patients in Kuwait [8–15]. In a previous study, we have reported species distribution and antifungal susceptibility profile of bloodstream isolates obtained during a 10-year period (1996–2005) [8]. Herein, we aimed to identify epidemiological changes that have occurred in the distribution and antifungal susceptibility pattern of six *Candida* species during a 12-year period since 2006.

Materials and methods

Bloodstream yeast isolates and phenotypic methods for identification

Candida species isolates were grown from blood collected after obtaining informed verbal consent from patients for routine diagnosis. The isolates were referred to Mycology Reference Laboratory (MRL) as a part of routine patient care for identification and susceptibility testing. Since this retrospective study did not involve human participants, no specific ethical approval was required. Patients details were fully anonymized and results on de-identified samples are presented in this manuscript. Six *Candida* species/species complexes viz. *C. albicans*, *C. parapsilosis* sensu lato, *C. tropicalis*, *C. glabrata*, *C. dubliniensis* and *C. krusei* were included. A total of 1448 culture confirmed candidemia cases due to six *Candida* species were recorded during the 12-year study period (2006 to 2017) across Kuwait. Repeat bloodstream isolates were also obtained from several patients with persistent candidemia. The bloodstream isolates were divided into two 6-year study periods that is 2006 to 2011 and 2012 to 2017 to compare relative shift in the prevalence of *Candida* species. The isolates were identified by phenotypic methods which included germ tube test, colony characteristics on CHROMagar Candida and VITEK2 yeast identification system.

Molecular identification

The identity of the isolates which showed unusual phenotypic and/or antifungal susceptibility pattern or resistance to antifungal drugs was confirmed by PCR amplification/PCR-sequencing of rDNA, performed as described in detail previously [16,17]. *C. parapsilosis* sensu stricto were differentiated from *C. orthopsilosis* and *C. metapsilosis* by a multiplex PCR, as described previously [18, 19]. The identity of the isolates was also confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; bioMérieux, Marcy l'Etoile, France) as described previously [20].

Antifungal susceptibility testing

The *in vitro* activity of the antifungal agents was determined by E-test (bioMérieux, Marcy l'Etoile, France). The test was performed in accordance with the manufacturer's instructions. Petri dishes (150 mm diameter) containing 60 ml RPMI agar supplemented with 2% glucose and buffered to pH 7.0 with MOPS were used. The inoculum was applied with cotton swabs using growth suspension prepared in 0.85% NaCl with turbidity adjusted to 0.5 McFarland standard. Plates were incubated at 35°C and read after 24 h. Reference strains of *C. albicans* (ATCC 90028) and *Candida parapsilosis* (ATCC 22019) were used for quality control. Susceptibility breakpoints used for interpretation of susceptible, intermediate/ susceptible dose-dependent and resistant strains were those recommended by the Clinical and Laboratory Standards Institute [21]. Due to lack of defined breakpoints for amphotericin B, isolates showing an MIC of ≤1.0/µg ml were taken as susceptible and those with MIC >1µg/ml as resistant [22]. The CLSI epidemiologic cutoff values (ECV) of fluconazole for *C. albicans*, *C. parapsilosis* and *C. tropicalis* were ≤0.5 µg/ml, ≤2 µg/ml and ≤2 µg/ml, respectively, as also proposed by Pfaller & Diekema [23].

Statistical analysis

Statistical analysis was performed by using chi-square test or Fisher's exact test as appropriate and probability levels <0.05 by the two-tailed test were considered as statistically significant. Statistical analyses were performed by using WinPepi software ver. 11.65 (PEPI for Windows, Microsoft Inc., Redmond, WA, USA).

Results

The species distribution of 2075 *Candida* species isolates obtained from 1448 candidemia patients during the 12-year study period are presented in Table 1. *C. albicans* was the most frequently isolated species accounting for 539 (37.22%) cases closely followed by 502 (34.67%) cases due to *C. parapsilosis* complex isolates including *C. orthopsilosis* (n = 5) and *C. metapsilosis* (n = 2). The remaining cases were due to *C. tropicalis* (n = 210, 14.5%), *C. glabrata* (n = 148, 10.22%), *C. krusei* (n = 27, 1.81%) and *C. dubliniensis* (n = 22, 1.5%) (Table 1). The comparative percent distribution of *Candida* species causing candidemia between 2006–2011 and 2012–2017 was as follows: *C. albicans* 41.8% and 33.1%, *C. parapsilosis* complex 32.01% and 37.04%, *C. tropicalis* 13.59% and 15.31%, and *C. glabrata* 8.77% and 11.51%, and *C. krusei*

Table 1. Distribution of 2075 *Candida* species isolated from 1448 candidemia patients obtained during 2006–2011 and 2012–2017.

Species/ complexes	Distribution of candidemia cases and isolates during				Total cases (%)
	2006–2011		2012–2017		
	Cases (%)	Isolates (%)	Cases (%)	Isolates (%)	
C. albicans	286 (41.8)	405 (41.75)	253 (33.11)	363 (32.85)	539 (37.22)
C. dubliniensis	12 (1.75)	16 (1.64)	10 (1.30)	11 (0.99)	22 (1.5)
*C. parapsilosis**	219 (32.01)	332 (34.22)	283 (37.04)	450 (40.72)	502 (34.66)
C. tropicalis	93 (13.59)	126 (12.98)	117 (15.31)	150 (13.57)	210 (14.5)
C. glabrata	60 (8.77)	76 (7.83)	88 (11.51)	115 (10.40)	148 (10.2)
C. krusei	14 (2.04)	15 (1.54)	13 (1.70)	16 (1.44)	27 (1.8)
Total	**684**	**970**	**764**	**1105**	**1448**

*Includes 7 *C. orthopsilosis* isolates from 5 cases and 2 *C. metapsilosis* isolates from 2 cases

https://doi.org/10.1371/journal.pone.0216250.t001

2.0% and 1.7%, respectively (Table 1). There was an overall increase of 8.8% candidemia cases caused by non-*albicans Candida* species during 2012–2017.

The year-wise distribution of *Candida* species isolates during the study period and the annual incidence rate of candidemia are presented in Table 2. Although *C. albicans* was the most frequently isolated species during 2006 to 2012, it was replaced by *C. parapsilosis* sensu stricto in the next four years (2013 to 2016) as the most frequently isolated *Candida* species from candidemia patients (numbers shown in bold). The isolation frequency of *C. albicans* varied from a low of ~29% in 2013 and 2016 to a high value of 52.2% in 2011, however, it showed a general declining trend over the study period. On the other hand, the isolation frequency of *C. parapsilosis* showed a declining trend during 2006 to 2011 and then an upward trend during 2012 to 2016 (Table 2). *C. tropicalis* and *C. glabrata* were recorded as the third and fourth most frequently isolated *Candida* species in Kuwait, respectively, and the isolation frequency of both the species generally showed an upward trend during the study period (Table 2). Consistent with these data, the ratio of isolation of *C. albicans* versus all six *Candida* species isolates declined from 0.46 in 2006 to 0.33 in 2017. The annual incidence rate (number of candidemia cases per 100, 000 population) showed a declining trend (from 4.45 to 3.5) in Kuwait over the study period (Table 2). One-third of candidemia cases (n = 480) occurred among neonates and there was a >2-fold increase in isolation of *C. parapsilosis* from neonates in Maternity Hospital during 2012–2017 (n = 115) as compared to 2006–2011 (n = 49). *C. glabrata* was isolated in greater number from elderly immunocompromised patients including those with cancer, that is, 23 isolates in 2012–2017 as compared to only 4 in 2006–2011. None of the bloodstream *C. glabrata* isolate was identified as *C. nivariensis* or *C. bracarensis* by PCR amplification and/or PCR-sequencing of rDNA.

The antifungal susceptibility profiles of *Candida* species isolates obtained during the study period were determined by Etest against four antifungal drugs, viz. fluconazole, voriconazole, amphotericin B and caspofungin and the data are presented in Tables 3 and 4. None of the *C. albicans* (n = 741), *C. parapsilosis* sensu lato (n = 763), *C. tropicalis* (n = 264), and *C. dubliniensis* (n = 26) isolates tested in this study was resistant to amphotericin B as their MIC values were ≤1.0/µgml. Only one of 31 *C. krusei* and 2 of 190 *C. glabrata* isolates were resistant to amphotericin B as their MIC values were >1.0 µgml. On the other hand, resistance to

Table 2. Year-wise distribution of *Candida* species and population-based incidence of candidemia in Kuwait (2006–2017).

Year	*C. albicans*	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. dubliniensis*	*C. krusei*	Total	Ratio of *C. albicans*/ all *Candida* species	Total Kuwait population	Annual incidence/10^5 population[*]
2006	**49**	41	9	4	2	1	106	0.46	2377258	4.45
2007	**44**	39	12	8	0	1	104	0.42	2503410	4.15
2008	**43**	35	21	12	0	5	116	0.37	2652340	4.37
2009	**43**	36	17	14	8	0	118	0.36	2818939	4.18
2010	**49**	45	18	11	2	4	129	0.38	2998083	4.3
2011	**58**	23	16	11	0	3	111	0.52	3191051	3.47
2012	**41**	26	16	15	1	0	99	0.41	3396556	2.91
2013	29	**37**	16	15	0	2	99	0.29	3598386	2.75
2014	46	**63**	17	16	3	2	147	0.31	3782450	3.88
2015	50	**55**	19	12	2	4	142	0.35	3935794	3.6
2016	39	**58**	20	13	0	2	132	0.29	4052584	3.25
2017	**48**	44	29	17	4	3	145	0.33	4136528	3.5

[*]Mean annual incidence is 3.69/10^5 population.

Numbers in bold indicate most common species in that year.

Table 3. Prevalence of antifungal drug resistance among bloodstream isolates of major *Candida* species/complexes tested during 2006–2011 and 2012 to 2017 in Kuwait[a].

Antifungal drug	Duration	*Candida albicans*		*Candida parapsilosis*		*Candida orthopsilosis*		*Candida tropicalis*		*Candida glabrata*		*Candida krusei*	
		No. tested	Resistant (%)	No. tested	Resistant (%)	No. tested	Resistant (%)	No. tested	Resistant (%)	No. tested	Resistant (%)	No. tested	Resistant (%)
Amphotericin B	2006–2011	378	0	310	0	5	0	114	0	75	1 (1.4)	15	1 (6.7)
	2012–2017	363	0	446	0	2	0	150	0	115	1 (1.1)	16	0
Fluconazole	2006–2011	371	3 (0.8)	310	1 (0.3)	5	0	112	1 (0.8)	74	3 (4.1)	13	0
	2012–2017	363	5 (1.4)	446	**21 (4.7)**[b]	2	0	150	1 (0.7)	115	14 (12.2)	16	0
Voriconazole	2006–2011	331	4 (1.2)	274	0	5	0	108	1 (0.9)	62	0	14	0
	2012–2017	304	4 (1.3)	360	**7 (1.9)**[b]	1	0	117	0	84	0	12	0

[a]Bloodstream isolates of all *Candida* species tested in this study were susceptible to caspofungin and all isolates of *C. dubliniensis* were additionally susceptible to amphotericin B, fluconazole and voriconazole

[b]Significantly higher rates of resistance to fluconazole and voriconazole observed for *C. parapsilosis* during 2012 to 2017 compared to 2006 to 2011 period are highlighted in boldface numbers

https://doi.org/10.1371/journal.pone.0216250.t003

fluconazole and cross-resistance to voriconazole was detected more frequently. Only eight *C. albicans* isolates were resistant to fluconazole and five of these were isolated during 2012–2017. Three of these isolates also showed cross-resistance to voriconazole. Although resistance to fluconazole in *C. albicans* increased during 2012 to 2017 compared to 2006 to 2011, it was not statistically significant ($P = 0.501$). However, resistance rate among *C. parapsilosis* sensu stricto isolates increased over time for both fluconazole and voriconazole and these differences were statistically significant ($P = 0.000$ and $P = 0.021$, respectively) (Table 3). Resistance to fluconazole was also detected more frequently among *C. glabrata* isolates during 2012 to 2017 compared to 2006 to 201, however, the difference was not statistically significant ($P = 0.070$) (Table 3).

The MIC_{50}, MIC_{90} and the geometric mean of the MICs for amphotericin B and caspofungin remained nearly same for different *Candida* species during the study period (Table 4). None of the bloodstream isolates of *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. dubliniensis* were resistant to amphotericin B and geometric mean of the MICs remained stable between the two study periods (Table 4). Only two isolates of *C. glabrata* were resistant to amphotericin B (MIC $\geq 1\mu g/ml$), one each among the 75 isolates tested during 2006–2011 and 115 isolates during 2012–2017. Three of 371 (0.8%) *C. albicans* isolates tested during 2006–2011 and 5 of 363 (1.37%) isolates during 2012–2017 were resistant to fluconazole (MIC ≥ 8 μg /ml) (Tables 3 and 4). Three of these isolates also showed cross-resistance to voriconazole.

The comparative distribution of MIC values for fluconazole for four major *Candida* species (viz. *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata*) isolates during the study period is presented in Table 5. The *C. albicans* and *C. parapsilosis* isolates showed an increasing trend in MICs to fluconazole. At an ECV of ≤ 0.5 μg/ml, 260 of 371 (70%) *C. albicans* isolates tested during 2006–2011 were wild-type for fluconazole as against 211 of 363 (58.1%) tested during 2012–2017 (Table 5). Emergence of fluconazole resistance was more pronounced among bloodstream isolates of *C. parapsilosis*. Of 310 *C. parapsilosis* isolates, only one isolate (0.3%) was resistant to this drug during 2006–2011 as against 21 of 446 (4.7%) isolates during 2012–2017. This observation was also supported by distribution of MIC values of the isolates

Table 4. Comparison of antifungal susceptibility of bloodstream isolates of five major _Candida_ species/complexes tested during 2006–2011 and 2012–2017.

Candida Species/ Antifungal agent	2006–2011						2012–2017					
	No. of isolates	Range (µg/ml)	MIC50	MIC90	GM (µg/ml)	% Resistance (n)**	No. of isolates	Range (µg/ml)	MIC50	MIC90	GM (µg/ml)	% Resistance (n)**
Amphotericin B												
C. albicans	378	0.002–0.75	0.047	0.125	0.04 ± 0.07	0	363	0.002–0.38	0.047	0.125	0.04 ± 0.05	0
C. dubliniensis*	15	0.002–0.125	0.012	0.047	0.011 ± 0.03	0	11	0.003–0.094	0.008	0.032	0.013 ± 0.025	0
C. parapsilosis	310	0.002–1	0.023	0.094	0.02 ± 0.11	0	446	0.002–0.75	0.023	0.094	0.02 ± 0.08	0
C. orthopsilosis*	5	0.008–0.125	0.023	0.125	0.02 ± 0.04	0	2	0.008–0.064	-	-	0.022 ± 0.039	0
C. tropicalis	114	0.002–0.75	0.047	0.19	0.04 ± 0.11	0	150	0.003–0.5	0.064	0.19	0.06 ± 0.07	0
C. glabrata	75	0.004–2	0.094	0.25	0.08 ± 0.24	1.4 (1)	115	0.002–32	0.125	0.38	0.10 ± 2.97	1.07 (1)
C. krusei	15	0.094–6	0.125	1	0.14 ± 1.51	6.66 (1)	16	0.012–0.5	0.19	0.25	0.13 ± 0.14	0
Fluconazole												
C. albicans	371	0.004-≥256	0.38	1	0.39 ± 22.91	0.8 (3)	363	0.094–24	0.5	1	0.55 ± 1.86	1.37 (5)
C. dubliniensis*	15	0.016–0.75	0.25	0.75	0.24 ± 0.22	0	11	0.125–1	0.38	0.75	0.34 ± 0.26	0
C. parapsilosis	310	0.016–32	0.38	1	0.33 ± 1.88	0.32 (1)	446	0.047-≥256	0.75	1.5	0.69 ± 41.24	4.7 (21)
C. orthopsilosis*	5	0.19–0.5	0.25	0.5	0.25 ± 0.15	0	2	0.25–0.38	-	-	0.308 ± 0.091	0
C. tropicalis	112	0.012–8	0.75	2	0.55 ± 1.05	0.8 (1)	150	0.023-≥256	0.38	1	0.43 ± 20.86	0.66(1)
C. glabrata	74	0.064-≥256	0.094	32	7.17 ± 49.68	4.05 (3)	115	0.75-≥256	12	64	12.43 ± 54.83	12.17 (14)
C. krusei	13	6–64	24	64	21.65 ± 21.57	-	16	0.38–48	16	32	14.11 ± 13.25	-
Voriconazole												
C. albicans	331	0.002-≥32	0.023	0.094	0.02 ± 3.03	1.2 (4)	304	0.002–1.5	0.023	0.125	0.02 ± 0.16	1.3 (4)
C. dubliniensis*	15	0.004–0.19	0.016	0.064	0.02 ± 0.04	0	11	0.006–0.094	0.008	0.023	0.011 ± 0.025	0
C. parapsilosis	274	0.002–0.38	0.016	0.047	0.015 ± 0.037	0	360	0.002-≥32	0.023	0.094	0.024 ± 2.39	1.94 (7)
C. orthopsilosis*	5	0.006–0.125	0.008	0.125	0.014 ± 0.05	0	1	0.012	-	-	-	0
C. tropicalis	108	0.003–2	0.047	0.125	0.046 ± 0.196	0.92 (1)	117	0.004–0.75	0.047	0.125	0.042 ± 0.079	0
C. glabrata	62	0.016–4	0.125	0.75	0.15 ± 0.58	-	84	0.032-≥32	0.25	1.5	0.31 ± 3.51	-
C. krusei	14	0.047–0.5	0.094	0.38	0.13 ± 0.14	0	12	0.047–0.38	0.125	0.38	0.16 ± 0.12	0
Caspofungin												
C. albicans	65	0.002–0.38	0.094	0.19	0.05 ± 0.07	0	363	0.002–0.38	0.094	0.19	0.08 ± 0.06	0
C. dubliniensis*	1	0.047	-	-	-	0	11	0.032–0.19	0.064	0.19	0.08 ± 0.06	0
C. parapsilosis	32	0.012–1.5	0.38	1	0.27 ± 0.34	0	446	0.002–1	0.38	0.5	0.29 ± 0.15	0
C. orthopsilosis*	1	0.38	-	-	-	0	2	0.38–0.5	-	-	0.43 ± 0.084	0

(Continued)

Table 4. (Continued)

Candida Species/ Antifungal agent	2006–2011						2012–2017					
	No. of isolates	Range (μg/ml)	MIC50	MIC90	GM (μg/ml)	% Resistance (n)**	No. of isolates	Range (μg/ml)	MIC50	MIC90	GM (μg/ml)	% Resistance (n)**
C. tropicalis	29	0.008–0.75	0.094	0.25	0.093 ± 0.147	0	150	0.002–0.38	0.125	0.19	0.10 ± 0.07	0
C. glabrata	9	0.047–0.38	0.125	0.25	0.129 ± 0.107	0	115	0.002–0.38	0.125	0.19	0.11 ± 0.07	0
C. krusei	5	0.125–0.38	0.38	0.38	0.26 ± 0.12	0	16	0.19–0.5	0.25	0.38	0.26 ± 0.104	0

*CLSI breakpoints as recommended for *C. albicans* and *C. parapsilosis* [19].

Fluconazole: *C. albicans*, *C. parapsilosis*, *C. tropicalis*: ≤ 2 μg/ml (S), 4 μg/ml (SDD), ≥ 8 μg/ml (R); *C. glabrata*: ≤ 32 μg/ml (SDD), ≥ 64 μg/ml (R)

Voriconazole: *C. albicans*, *C. parapsilosis*, *C. tropicalis*: ≤ 0.12 μg/ml (S), 0.25–0.5 μg/ml (I), ≥1 μg/ml; *C. krusei*: ≤ 0.5 μg/ml (S), 1 μg/ml (I), ≥ 2 μg/ml (R)

**Figures in parentheses indicate number of patients yielding resistant isolates.

obtained during the two periods (Table 5). At an ECV of ≤2 μg/ml, 304 of 310 (98.0%) isolates obtained during 2006–2011 were wild-type for fluconazole as against 417 of 446 (93.4%) isolates tested during 2012–2017 (Table 5). Among 21 fluconazole-resistant *C. parapsilosis* isolates, cross-resistance to voriconazole (MIC >1μg/ml) was observed in 7 isolates. One isolate each of *C. tropicalis* from both the periods (1 of 112 during 2006–2011 and 1 of 150 during 2012–2017) was resistant to fluconazole. None of the *C. dubliniensis* (n = 27), *C. orthopsilosis* (n = 7) and *C. metapsilosis* (n = 2) isolates were resistant to the four antifungal agents tested including fluconazole (Tables 3 and 4). Although *C. glabrata* intrinsically is less susceptible to azoles, a greater number of fluconazole-resistant isolates (14 of 115, 12%) were encountered in 2012–2017 than in 2006–2011 (3 of 74, 4.0%).

Discussion

Invasive fungal infections (IFI) are regarded as the diseases of medical progress. With the changes in clinical practice, the spectrum of fungi capable of causing IFI is also changing, and so is the epidemiology of invasive *Candida* infections. *Candida* infections form a major component of healthcare-associated IFIs and are associated with considerable mortality, varying from 29% to 72% in different patient populations representing different geographic regions/ countries [24]. Like-wise, annual incidence of candidemia is also quite variable in different populations and age groups [24]. Surprisingly, the annual incidence rate (number of candidemia cases per 100,000 population) showed a declining trend (from 4.45 to 3.5) in Kuwait over the study period. One likely explanation for this decline is the rapid increase (from 2.377 million in 2006 to 4.136 million in 2017) in the total population of Kuwait particularly during 2010–2014, mostly due to influx of large number of younger (<40 years of age) and healthy expatriate workers who are less likely to have IFIs. In our previous study from Kuwait, we have reported *C. albicans* as the predominant species accounting for 39.5% of isolates over a 10-year period (1996–2005), followed by *C. parapsilosis* (30.0%), *C. tropicalis* (12.5%) and *C. glabrata* (5.6%) [8]. While *C. albicans* remained as the most frequently isolated species from candidemia patients during 2006 to 2012, it was replaced by *C. parapsilosis* sensu stricto during 2013 to 2016 as the most frequently isolated *Candida* species. Furthermore, the isolation frequency of *C. tropicalis* and *C. glabrata* also showed an upward trend during the study period. Consistent with these data, the ratio of isolation of *C. albicans* versus all six *Candida* species isolates declined nearly consistently from a high of 0.46 in 2006 to a low of 0.33 in 2017. Thus, the proportion of *Candida* species causing candidemia has significantly changed in favor of non-

Table 5. Comparative distribution of four major *Candida* species obtained during 2006–2011 and 2012–2017.

Candida species / Antifungal agent	Year of testing	No. of isolates	MIC distribution of isolates (µg/ml by Etest)																						
			≤0.023	0.03	0.047	0.06	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	6	8	12	16	24	32	48	≥64
Amphotericin B																										
C. albicans	2006–2011	378	120	65	64	53	31	24	9	5	5		2													
	2012–2017	363	112	50	56	55	51	20	14	3	2															
C. parapsilosis	2006–2011	310	157	42	42	24	14	13	7	2	4	1	2	2												
	2012–2017	446	229	65	57	40	29	8	7	2	3	3	3													
C. tropicalis	2006–2011	114	33	10	18	13	14	11	6	4	1	3	1													
	2012–2017	150	21	16	19	20	29	24	13	6	1	1														
C. glabrata	2006–2011	75	18	4	8	4	4	4	16	10	3	3			1											
	2012–2017	115	13	2	11	12	15	22	12	15	5	7												1		
Fluconazole																										
C. albicans	2006–2011	371	4	2		7	5	33	49	49	63	48	54	21	21	7	2	3								3
	2012–2017	363					2	8	22	37	62	80	65	60	17	2	1	2	3	1	1					
C. parapsilosis	2006–2011	310	13	3	2	2	7	19	31	52	49	58	34	18	13	3	2	2	1						1	
	2012–2017	446			1		10	10	34	35	66	66	86	66	28	15	5	2	1	2	1		2	2		14
C. tropicalis	2006–2011	112	3				4	4	5	10	15	10	24	18	6	9	1	1	1	1						
	2012–2017	150	1			2		4	16	20	33	31	24	14	3		1									1
C. glabrata	2006–2011	74				1		1	1			1	1		4	1	3	13	6	13	11	4	6	4	1	3
	2012–2017	115											1		1	2		22	13	17	13	17	3	9	3	14
Voriconazole																										
C. albicans	2006–2011	331	190	31	48	17	18	17	3	2		1		1										3		
	2012–2017	304	173	24	22	33	15	10	13	3	6		1		2	2										
C. parapsilosis	2006–2011	274	209	25	14	7	10	5	2	1	1															
	2012–2017	360	224	48	27	18	7	7	3	5	8	3	3		1	2	2							2		
C. tropicalis	2006–2011	108	34	9	13	10	22	10	5	3	1				1											
	2012–2017	117	36	18	13	20	17	6	5	1			1													
C. glabrata	2006–2011	51	4		1	5	8	6	5	7	5	2	3	1	3			1								
	2012–2017	84		3	2	3	4	6	15	10	19	4	5	2	3	4	1	2						1		

(*Continued*)

Table 5. (Continued)

Candida species / Antifungal agent	Year of testing	No. of isolates	MIC distribution of isolates (µg/ml by Etest)																							
			≤0.023	0.03	0.047	0.06	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	6	8	12	16	24	32	48	≥64
Caspofungin																										
C. albicans	2006–2011	65	14	3	5	10	13	13	2	3	2																
	2012–2017	363	35	18	24	56	77	64	75	13	1																
C. parapsilosis	2006–2011	32	3			1		4	3	2	5	7	3	3	1												
	2012–2017	446	5	1	3	6	9	11	73	82	154	83	17	2													
C. tropicalis	2006–2011	29	3		4	6	4	3	3	4	1		1														
	2012–2017	150	8	3	13	9	31	32	43	6	5																
C. glabrata	2006–2011	9			1	2		2	2	1	1																
	2012–2017	115	8	1	2	12	20	25	39	6	2																

Note: Number of resistant isolates shown in bold.

albicans Candida species. During the first half of the study (2006–2011), C. albicans accounted for 41.8% of bloodstream infections, followed by C. parapsilosis (32.01%), C. tropicalis (13.59%), and C. glabrata (8.77%). During the next six-year (2012–2017) period, the proportion of C. albicans decreased about 9% (33.11%), whereas C. parapsilosis showed an increase of >5% and the other two species, C. tropicalis and C. glabrata of about 2% each.

Our data are consistent with world reports showing that the prevalence of non-albicans Candida species is gradually increasing and other Candida species such as C. parapsilosis, C. glabrata or C. tropicalis are emerging as more frequent cause of bloodstream infections [1, 25–28]. C. glabrata has now become the dominant species in some centers of USA, Canada and Europe [4, 6] while C. parapsilosis has also become the dominant species in some centers/geographical locations [4, 28–30]. Additionally, many less common and newly recognized Candida species including Candida auris are being isolated with increasing frequency [31–35]

A comparative distribution of bloodstream isolates of Candida species from the Middle East is presented in Table 6 [8, 12, 36–47]. Recent studies from Qatar [43], Israel [44] and Iran [45] also indicate predominance of non-albicans Candida species as compared to C. albicans. Generally, the prevalence of C. tropicalis and C. glabrata has been reported to be ≤20% with one exception from Israel, where a higher rate of C. glabrata (23.7%) was reported in adult patients (Table 6) [8, 12, 36–61]. This shift towards non-albicans Candida species is consistent with many previous reports [1, 6, 24]. However, this shift is also somewhat species-specific. The largest proportional increase in C. glabrata has been observed in USA [4, 29], Australia [48] and Belgium [49]. In contrast, several surveillance and multicenter studies in other countries, such as Spain [50], Latin America [26, 28] and South Africa [51] have indicated the emergence of C. parapsilosis as the second most important cause of bloodstream infection after C. albicans. Consistent with many other studies from North America, Latin America, China and Europe [26, 28, 29, 52], C. tropicalis is the third most common cause of bloodstream infection in Kuwait. This is in contrast to countries of Asian Pacific region [53] as well as India [54] and

Table 6. Distribution of *Candida* species isolated from candidaemia patients in different studies reported from the Middle East.

Reference	Study period	Country	No. of isolates	% Distribution of Candida species						% Ratio *C. albicans*/ non *albicans*
				C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. krusei*	Others	
Rennert *et al.* [36]	1994	Israel	298	53.6	11.9	10.9	6.5	0.7	15.9	53.6/45.9
Bukharie [37]	1995–2000	Saudi Arabia	32	19	44	25	3	6	3	19/81
Ellis *et al.* [38]	1995–2001	UAE	60	45	5	15	5	0	30	45/55
Al-Jasser & Elkhizzi [39]	1996–2002	Saudi Arabia	294	50.7	10.9	20.7	7.1	7.8	3.1	50.7/49.6
Al-Sweih *et al.* [12]	1997–2006	Kuwait	153	41.2	49	-	1.3	-	8.49	41.2/58.79
Osoba *et al.* [40]	1998–2002	Saudi Arabia	83	46	10.8	10.8	4.8	6	21.6	46/54
Al-Essa *et al.* [41]	1997	Kuwait	22	31.8	13.7	-	-	-	54.5	31.8/68.2
Mokaddas *et al.* [42]	1994–1998	Kuwait	25	56	28	8	8	-	10.8	56/54.8
Mokaddas *et al.* [8]	1996–2005	Kuwait	607	39.5	30.6	12.4	5.6	1.6	11.8	39.5/62
Taj-Aldeen *et al.* [43]	2004–2010	Qatar	201	33.8	20.8*	17.9	18.9	-	8.5	33.8/45.3
Eliakim-raz *et al.* [44]	2007–2014	Israel	118**	44	15.2	13.5	23.7	0.8	2.54	44/55.74
Vaezi *et al.* [45]	until 2015	Iran	55	27.2	30.9	14.5	18.1	-	9	27.2/72.5
Obaid & Khan [46]	2014–2016	Kuwait	82	32	32	20	13	-	3.65	32/68.65
Taj-Aldeen *et al.* [47]	2009–2014	Qatar	301	30.2	17.9	17.9	25.5	-	8.3	30.2/69.6
Present study	2006–2011	Kuwait	970	41.75	34.2	12.98	7.8	1.54	-	41.75/56.52
	2012–2017		1105	32.8	40.72	13.57	10.4	1.44	-	32.8/66.13

*Includes 8 isolates of *C. orthopsilosis*;

**Includes population of >18 years; United Arab Emirates (UAE)

Pakistan [55] where *C. tropicalis* is the second most common bloodstream pathogen after *C. albicans*. The epidemiologic factors that might have contributed to predominance of *C. tropicalis* in these countries are not fully understood.

Our data of antifungal susceptibility suggest that resistance is still uncommon (<5%) but it is beginning to emerge against fluconazole particularly in *C. albicans* and *C. parapsilosis*. The comparison of MIC data clearly demonstrates that there is a noticeable increase in fluconazole MICs of *C. albicans* as well as of *C. parapsilosis* among the isolates obtained during 2012–2017 as compared to 2006–2011. The first fluconazole-resistant *C. albicans* isolate from blood was detected in March 2011. Subsequently, the number of resistant isolates recorded during 2006–2011 and 2012–2017 increased to three (0.8%) and five (1.37%), respectively. The reduction in fluconazole susceptibility was also reflected by MIC distribution. At an ECV of ≤0.5 μg/ml, 260 of 371 (70.1%) *C. albicans* isolates obtained during 2006–2011 were wild-type for fluconazole as compared to 211 of 363 (58.1%) isolates during 2012–2017. Emergence of fluconazole resistance was more conspicuous and statistically significant among *C. parapsilosis* isolates, where 21 (4.7%) of the isolates were found to be resistant to this drug during 2012–2017. This trend was also supported by the fact that at an ECV of ≤2 μg/ml, 304 of 310 (98.0%) isolates obtained during 2006–2011 were wild-type for fluconazole as compared to 93.4% (417 of 446) during 2012–2017 (Table 5). Notably, none of our fluconazole-resistant isolate came from a neonate. The first fluconazole-resistant bloodstream isolate of *C. parapsilosis* was detected in April 2010 and gradually resistant strains have spread to other hospitals. In a study of 442 *C. parapsilosis* sensu stricto isolates, 5 of 11 (45%) fluconazole-resistant isolates had Y132F

mutation in *ERG*11 and originated from a single hospital [56]. Four of these isolates came from blood and one from sputum. It is not known if these patients had fluconazole exposure prior to developing resistance. Since two of the patients were females, it is possible that they might have used fluconazole for vaginal symptoms. In a previous study, Grossman et al. (2015) [57] reported two mechanisms that contribute to fluconazole resistance in *C. parapsilosis*, that is by an amino acid substitution in *ERG*11 gene and by overexpression of the efflux pump MDR1, possibly due to point mutations in the MRR1 transcription factor that regulates MDR1. Although there is no clear evidence that prior exposure to fluconazole can be linked to emergence of resistance [51, 58], nonetheless, reports of outbreak of candidemia caused by resistant strain following prolonged antifungal therapy have begun to emerge [59, 60], thus limiting treatment of choice to amphotericin B or an echinocandin, although the latter drug is relatively less active but still therapeutically used against *C. parapsilosis* candidemia. Our data seem to suggest that *C. parapsilosis* may have a greater propensity to develop acquired resistance to azoles than *C. albicans* or *C. tropicalis*. The report highlights the need of strict compliance of hand sepsis protocols to prevent transmission of fluconazole-resistant strains of *C. parapsilosis* through healthcare staff. It is worth mentioning that in a recent study, 11 (52%) of 21 fluconazole-resistant bloodstream isolates came from a single hospital and belonged to a single clone (unpublished data).

In most of the published studies resistance to fluconazole has been <10% with few exceptions [24]. In a study from Spain, a high rate of fluconazole resistance (27.6%) has been observed among *Candida* isolates obtained from hematology and oncology patients [50]. Similarly, among *C. parapsilosis* isolates from South Africa, only 37% were found susceptible to fluconazole and voriconazole and 44% of fluconazole-resistant isolates also showed cross-resistance to voriconazole. Unlike higher rates of fluconazole resistance among *C. tropicalis* isolates in China [62, 63] and Australia [48], it was uncommon/rare (0.38%) in Kuwait. In India, also where *C. tropicalis* has emerged as the most common bloodstream pathogen (n = 382; 41.6%), resistance to fluconazole was uncommon (2.6%) and same is true in the Asia Pacific region [63]. *C. glabrata* is known to be intrinsically less susceptible to fluconazole, however, there was an indication that this species is also becoming less susceptible to this drug. Of 74 *C. glabrata* isolates tested during 2006–2011, 10.8% isolates were inhibited at MICs of 32 μg/ml or more as compared to 22.6% (n = 115) tested during 2012–2017 and resistance to fluconazole was restricted to only 12%. In Kuwait, caspofungin is in clinical use for several years. However, none of the *Candida* species isolates developed resistance to caspofungin, so far.

Our study has two major limitations, firstly, because of retrospective nature, clinical details of the patients including antifungal treatment received are not available, and secondly, it is possible that not all *Candida* species isolates from candidemia cases were referred for identification and antifungal susceptibility testing to MRL by different hospitals.

In conclusion, our data indicate that during the two study periods there is a shift of 8.8% in favor of non-*albicans Candida* species causing candidemia in Kuwait. Additionally, our antifungal susceptibility data suggest a gradual decrease in susceptibility to fluconazole, particularly among *C. albicans* and *C. parapsilosis*, which may be a prelude of emergence of greater number of resistant strains in future. While resistance to fluconazole and other antifungal agents is still uncommon, it calls for a continued need of surveillance and strengthening of antifungal stewardship policies to minimize acquisition of acquired resistance.

Acknowledgments

Author Contributions

Conceptualization: Ziauddin Khan, Suhail Ahmad, Noura Al-Sweih, Eiman Mokaddas, Khalifa Al-Banwan, Wadha Alfouzan, Inaam Al-Obaid, Khaled Al-Obaid, Mohammad Asadzadeh, Ahlam Jeragh.

Data curation: Ziauddin Khan, Suhail Ahmad, Eiman Mokaddas, Khalifa Al-Banwan, Wadha Alfouzan, Leena Joseph, Omar Al-Musallam.

Formal analysis: Ziauddin Khan, Suhail Ahmad, Leena Joseph.

Investigation: Ziauddin Khan, Suhail Ahmad, Noura Al-Sweih, Khalifa Al-Banwan, Khaled Al-Obaid, Ahlam Jeragh.

Methodology: Ziauddin Khan, Mohammad Asadzadeh, Leena Joseph, Soumya Varghese, Sandhya Vayalil, Omar Al-Musallam.

Project administration: Ziauddin Khan, Suhail Ahmad.

Resources: Ziauddin Khan.

Software: Mohammad Asadzadeh, Leena Joseph, Soumya Varghese, Sandhya Vayalil.

Supervision: Eiman Mokaddas, Khalifa Al-Banwan, Wadha Alfouzan, Inaam Al-Obaid, Mohammad Asadzadeh, Leena Joseph, Sandhya Vayalil.

Validation: Ziauddin Khan, Noura Al-Sweih, Eiman Mokaddas, Khalifa Al-Banwan, Wadha Alfouzan, Inaam Al-Obaid, Khaled Al-Obaid, Ahlam Jeragh, Soumya Varghese, Sandhya Vayalil, Omar Al-Musallam.

Visualization: Khalifa Al-Banwan, Wadha Alfouzan, Ahlam Jeragh, Soumya Varghese, Omar Al-Musallam.

Writing – original draft: Ziauddin Khan, Suhail Ahmad, Noura Al-Sweih, Eiman Mokaddas, Khalifa Al-Banwan, Wadha Alfouzan, Inaam Al-Obaid, Khaled Al-Obaid, Mohammad Asadzadeh, Ahlam Jeragh, Leena Joseph, Soumya Varghese, Sandhya Vayalil, Omar Al-Musallam.

Writing – review & editing: Ziauddin Khan, Suhail Ahmad, Noura Al-Sweih, Eiman Mokaddas, Khalifa Al-Banwan, Wadha Alfouzan.

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