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Species distribution and antifungal susceptibility of *Candida* bloodstream isolates in Kuwait: a 10-year study

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Bloodstream infections due to *Candida* species are important complications in severely ill hospitalized patients. This study presents data on species distribution and antifungal susceptibility profiles of *Candida* bloodstream isolates obtained from Kuwait during a 10-year period. All the bloodstream isolates were identified to species level by the germ tube test and carbohydrate assimilation profile using the VITEK 2 yeast identification system. Using E-test strips for amphotericin B, fluconazole, 5-flucytosine and voriconazole, MICs were determined on RPMI agar supplemented with 2 % glucose. The MIC breakpoints for resistance were based on Clinical and Laboratory Standards Institute criteria or those published by reference laboratories, and were as follows: amphotericin B, >1 μg ml^{-1}; fluconazole, $\geqslant 64$ μg ml^{-1}; 5-flucytosine, $\geqslant 32$ μg ml^{-1}; and voriconazole, 4 μg ml^{-1}. In all, 607 bloodstream yeast isolates were obtained over the past 10 years in Kuwait. *Candida albicans* was the predominant species (39.5 %), followed by *Candida parapsilosis* (30.6 %), *Candida tropicalis* (12.4 %), *Candida glabrata* (5.6 %) and *Candida krusei* (1.6 %). All *C. albicans*, *C. tropicalis* and *C. glabrata* isolates were susceptible to amphotericin B. Of 186 isolates of *C. parapsilosis* tested, only four (2 %) exhibited an MIC for amphotericin B of >1 μg ml^{-1}. Resistance to fluconazole was observed in nine (3.8 %) *C. albicans* isolates, two (5.8 %) *C. glabrata* isolates and four (40 %) *C. krusei* isolates. Resistance to 5-flucytosine was observed in two (0.8 %) *C. albicans* isolates, seven (9.3 %) *C. tropicalis* isolates, three (1.6 %) *C. parapsilosis* isolates and all ten (100 %) *C. krusei* isolates. All the isolates of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei* were susceptible to voriconazole, including those resistant to fluconazole. Although amphotericin B and fluconazole are widely used in clinical practice in Kuwait, resistance to these drugs remained low.

INTRODUCTION

Candida species are one of the major causes of nosocomial bloodstream infections worldwide (Jarvis, 1995; Pfaller *et al.*, 2001). Despite the availability of an expanded antifungal armamentarium, the mortality associated with invasive *Candida* infections remains high, ranging between 19 and 49 % (Blot *et al.*, 2002; Alonso-Valle *et al.*, 2003; Gudlaugsson *et al.*, 2003; Morgan, 2005). The incidence and associated mortality due to candidaemia can be influenced by several factors including characteristics of the population at risk, standard of the healthcare facilities available, distribution of *Candida* species and prevalence of resistance (Hobson, 2003; Eggimann *et al.*, 2003). Hence, epidemiological information available for one centre or geographical region may not be applicable to others (Hobson, 2003). The increased isolation rates of non-*albicans Candida* species and a gradual shift in the antifungal susceptibility profile, especially against azole antifungal agents, have underlined the need to monitor laboratory data for possible emergence of resistance and to select the most appropriate antifungal agent for therapy (Sanglard & Odds, 2002; Eggimann *et al.*, 2003). *Candida* species have been reported as important nosocomial pathogens in several studies reported from the Middle East (Nampoory *et al.*, 1996; Khan & Chugh, 2000; Al-Essa *et al.*, 2000; Mokaddas *et al.*, 2000; Rennert *et al.*, 2000; Bukharie, 2002; Ahmad *et al.*, 2002, 2003; Ellis *et al.*, 2003; Al-Jasser & Elkhizzi, 2004). However, comprehensive studies on antifungal susceptibility of *Candida* species from this region are lacking. In this communication, we present data on five major *Candida* species isolated from candidaemic patients during 1996–2005 and their antifungal susceptibility profiles.

Abbreviation: CLSI, Clinical and Laboratory Standards Institute.

METHODS

Bloodstream yeast isolates. The bloodstream yeast isolates referred to the Mycology Reference Laboratory for identification and

antifungal susceptibility testing from January 1996 to December 2005 were included in the study. These isolates originated from different categories of patients admitted to tertiary care hospitals in Kuwait. The isolates were initially tested with the germ tube test. All the germ tube positive isolates were provisionally identified as *Candida albicans* or *Candida dubliniensis*. The germ tube negative isolates were identified by carbohydrate assimilation tests using the VITEK 2 yeast identification system (bioMérieux). None of the isolates was identified as *C. dubliniensis* by the VITEK 2 system or morphological characteristics on sunflower seed agar (Khan *et al.*, 2004).

Antifungal susceptibility by the E-test. The *in vitro* activity of the antifungal agents was determined by the E-test (AB Biodisk), in accordance with the manufacturer's instructions. The E-test was performed by inoculating a 150 mm Petri dish containing 60 ml RPMI agar supplemented with 2 % glucose and buffered to pH 7.0 with MOPS. 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 for 24 h at 35 °C and read after 24 h. For *Candida glabrata*, the incubation time was 48 h. Reference strains *C. albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019 were used for quality control. Interpretive susceptibility criteria for fluconazole and 5-flucytosine were those recommended by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 2002). For fluconazole, isolates showing MICs $\leqslant$8.0 μg ml^{-1} were regarded as susceptible, 16–32 μg ml^{-1} as dose-dependent susceptible and $\geqslant$64 μg ml^{-1} as resistant. For 5-flucytosine, isolates showing MICs $\leqslant$4 μg ml^{-1} were considered as susceptible, 8–16 μg ml^{-1} as intermediate and $\geqslant$32 μg ml^{-1} as resistant. For voriconazole, recently approved CLSI breakpoints were followed: $\leqslant$1.0 μg ml^{-1} regarded as susceptible, 2.0 μg ml^{-1} as dose-dependent and $\geqslant$4 μg ml^{-1} as resistant (Pfaller *et al.*, 2003, 2005). Due to the lack of defined breakpoints for amphotericin B, isolates showing an MIC of $\leqslant$1.0 μg ml^{-1} were taken as susceptible and those with MIC $>$1 μg ml^{-1} were considered as resistant (Rex *et al.*, 1997; Ostrosky-Zeichner *et al.*, 2003a).

RESULTS AND DISCUSSION

During the 10-year study period, a total of 607 bloodstream yeast isolates were received in the Mycology Reference Laboratory for identification. The species distribution of *Candida* species was as follows: *C. albicans*, 39.5 % (n=240); *C. parapsilosis*, 30.6 % (n=186); *Candida tropicalis*, 12.4 % (n=75); *C. glabrata*, 5.6 % (n=34); *Candida krusei*, 1.6 % (n=10); and other yeast species, 10.2 % (n=62) (Table 1). Of these, 198 (32.6 %) isolates were received for identification during 1996–2000 as compared to 409 (67.4 %) during 2001–2005, representing a more than twofold increase in the latter period. The most prominent increase was observed in the isolation rate of *C. tropicalis* (3.4-fold), followed by *C. albicans* (3.1-fold), *C. glabrata* (1.8-fold) and *C. parapsilosis* (1.4-fold). The ratio of *C. albicans* to non-*albicans* isolates in the two study periods was 59 : 139 and 181 : 228, respectively. The greater than twofold increase in candidaemia cases detected between the two study periods may be a reflection of its rising incidence. Recent epidemiological studies from the USA and Europe have suggested that the annual incidence of candidaemia in some centres might have stabilized or decreased (Trick *et al.*, 2002; Hobson, 2003; Eggimann *et al.*, 2003; Morgan, 2005). However, this does not appear to be the case in Kuwait. Here it may be mentioned that our data are based on the number of bloodstream isolates received in the Reference Laboratory for identification and susceptibility testing from different tertiary care hospitals and not on isolation rates/1000 admissions.

Table 1. Species distribution of bloodstream yeast isolates received in 1996–2000 and 2001–2005

Species	*Candida* isolates received:		Total (%)
	1996–2000	2001–2005	
C. albicans	59	181	240 (39.5)
C. parapsilosis	78	108	186 (30.6)
C. tropicalis	17	58	75 (12.4)
C. glabrata	12	22	34 (5.6)
C. krusei	4	6	10 (1.6)
Others	28	34	62 (10.2)
Total	198 (32.6)	409 (67.4)	607 (100)

Consistent with the published reports from different parts of the world (Eggimann *et al.*, 2003; Hobson, 2003), *C. albicans* was the predominant species, followed by *C. parapsilosis*. A relatively greater proportion of *C. parapsilosis* isolates in our study may be related to the large number of isolates received from paediatric patients where small outbreaks of candidaemia due to this species had occurred. Of 138 candidaemia cases recorded during 1998–2003 in a neonatal intensive care unit of the maternity hospital, Kuwait, 48.2 % were caused by *C. parapsilosis* (N. A. Al-Sweih, unpublished results). Considerable differences in the distribution of *Candida* species were also reported in different published studies from the region (Table 2). These differences may be attributed to several factors including the type of patient population studied, sample size, duration of study and presence of intravascular catheters. In a solitary report from Saudi Arabia based on 32 candidaemic patients, *C. parapsilosis* was the predominant species; notably 66 % of these patients had a central venous catheter in place (Bukharie, 2002).

The data on E-test susceptibility for amphotericin B, fluconazole, 5-flucytosine and voriconazole against five *Candida* species are presented in Table 3. Except for four (2.2 %) isolates of *C. parapsilosis* which showed an MIC of $\geqslant$1 μg ml^{-1}, all *Candida* isolates were susceptible to amphotericin B. Resistance against 5-flucytosine was observed in 0.8 % of *C. albicans* isolates, 9.3 % of *C. tropicalis* isolates, 1.6 % of *C. parapsilosis* isolates and all the isolates of *C. krusei*. Nine (3.8 %) *C. albicans* isolates and two (5.8 %) *C. glabrata* isolates exhibited resistance to fluconazole. None of the isolates was found to be resistant to voriconazole, including those that showed resistance to fluconazole (Table 3).

A comparison of the MIC_{90} values for amphotericin B for different *Candida* species suggested that *C. albicans* tended

Table 2. Distribution of *Candida* species isolated from candidaemic patients in different studies reported from the Middle East

Reference	Study period (years)	Country*	No. of isolates	Distribution of *Candida* (%)					
				albicans	*parapsilosis*	*tropicalis*	*glabrata*	*krusei*	Others
Rennert *et al.* (2000)	1994	Israel	298	53.6	11.9	10.9	6.5	0.7	15.9
Bukharie (2001)	1995–2000	SA	32	19.0	44.0	25.0	3.0	6.0	3.0
Ellis *et al.* (2003)	1995–2001	UAE	60	45.0	5.0	15.0	5.0	0	30.0
Al-Jasser & Elkhizzi (2004)	1996–2002	SA	294	50.7	10.9	20.7	7.1	7.8	3.1
Osoba *et al.* (2003)	1998–2002	SA	83	46.0	10.8	10.8	4.8	6.0	21.6
Al-Essa *et al.* (2000)	1997	Kuwait	22	31.8	13.7	–	–	–	54.5
Mokaddas *et al.* (2000)	1994–1998	Kuwait	25	56.0	28.0	8.0	8.0	–	10.8
Present study	1996–2005	Kuwait	607	39.5	30.6	12.4	5.6	1.6	11.8

*SA, Saudi Arabia; UAE, United Arab Emirates.

to have higher susceptibility (0.25 μg ml^{-1}) than *C. tropicalis* (0.38 μg ml^{-1}), *C. krusei* (0.38 μg ml^{-1}), *C. parapsilosis* (0.5 μg ml^{-1}) and *C. glabrata* (0.38 μg ml^{-1}). These observations were consistent with several studies comparing MICs for bloodstream isolates of *C. albicans* with those for non-*albicans Candida* species (Simor *et al.*, 1997; Ostrosky-Zeichner *et al.*, 2003a; Lu *et al.*, 2004). While resistance to 5-flucytosine for *C. albicans*, *C. parapsilosis* and *C. glabrata* was generally low (<2 %), 9.3 % of *C. tropicalis* isolates and all the *C. krusei* isolates were found to be resistant to this drug. In early studies, up to 6.5 % of *C. albicans* isolates in Europe and 33 % in the USA were found to be intrinsically resistant (Scholer, 1980), which apparently precluded use of this drug as a single therapeutic agent. However, more recent surveys based on CLSI methodology demonstrated much lower levels of 5-flucytosine resistance (Pfaller *et al.*, 1998, 2002; Barchiesi *et al.*, 2000). Pfaller *et al.* (2002) determined the *in vitro* activity of 5-flucytosine

Table 3. *In vitro* susceptibility of *Candida* bloodstream isolates to amphotericin B, 5-flucytosine, fluconazole and voriconazole

Antifungal agents and *Candida* spp. (no. tested)	MIC (μg ml^{-1})			Percentage resistant
	50 %	90 %	Range	
Amphotericin B				
C. albicans (240)	0.094	0.25	0.002–0.75	0
C. parapsilosis (186)	0.125	0.5	0.004–2	2.2
C. tropicalis (75)	0.125	0.38	0.002–0.75	0
C. glabrata (34)	0.19	0.75	0.016–1	0
C. krusei (10)	0.25	0.38	0.047–1	0
5-Flucytosine				
C. albicans (240)	0.047	0.125	0.004–⩾32	0.8
C. parapsilosis (186)	0.047	0.19	0.003–⩾32	1.6
C. tropicalis (75)	0.032	0.75	0.002–⩾32	9.3
C. glabrata (34)	0.016	0.032	0.006–4	0
C. krusei (10)	>32	>32	>32	100
Fluconazole				
C. albicans (240)	0.5	1.5	0.047–>256	3.8
C. parapsilosis (186)	0.5	1.5	0.016–12	0
C. tropicalis (75)	0.5	1.5	0.016–3	0
C. glabrata (34)	12	24	1–>256	5.8
C. krusei (10)	32	>256	24–>256	40
Voriconazole				
C. albicans (104)	0.023	0.064	0.004–0.38	0
C. parapsilosis (58)	0.016	0.047	0.002–0.75	0
C. tropicalis (39)	0.032	0.094	0.002–0.094	0
C. glabrata (20)	0.19	0.5	0.012–1.5	0
C. krusei (10)	0.094	0.125	0.064–0.25	0

against 8803 clinical isolates, representing 18 *Candida* species, which were obtained from 200 medical centres worldwide. Regardless of the *Candida* species tested, 90 % of isolates showed MICs of $\leqslant 1$ μg ml^{-1}. While 97 % of *C. albicans* isolates and 92 % of *C. tropicalis* isolates were susceptible to 5-flucytosine, 28 % of 184 *C. krusei* isolates were resistant and 67 % showed intermediate susceptibility. In another study of 2000 *Candida* bloodstream isolates from the USA, Ostrosky-Zeichner *et al.* (2003a) found that 6 % of *C. tropicalis* and 12 % of *C. krusei* isolates were resistant to this drug, whereas resistance among other *Candida* species was less than 5 %. It is apparent that *Candida* species have variable susceptibilities to 5-flucytosine, and *C. krusei* is relatively less susceptible with resistance rates ranging between 7 and 44 % in different studies (Medoff & Kobayashi, 1980; Pfaller *et al.*, 2002; Quindos *et al.*, 2004). In this context, demonstration of 100 % resistance to 5-flucytosine in our *C. krusei* isolates is noteworthy.

Susceptibility to fluconazole was similar to that seen in other major surveillance studies reported from Europe and the USA (Sanglard & Odds, 2002; Ostrosky-Zeichner *et al.*, 2003a). In our isolates, with the exception of *C. krusei* where resistance to fluconazole was 40 % (4/10), the other *Candida* species were found to be quite susceptible (Table 3). Although *C. krusei* had a low prevalence (1.6 %) among bloodstream isolates, its resistance to fluconazole may have therapeutic implications. Since some of the isolates of *C. krusei* could be intrinsically resistant to fluconazole, it is not clear whether these isolates developed resistance during chemoprophylaxis. Ostrosky-Zeichner *et al.* (2003a) found that 34 % of 50 *C. krusei* bloodstream isolates were resistant to fluconazole, which is similar to what we observed in Kuwait. Interestingly, *Candida* species isolates exhibiting resistance to fluconazole were not resistant to voriconazole. Hence voriconazole, due to its wider species coverage, could be used in the treatment of candidaemia cases caused by fluconazole-resistant strains of *C. krusei* (Ostrosky-Zeichner *et al.*, 2003b).

In summary, we have presented data on species distribution and antifungal susceptibility profiles of *Candida* bloodstream isolates received in the Mycology Reference Laboratory over a 10-year period. The percentage prevalence of different *Candida* species was largely similar to what has been reported in most published studies (Eggimann *et al.*, 2003). Non-*C. albicans* yeast species constituted 60.5 % of the isolates. Although amphotericin B and fluconazole are widely used in clinical practice in Kuwait, there was no evidence of enhanced resistance.

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