

Epidemiology of nosocomial candidaemia in a university hospital: a 12-year study

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SUMMARY

The incidence of nosocomial candidaemia was evaluated in a retrospective study in a Turkish tertiary-care hospital. Over a 12-year period (1996–2007), a total of 743 episodes of candidaemia occurred in 743 patients, accounting for an average incidence of 1·9 episodes/1000 admissions and 2·9 episodes/10 000 patient-days per year. The annual incidence was almost constant during the study period except for 1996 when it was significantly higher in comparison with other years ($P < 0\cdot05$). The most common species isolated was *Candida albicans* (45%), followed by *C. parapsilosis* (26%), *C. tropicalis* (7%), *C. krusei* (7%), and *C. glabrata* (3·5%). A significant increase in *C. albicans* isolates causing candidaemia linked to a decrease in *C. parapsilosis* isolates in adult patients and *C. krusei* isolates in children was found between the two 6-year study periods. This trend reflects improved infection control at Uludağ University Hospital. Ninety percent of isolates were susceptible to fluconazole ($\leq 8 \mu\text{g/ml}$) and resistance was found only in *C. glabrata* and *C. parapsilosis* isolates. Regular local surveillance of *Candida* spp. is important in order to develop empirical treatment protocols to reduce the incidence and mortality of candidaemia.

Key words: Candidaemia, epidemiology, nosocomial, university hospital.

INTRODUCTION

Candida spp. are common inhabitants of the mucosal membranes of the gastrointestinal tract of mammals. The prevalence of *Candida* spp. colonization in the gastrointestinal tract ranges from 25–50% in healthy individuals, but *C. albicans* may reach up to 80% in hospitalized patients [1–3].

Candida spp. can cause invasive as well as non-invasive infections and have been implicated in

10–15% of all nosocomial infections [2, 3]. Blood-stream infection (BSI) is a life-threatening invasive disease associated with significant mortality and morbidity. Data from North American and European surveillance programmes of hospital-acquired bacteraemia have revealed that *Candida* spp. are the fourth most common cause, accounting for 8–10% of nosocomial BSIs [4–7]. *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* are the most frequently encountered causative agents of candidaemia. More rarely, *C. krusei*, *C. lusitaniae*, *C. guilliermondii*, and *C. rugosa* are detected. The mortality associated with *Candida* spp. BSI is consistently high, with estimated figures between 40% and 50% in adults and 20% in children [5]. Candidaemia can vary from a self-limiting

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infection to a severe clinical picture leading to sepsis and multiple organ failure [8, 9].

The frequency of isolation and the relative proportion of *C. albicans* to non-*albicans* *Candida* spp. are highly divergent in different countries and hospitals and knowledge of their distribution are crucial for the choice of empirical antifungal treatment. Fluconazole and amphotericin B are the most widely used drugs worldwide but the side-effects associated with amphotericin B limits its use. The azoles are much safer but resistance has been seen towards azoles in *Candida* [10]. The objective of this retrospective investigation was to characterize the incidence and epidemiology of candidaemia in adults and paediatric patients hospitalized in a large university-affiliated hospital over a 12-year period and to determine the fluconazole susceptibility pattern of some isolates. The epidemiology of candidaemia in adult intensive-care units (ICUs) was additionally investigated as critically ill patients are at particular risk for candidaemia because of their debilitated condition and frequent need for invasive procedures [11, 12].

MATERIALS AND METHODS

Setting and study design

Uludağ University Hospital is a tertiary-care 800-bed hospital that includes six ICUs (resuscitation, surgical, burn, stroke, neurosurgery, cardiovascular), haematology and oncology clinics, and a renal transplantation unit. Candidaemia cases were identified through the surveillance of all blood culture isolates in the hospital microbiology database from 1996 to 2007. The incidence of candidaemia was calculated as the ratio of total number of patients to 1000 admissions and to 10 000 patient-days. The 12-year survey was divided into two 6-year periods (1996–2001 and 2002–2007) for ease of comparison.

Definition of candidaemia

Blood cultures are taken routinely when patients deteriorate and/or have fever. A nosocomial episode of candidaemia occurring 48 h after admission was defined as at least one positive blood culture yielding *Candida* spp. during a single hospitalization. Subsequent positive cultures were defined as new episodes only if at least 12 weeks had lapsed since the previous isolate and at least 14 days of treatment with an

antifungal agent had been completed with resolution of symptoms.

Identification of organisms

Clinical isolates were detected using an automated continuous monitoring blood culture system (Bactec 9240, Becton Dickinson Inc., USA). No special media for fungal blood cultures was used. Blood culture bottles positive for yeasts following a Gram stain were cultured on Emmon's modified Sabouraud dextrose agar (SDA 2%) and inhibitory mould agar (IMA) and incubated for 24–72 h at 35 °C. The isolates were identified by standard procedures (germ tube production, morphology on cornmeal Tween-80 agar, and/or chromogenic agar) and analysis of biochemical patterns by ID 32C (bioMérieux, France).

Antifungal susceptibility testing

Routine antifungal susceptibility tests are not performed in our hospital. To determine fluconazole susceptibility, 214 isolates of *Candida* spp. other than *C. krusei* (one isolate per infection episode), were randomly selected. Of these isolates 92, 86 and 45 were recovered in the years 1996–1998, 2000–2001, and 2007, respectively. Fluconazole susceptibility was determined using the CLSI M27-A2 reference method for broth microdilution antifungal susceptibility testing of yeasts [13]. The control strains *C. parapsilosis* (ATCC 22016) and *C. krusei* (ATCC 6258) were run in parallel with the test isolates. Minimum inhibitory concentrations (MICs) were read after 48 h of incubation at 35 °C and interpretative breakpoints were determined according to the CLSI guidelines [13]. Resistant, susceptible dose-dependent, and susceptible to fluconazole were defined as MICs ≥ 64 $\mu\text{g/ml}$, 16–32 $\mu\text{g/ml}$, and ≤ 8 $\mu\text{g/ml}$, respectively.

Statistical analysis

Statistical analysis was performed with SPSS software, version 13.0 for Windows (SPSS Inc., USA). Categorical variables were given with number and percent values, and χ^2 analysis was used to test for differences in the proportions of categorical variables between two or more groups. Fisher's exact test (two-tailed) was used in 2×2 tables instead of a χ^2 test when the sample size was small. A value of $P < 0.05$ was accepted as statistically significant.

Table 1. Average rates of candidaemia during the two study periods

Year	Candidaemia	Number of hospitalized patients	Days of hospital stay	Candidaemia/ number of hospitalized patients (1/1000)	Candidaemia/ days of hospital stay (1/10 000)
1996–2001	350	159 552	1 151 496	2·194	3·039
2002–2007	393	233 172	1 436 096	1·685	2·737
Total	743	392 724	2 587 592	1·892	2·871

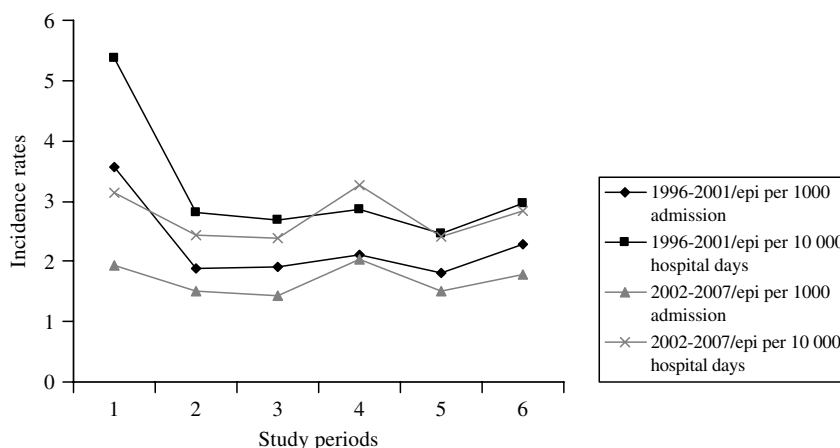


Fig. 1. Incidence of candidaemia during 1996–2007.

RESULTS

Candidaemia incidence

The number of annual admissions ranged from 18 823 in 1996 to 42 987 in 2005, and over the 12-year period, 743 episodes of candidaemia (457 adult, 286 paediatric patients) were detected in 392 724 patients on a total of 2 587 592 patient-days (Table 1). The annual incidence of candidaemia ranged from 1·4 to 3·6 (average mean 1·9) cases/1000 hospital admissions and the incidence per 10 000 patient-days per year ranged from 2·4 to 5·4 (average mean 2·9). The annual incidence was almost constant during the study period except for 1996 (Fig. 1) when it was significantly higher in comparison to other years, with 3·559/1000 admissions and 5·374 for 10 000 patient-days ($P < 0\cdot001$). A significant reduction of incidence was also found between the two study periods regarding 1000 patients admission and 10 000 hospital days ($P < 0\cdot001$) (Table 1).

Epidemiology of candidaemia

The species distribution in adult patients and children aged <18 years during the two study periods is shown

in Table 2. In the first period (1996–2001), there were no episodes of candidaemia involving more than one species of *Candida*, but 18 patients (4·6% of all patients) harboured two different species in the second period (2002–2007). In total, 411 isolates were obtained from 393 patients. *C. albicans* remained the predominant species, 39% and 50% of all isolates recovered, in the first and second period, respectively, and *C. parapsilosis* was the second most common. Compared to the first period, a significant increase in *C. albicans* isolates causing candidaemia was observed in the second period, mainly because of a decrease in *C. parapsilosis* isolates in adult patients and *C. krusei* isolates in children ($P < 0\cdot05$). There were no definitive differences in *C. glabrata* isolation between the two study periods both in adult patients and children, and significantly increased isolation of *C. parapsilosis* was observed in children during the second period ($P < 0\cdot05$). *C. kefyr* was predominantly isolated from adult patients, whereas *C. lipolytica* was isolated from the paediatric group.

Table 3 details the species distribution of candidaemia in the adult ICUs; 36% of all episodes occurred in adult patients (166/457) hospitalized in the medical and surgical ICUs. *C. parapsilosis* was the

Table 2. Distribution of *Candida* spp. isolated from blood during the two study periods

	1996–2001			2002–2007		
	Adults	Children*	Total	Adults	Children*	Total
<i>C. albicans</i>	78 (39.4)	59 (38.8)	137 (39.1)	140 (52.2)	66 (46.1)	206 (50.2)
<i>C. parapsilosis</i>	68 (34.3)	27 (17.8)	95 (27.1)	58 (21.6)	45 (31.5)	103 (25.1)
<i>C. tropicalis</i>	15 (7.6)	12 (7.9)	27 (7.7)	22 (8.2)	3 (2.1)	25 (6)
<i>C. krusei</i>	3 (1.5)	34 (22.4)	37 (10.6)	11 (4.1)	5 (3.5)	16 (4)
<i>C. glabrata</i>	8 (4)	3 (2)	11 (3.1)	10 (3.7)	6 (4.2)	16 (4)
<i>C. kefyr</i>	6 (3)	1 (0.7)	7 (2)	11 (4.1)	1 (0.7)	12 (2.9)
<i>C. guilliermondii</i>	1 (0.5)	0	1 (0.3)	5 (1.7)	3 (2.1)	8 (1.9)
<i>C. lipolytica</i>	0	1 (0.7)	1 (0.3)	1 (0.4)	4 (2.8)	5 (1.2)
Other <i>Candida</i> spp.†	19 (9.6)	15 (9.9)	34 (9.7)	9 (3.4)	10 (7)	19 (4.6)
Total/average	198	152	350	268	143	411 (100)

Values are number (%).

* Patients aged <18 years.

† *C. pelliculosa*, *C. lusitanae*, *C. zeylanoides*, *C. inconspicua*, *C. dubliniensis*, and non-specified non-*albicans* *Candida* spp.

Table 3. *Candida* spp. in adult intensive care unit patients

1996–2001	n (%)	2002–2007	n (%)
<i>C. albicans</i>	31 (34.4)	<i>C. albicans</i>	42 (55.3)
<i>C. parapsilosis</i>	35 (38.9)	<i>C. parapsilosis</i>	18 (23.7)
<i>C. tropicalis</i>	5 (5.7)	<i>C. tropicalis</i>	8 (10.6)
<i>C. glabrata</i>	4 (4.4)	<i>C. glabrata</i>	2 (2.6)
<i>C. krusei</i>	1 (1.1)	<i>C. krusei</i>	2 (2.6)
<i>C. kefyr</i>	2 (2.2)	<i>C. kefyr</i>	2 (2.6)
Other	12 (13.3)	Other	2 (2.6)
<i>Candida</i> spp.*		<i>Candida</i> spp.*	
Total	90 (100)	Total	76 (100)

* *C. lipolytica*, *C. lusitanae*, *C. guilliermondii*, and non-specified non-*albicans* *Candida* spp.

most common species in these patients with an approximate rate of 40% in the first period, whereas in the second period, *C. albicans* was the most common aetiological agent. Thus, *C. albicans* increased by 20.9% ($P=0.003$) during 2002–2007 compared to 1996–2001, whereas *C. parapsilosis* decreased by 15.2% ($P=0.0015$).

Fluconazole susceptibility

The MICs for the two control organisms tested by the reference method in all the sets of experiments consistently agreed with those from the CLSI reference results, confirming both the reproducibility of the results and the correct drug concentrations. The fluconazole susceptibility of the 214 *Candida* spp.

isolates tested is presented in Table 4; the overall resistance rate was 1.9% (MIC ≥ 64 $\mu\text{g/ml}$). All *C. albicans* and *C. tropicalis* isolates were susceptible to fluconazole (MIC ≤ 8 $\mu\text{g/ml}$) whereas 2.6% and 14.3% of *C. parapsilosis* and *C. glabrata* isolates, respectively, were resistant. Reduced susceptibilities (16–32 $\mu\text{g/ml}$) were obtained in some *C. parapsilosis*, *C. glabrata*, *C. kefyr* and *C. pelliculosa* isolates. No significant changes in susceptibility to fluconazole were seen over time in the *C. albicans*, *C. parapsilosis* and *C. tropicalis* strains tested between 1996 and 2007 (data not shown). Due to the limited number of isolates, similar comparisons with other *Candida* spp. were not calculable.

DISCUSSION

Invasive candidiasis is the most frequent life-threatening fungal disease, and candidaemia represents 20–30% of all invasive cases. According to the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infection Group, a single positive blood culture is considered sufficient to prove invasive fungal infection [14]. Several recent studies have reported a steady increase in the incidence of *Candida* BSIs over the last two decades [15]. This increase is multifactorial in origin and reflects advances in medical and surgical technology. Improved chemo- and immunosuppressive therapy, transplant medicine, and intensive-care technology have decreased the mortality of many life-threatening diseases but have also led to an increase in patients

Table 4. *Fluconazole susceptibility of Candida spp.*

Species (no. of isolates tested)	Number of isolates (%)		
	Susceptible (MIC \leq 8 μ g/ml)	Susceptible dose-dependent (MIC 16–32 μ g/ml)	Resistant (MIC \geq 64 μ g/ml)
<i>C. albicans</i> (83)	83 (100)	0	0
<i>C. parapsilosis</i> (78)	65 (83.3)	11 (14.1)	2 (2.6)
<i>C. tropicalis</i> (24)	24 (100)	0	0
<i>C. kefyr</i> (9)	8 (88.9)	1 (11.1)	0
<i>C. glabrata</i> (7)	2 (28.6)	4 (57.1)	1 (14.3)
<i>C. pelliculosa</i> (7)	5 (71.4)	2 (28.6)	0
Other <i>Candida</i> spp.* (6)	5 (83.3)	0	1 (16.7)
Total (214)	192 (89.7)	18 (8.4)	4 (1.9)

MIC, Minimum inhibitory concentration.

* *C. guilliermondii* (3 isolates), *C. dubliniensis*, *C. lusitanae* and *C. zeylanoides* (1 isolate each).

vulnerable to infections. In the USA, *Candida* spp. are now the fourth most common cause of nosocomial BSIs, and in many countries these organisms account for 5–8% of all blood culture isolates [4, 11, 15].

Surveys performed at a single hospital have tremendous value for therapeutic decision-making and prevention measures at the local level. As only a single report documenting the incidence and spectrum of organisms responsible for candidaemia in a Turkish hospital has been published [16], we performed a hospital-wide survey that included all hospital units. Although this study was restricted to a single hospital, a large sample size (2.5 million patient-days) was attained. The incidence of candidaemia (1.9/1000 patients and 2.9/10 000 patient-days) in our centre is higher than the rates reported from centres in the USA and other European countries, including the above-mentioned report from our country [4, 6, 15, 16]. Morgan [17] cites the incidence of candidaemia from different countries to be between 0.2 and 2.8/1000 admissions and 0.3 and 1.14/10 000 patient-days. The incidence rates most similar to those found here (above 1.5/10 000 patient-days) have been reported from Italy, Spain, Belgium, Brazil, and Taiwan [6, 18–21]. It is well documented that the frequency of isolation of *Candida* spp. varies widely in different countries and hospitals. Numerous factors contribute to this such as differences in patient demographics and comorbidities, as well as medical practices, especially the use of long-term vascular catheters and antibacterial and antifungal prescribing patterns. We speculate that the high incidence of candidaemia in our tertiary-care teaching hospital of

800 beds is probably due to the large patient population at risk for infection. The surveillance system (NNIS) in the USA showed a clear association between the incidence of candidaemia and the number of hospital beds and/or the academic affiliation [22] and corroborated the European Confederation of Medical Mycology survey [6].

Much higher rates are observed when paediatric age groups are evaluated separately [23]. An earlier study of paediatric patients in our hospital, found the incidence of candidaemia to be 5.1/1000 admissions [24], and this undoubtedly impacts on the total incidence observed in this survey. Our hospital has a large-capacity neonatal ICU that cares for most of the premature infants and newborns in the region.

As seen in Table 1, the significant reduction in the incidence of *Candida* infections is marked in the second period (2002–2007) of the study. Improvements in the application of infection control precautions, more conscious and appropriate use of parenteral nutrition, improvements in the care of central venous catheters, limited use of the broad spectrum antibiotics since 2002, and widespread infectious diseases consultation are factors considered to have played a role in this decrease, and it is believed that this decreasing trend will continue.

The frequency of diagnostic test ordering, especially of blood cultures, and the type of blood culture systems employed might also impact on the incidence rates in laboratory-based surveillance [15, 25]. Good laboratory practice and clinical cooperation and training, a single type of blood culture system during the study period, and physicians

accustomed to frequently ordering blood cultures at our institute might be other reasons for this high incidence.

More than 17 species of *Candida* have been reported to be the aetiological agents of candidaemia in humans, but most invasive infections are attributed to *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. In both study periods, *C. albicans* remained the predominant species, but the 13% increase ($P=0.006$) in the frequency of isolation of this species in the second period appears to be an unusual finding. Although most countries are experiencing a decrease or stable incidence of *C. albicans* BSIs over time, one report from the USA has noted a rise in the frequency of isolation of *C. albicans* [26]. The increased isolation of *C. albicans* might be a consequence of the decreased isolation of *C. parapsilosis* and *C. krusei* in adults and paediatric patients, respectively. Nevertheless, *C. albicans* remains the dominant species causing BSIs throughout the world with low frequency (37%) in Latin America to 70% in Norway [15]. We found the rate of *C. albicans* to be 45% over the 12-year study period, which is similar to frequencies reported from the USA and some European countries [27, 28].

The proportion of *Candida* bloodstream isolates due to non-*albicans* species appears to be on the increase [15]. This change is believed to be related to the introduction of fluconazole prophylaxis, but the reasons for the variability in the frequency of the different species from diverse studies remain controversial [29, 30]. *C. glabrata* has undoubtedly emerged as an important opportunistic pathogen in the USA, and virtually every US-based survey has shown *C. glabrata* to rank second to *C. albicans* as a cause of BSI, accounting for 20–24% of all *Candida* BSIs [15]. In contrast, *C. glabrata* is much less common as a cause of BSI in most other countries, and the lowest frequency of *C. glabrata* as a cause of BSI has been reported in Latin America, where only 4–7% of *Candida* BSIs are attributed to this species [20]. In our study, the total frequency of *C. glabrata* was found to be 3.5% over the 12-year period, which is similar to the surveys in Latin America and Turkey [16]. The reasons for such marked variation in the frequency of *C. glabrata* as a cause of BSIs are unclear but may include exposure to azoles, patients' age, underlying disease, geographic location, or other, unknown factors [15]. In our centre, haematopoietic stem cell transplantation has not yet been performed, and liver transplantation has only recently begun (in 2008).

Prophylactic fluconazole use has not been widespread in this hospital, and the lower frequency of *C. glabrata* might be attributed to this. Throughout this survey, we used the same blood culture system and a longer incubation period (72 h), which is thought to be efficient for the isolation of *C. glabrata*; therefore, the possibility of false-negative culture results can be discounted [25].

Similar to *C. glabrata*, the isolation of *C. krusei* can be related to fluconazole usage. This species has emerged in blood and marrow transplant recipients receiving fluconazole prophylaxis but fluconazole exposure alone does not explain the reported increase in infections caused by this species [31]. In most of the surveys, *C. krusei* accounts for 2–4% of all *Candida* BSIs [15]. Significant differences were observed here between the two periods with higher frequencies of isolation of *C. krusei* recorded in the first period ($P<0.05$). Although elevated frequencies of *C. krusei* have been reported in cancer patients, our high rate was clearly due to the paediatric age group (Table 2) where an outbreak in the first study period was thought to be responsible for this high rate.

C. parapsilosis is an exogenous pathogen that may be found on skin rather than mucosal surfaces. This species is notorious for its ability to form biofilms on catheters, for nosocomial spread by hands and for persistence in the hospital environment [15]. Here, *C. parapsilosis* was found to be the second most common isolate after *C. albicans*, accounting for 27.1% and 25.1% of all *Candida* BSIs, in the first and second period, respectively. *C. parapsilosis* is also well-known for causing infections in infants, neonates and ICU patients. In a report from Turkey, the ratio of *C. parapsilosis* was found to be 37.2% in paediatric patients, which is also similar to our rates for the second period [32]. In contrast, the frequency of *C. parapsilosis* isolation significantly decreased in adult patients hospitalized in ICUs and clinical wards (Tables 2 and 3). Parallel to the decrease of incidence in the second period, the lower isolation of *C. parapsilosis* also reflects improvements in the infection control strategies at our centre. The total frequency of *C. tropicalis* was 6.8% over the 12-year study period, which is similar to rates in different countries and hospitals except in Latin America (~20%) [15].

Fluconazole and amphotericin B are the most widely used drugs worldwide for *Candida* infections. The side-effects associated with amphotericin B limits its use and detection of resistance to amphotericin B by the CLSI M27-A2 method has been problematic

due to the very narrow range of MICs obtained [15]. Thus, fluconazole susceptibility testing is considered more important in epidemiological studies. Moreover, decreased susceptibility to fluconazole may precede decreased susceptibility to voriconazole [15]. Clinical laboratories performing antifungal susceptibility testing of fluconazole against *Candida* spp. can reliably use these results as a surrogate marker for voriconazole [33]. Here, ~90% of isolates were susceptible to fluconazole and none of the *C. albicans* and *C. tropicalis* isolates exhibited *in vitro* resistance confirming the rarely seen fluconazole resistance in *C. albicans* isolates outside of AIDS patients with recurrent oropharyngeal candidiasis [34, 35]. According to population-based and sentinel surveillance programmes, *C. glabrata* and *C. parapsilosis* organisms resistant to fluconazole have been noted in ~10% and 1% of BSI isolates, respectively, with the exception of higher frequencies (40% and 15%) from Sweden [15].

In conclusion, candidaemia and invasive candidiasis are persistent health problems. Epidemiological studies have revealed differences between countries and centres and emerging species that may vary geographically in terms of frequency of isolation. It is therefore essential that laboratories identify clinical isolates of *Candida* to the species level. The choice of appropriate antifungal treatment based on local epidemiological data is very important, and epidemiological changes indirectly reflect antifungal policy and efficacy of catheter care and infection control measures.

DECLARATION OF INTEREST

None.

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