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MYCOLOGY

Aetiology and antifungal susceptibility of yeast bloodstream infections in a Hungarian university hospital between 1996 and 2000

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The purpose of this study was to evaluate the aetiology and susceptibility of different *Candida* species originating from blood cultures received from different clinical wards of the University Hospital in Szeged, Hungary, from 1996 to 2000. A total of 145 episodes of fungaemia occurred in 68 patients. In 73.5% of the patients the infections were due to *Candida albicans*, 7.3% to *C. parapsilosis*, 5.9% to *C. krusei*, 4.4% to *C. tropicalis* and 3% each to *C. glabrata*, other *Candida* spp. and *Cryptococcus neoformans*. There were no appreciable differences in the distribution of yeast species during the 5-year period: *C. albicans* remained the predominant species causing bloodstream infections in this hospital, similar to the results of other studies (Norway, SENTRY Program in USA, Canada and South America). Most of the *Candida* isolates (39.3%) were from blood cultures of patients hospitalised in surgical wards, 28.3% were from adult intensive care units (ICUs), 13.8% from paediatric ICUs, 11% from haematology and 7.6% from cardiology departments. MICs for amphotericin B, fluconazole and itraconazole were determined for 83% of the isolates. All isolates were susceptible to amphotericin B. The percentage of yeast isolates with decreased susceptibility or resistance to fluconazole was smaller (15.7%) than that for itraconazole (24%).

Introduction

Yeasts are emerging as important aetiological agents of nosocomial infections and infections in immunocompromised patients [1–4]. Colonisation of the gastrointestinal tract and other body sites is likely to be the initial step preceding systemic yeast invasion.

Blood cultures can be used to diagnose invasive candida infections [5]. Besides conventional methods, several fully automated blood culture systems (Bact/Alert, Organon Technica; Bactec, Becton Dickinson; Vital, bioMérieux) have recently become available for the isolation of fungi. Despite the fact that blood cultures remain the basic tool for the diagnosis of fungaemia, this method may fail to detect as many as 50% of disseminated candidosis cases. The concomitant colonisation of mucosal surfaces by the same *Candida* species may be the only laboratory sign of a systemic fungal infection in immunocompromised patients [6–8].

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Candida spp. should be identified to species level, as some of the non-*albicans Candida* spp. have proved to be resistant to antimycotic agents, such as azoles, used in the empirical therapy of invasive fungal infection [9, 10]. In bloodstream infections, the breakpoints or MICs of antifungal agents must be determined to identify risk factors for infections by resistant yeasts and to assess the clinical relevance of the treatments. Similar reports in Europe have shown that bloodstream infections caused by yeasts are increasing [11–13].

The aims of this study were to establish the occurrence of different *Candida* spp. originating from blood cultures received from different clinical wards of the University Hospital in Szeged, Hungary, during a 5-year period. The presence of the same species within a 1-week interval in samples other than blood cultures was also evaluated. The susceptibility of these isolates to antifungal agents and the aetiology of bloodstream infections were also evaluated in this retrospective study.

Materials and methods

The automated Vital blood culture system (bioMérieux, Marcy-l'Etoile, France) was used to diagnose bloodstream infections. Aerobic and anaerobic blood culture bottles were used in parallel for all patients. In accordance with the manufacturer's instructions, separate blood culture bottles were not used in the event of the suspicion of candidaemia. If a positive signal was noticed, Gram's or acridine orange staining, or both, was done. All positive bottles in which yeasts were detected by direct microscopy were subcultured on CHROM agar (Becton-Dickinson, Budapest, Hungary) for direct isolation of the yeasts, in parallel with Sabouraud agar and Columbia blood agar. *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* were identified on the basis of the typical colours of their colonies on CHROM agar. All other species were identified with ATB 32C (bioMérieux).

The in-vitro susceptibilities of various *Candida* isolates to antifungal agents were determined by the Etest method (AB Biodisk, Solna, Sweden) [14]. This was performed in accordance with the manufacturer's instructions with the use of RPMI media. The drug concentration ranges used were 0.016–256 mg/L for fluconazole, and 0.002–32 mg/L for itraconazole and amphotericin B. The MICs for amphotericin B were taken as the drug concentrations causing 95% inhibition. MICs for azoles were read at the visually selected end-points of 80% inhibition of growth [14]. Interpretative susceptibility criteria for fluconazole, itraconazole and amphotericin B were used as published by the NCCLS [15].

Results

During the 5-year study period (1996–2000), the number of blood cultures in this 1240-bed university hospital varied between 6762 and 8845 per year (Table 1). The overall percentage of positive blood cultures was 14.5–18.8%, with little year-to-year variation and with 1.4–2.6% of all positive blood cultures yielding yeasts.

The 143 yeast isolates among a total of 145 isolates obtained from bloodstream infections during this period were identified to species level (Table 2): 112 (77.2%) isolates were *C. albicans*, 3 (2.1%) *C. glabrata*, 13 (9%) *C. krusei*, 7 (4.8%) *C. parapsilosis*, 5 (3.4%) *C. tropicalis*, 2 (1.4%) *Candida* spp. and 3 (2.1%) *Cryptococcus neoformans*. In some cases, the same species was isolated from more than one blood culture bottle from the same patient. The distribution of the species isolated from the positive blood cultures and the distribution of the species among separate episodes of candidaemia did not differ markedly (Table 2).

Table 3 details the distribution of these isolates in the various departments. Most yeast isolates (57, 39.3%) were from the blood cultures of patients in surgical wards. Fewer isolates were found in blood cultures from patients in adult intensive care units (ICUs) (41, 28.3%) and 20 (13.8%) were from paediatric ICUs. Relatively few isolates were from patients hospitalised in haematology (16, 11%) or cardiology (11, 7.6%) departments. Altogether, 68 patients had a bloodstream infection caused by yeasts during this period: 45.6% of all episodes occurred in patients hospitalised in ICUs and 16.2% of the cases were diagnosed among patients with haematological disorders. Two patients had *Cr. neoformans* sepsis: one was an immunosuppressed patient from the paediatric ICU and one had received long-term treatment with high-dose steroids because of dermatomyositis. (Differences between species distributions and departments were not statistically significant by the χ^2 test, $p > 0.05$.)

Of the 68 patients, 26 had yeast only in their blood cultures. In the other 42 patients, the same yeast was also found in other samples: 19 patients in one other sample (urogenital tract, faeces, respiratory tract or wound), 12 in two other samples (gastrointestinal-urogenital tracts, CVC-respiratory tract, CVC-urogenital tract, urogenital-respiratory tracts, wound-respiratory tract, wound-urogenital tract or CSF-respiratory tract), 10 in three other samples (CVC-gastrointestinal-respiratory tracts, CVC-gastrointestinal-urogenital tracts, gastrointestinal-urogenital-respiratory tracts, CVC-urogenital-respiratory tracts, CVC-wound-urogenital tract, wound-urogenital-respiratory tracts or CSF-wound-urogenital tract) and 1 patient in 4 other samples (CVC-wound-urogenital-respiratory tracts) besides the blood cultures. (Only samples taken within 1 week were considered.)

The susceptibilities of the blood culture isolates were evaluated retrospectively. Before 1997, resistance was not determined routinely for yeasts isolated from systemic infections. After 1997, isolates were tested

Table 1. Blood culture results at the University Hospital in Szeged between 1996 and 2000

	Blood culture bottles		Positive bottles with		
Year	tested	positive (% of all tested)	aerobic bacteria	anaerobic bacteria	yeasts (% of all positive)
1996	6782	1074 (15.9)	1024	26	24 (2.2)
1997	8569	1612 (18.8)	1539	31	42 (2.6)
1998	8759	1290 (14.7)	1239	33	18 (1.4)
1999	8845	1279 (14.5)	1226	22	31 (2.4)
2000	8695	1266 (14.6)	1206	30	30 (2.4)

Table 2. Species distribution of yeasts isolated from blood cultures between 1996 and 2000

Species	Number of isolates (number of patients)					Total number (%) of isolates, 1996–2000
	1996	1997	1998	1999	2000	
C. albicans	14 (12)	40 (10)	7 (5)	25 (11)	26 (12)	112 (77.2)
C. glabrata	0	0	2 (1)	1 (1)	0	3 (2.1)
C. krusei	6 (3)	0	7 (1)	0	0	13 (9)
C. parapsilosis	2 (1)	1 (1)	0	2 (1)	2 (2)	7 (4.8)
C. tropicalis	1 (1)	1 (1)	0	3 (1)	0	5 (3.4)
Candida spp.	1 (1)	0	1 (1)	0	0	2 (1.4)
Cr. neoformans	0	0	1 (1)	0	2 (1)	3 (2.1)
Total	24 (18)	42 (12)	18 (9)	31 (14)	30 (15)	145 (100)

Table 3. Species distribution of yeasts isolated from different departments between 1996 and 2000

Species	Number of isolates (number of patients)					Total number (%) of episodes, 1996–2000
	Cardiology	Haematology	Adult ICUs*	Pediatric ICUs†	Surgery	
C. albicans	9 (3)	10 (8)	37 (16)	16 (8)	40 (15)	50 (73.5)
C. glabrata	0	0	2 (1)	0	1 (1)	2 (2.9)
C. krusei	0	4 (2)	0	0	9 (2)	4 (5.9)
C. parapsilosis	1 (1)	2 (1)	2 (2)	0	2 (1)	5 (7.3)
C. tropicalis	0	0	0	2 (2)	3 (1)	3 (4.4)
Candida spp.	1 (1)	0	0	1 (1)	0	2 (2.9)
Cr. neoformans	0	0	0	1 (1)	2 (1)	2 (2.9)
Total	11 (5)	16 (11)	41 (19)	20 (12)	57 (21)	68 (100)

*Including surgical and internal ICUs.
†Including neonatal and paediatric ICUs.

for their susceptibilities to amphotericin B, fluconazole and itraconazole (Table 4). All the yeasts tested (98 *C. albicans*, 3 *C. glabrata*, 7 *C. krusei*, 5 *C. parapsilosis*, 4 *C. tropicalis*, 1 *Candida* spp. and 3 *Cr. neoformans*) were susceptible to amphotericin B, with MICs ≤1 mg/L. Among the *C. albicans* isolates, 89% and 79% were susceptible to fluconazole and itraconazole, respectively, 6% and 16% of them in a dose-dependent way; five of these isolates (5%) were resistant to these antimycotic agents. Two *C. glabrata* isolates were susceptible to fluconazole and itraconazole. One *C. glabrata* isolate was susceptible to fluconazole in a dose-dependent way, but resistant to itraconazole. The seven *C. krusei* isolates that were resistant to itraconazole with an MIC ≥1 mg/L were from one patient. All five *C. parapsilosis* isolates were susceptible to both drugs, as were all four *C. tropicalis* isolates and the one *Candida* sp. isolate. All three *Cr. neoformans* isolates were susceptible to all antimycotic agents examined.

Table 4. Antifungal susceptibilities of yeasts isolated from blood cultures

Species (number of isolates tested)	Number of isolates (%) classified as indicated by fluconazole Etest		
	Susceptible (MIC ≤8 mg/L)	Susceptible dose-dependent (MIC ≥16 ≤32 mg/L)	Resistant (MIC ≥64 mg/L)
C. albicans (98)	87 (88.8)	6 (6.1)	5 (5.1)
C. glabrata (3)	2 (66.7)	1 (33.3)	0
C. krusei (7)	0	0	7 (100)
C. parapsilosis (5)	5 (100)	0	0
C. tropicalis (4)	4 (100)	0	0
Candida spp. (1)	1 (100)	0	0
Cr. neoformans (3)	3 (100)	0	0
	Number of isolates (%) classified as indicated by itraconazole Etest		
	Susceptible (MIC ≤0.125 mg/L)	Susceptible dose-dependent (MIC ≥0.25 ≤0.5 mg/L)	Resistant (MIC ≥1 mg/L)
C. albicans (98)	77 (78.6)	16 (16.3)	5 (5.1)
C. glabrata (3)	2 (66.7)	0	1 (33.3)
C. krusei (7)	0	0	7 (100)
C. parapsilosis (5)	5 (100)	0	0
C. tropicalis (4)	4 (100)	0	0
Candida spp. (1)	1 (100)	0	0
Cr. neoformans (3)	3 (100)	0	0

Discussion

This retrospective study evaluated the occurrence of bloodstream infections caused by yeasts among patients hospitalised in different clinical wards of the University Hospital of Szeged. As compared with international studies (5–10 infections per 10 000 admissions [16]), overall fewer bloodstream infections were caused by *Candida* spp. during this period, with a rate of 2–4.1 per 10 000 admissions. Much higher rates are observed when surgical ICUs (9.8 per 1000 admissions) or neonatal ICUs (12.3 per 1000 admissions) are evaluated separately [17]. However, a recent epidemiological survey on candidaemia in Europe, involving four countries and 76 hospitals, revealed a rate of 0.1–4.3 per 10 000 patients hospitalised for more than 1 day [18].

Several studies from different parts of the world have shown that the number of bloodstream infections caused by yeasts is increasing. The SENTRY Antimicrobial Surveillance Program confirmed that *Candida* spp. remain the fourth most common cause of nosocomial bloodstream infections, but the frequency may vary widely in different institutions [19].

There have been similar reports in Europe. Studies from a Danish university hospital showed a gradual increase in the annual incidence of fungaemia from 1989 to 1994 [11]. During the study period the most frequently isolated yeast in Hungary was *C. albicans* (77.2%), which is similar to results in other European (54–66%) and American (45–55%) countries [13, 19–21]. The highest rate of *C. albicans* isolates in any country was reported from Hungary. The highest numbers of isolates of non-*albicans Candida* spp. were *C. glabrata* in Norway and the USA, *C. parapsilosis* in the Slovak Republic, Italy, Canada and Latin America, and *C. krusei* in Hungary. With respect to the number of episodes (Table 3), *C. parapsilosis* was the second most frequent non-*albicans Candida* sp. causing bloodstream infections, with 5 episodes out of 68. There were more *C. glabrata* than *C. krusei* isolates in America, but this rate varies widely in Europe [13, 19–21].

Bloodstream infections caused by yeasts usually originate from the gastrointestinal tract in immunocompromised patients. Risk factors for candida bloodstream infections include colonisation with the organism in other body sites associated with the presence of intravascular catheters. Carriage of the infecting strain on the hands of health-care workers may also cause nosocomial infections [22]. In patients who have candidaemia, the same species may be isolated from specimens other than blood cultures at the same time (within 1 week), and colonisation of different body sites may be the first sign of a systemic candida infection. Among the 68 patients whose blood cultures proved the existence of candidaemia, 42 were colonised with the same species in other specimens. The most frequent sites of colonisation with the same *Candida* sp. were the urogenital tract, the CVC and the respiratory tract. The colonisation in the different body sites was naturally influenced by various factors, such as the location of hospitalisation, the underlying disease and previous antibiotic treatment.

The clinical outcome in these yeast-infected patients was also evaluated retrospectively in this study. Of 50 patients infected by *C. albicans*, 23 (46%) died as a result of bloodstream infection and 16 (32%) survived. (Information concerning the outcome of 11 patients is not available.) Among patients with bloodstream infection due to non-*albicans Candida* spp., the lowest mortality rate was in cases of *C. tropicalis*: two patients (66.7%) survived and one (33.3%) died. The second lowest mortality rate was in *C. parapsilosis* bloodstream infection: three (60%) patients survived and two (40%) died. The highest mortality was due to *C. krusei*: all four infected patients died. Each patient infected by *C. glabrata* (two patients) or other *Candida* spp. (two patients) died from their bloodstream infections. Two patients with *Cr. neoformans* infections survived. In summary, 40% of these patients are known to have survived their bloodstream infections caused by yeasts. Differences relating to outcome and yeast species (*C. albicans* vs. non-*albicans Candida* spp.) were not statistically significant (χ^2 test, $p > 0.05$).

In conclusion, these results demonstrate that there were no statistically significant differences in the distribution of these species during the 5-year period studied. *C. albicans* remained the predominant species causing bloodstream infections in this hospital. In comparison with international reports [16], the number of bloodstream infections caused by yeasts per 10 000 admissions was smaller in the present study. However, there are similar data from other European surveys [18]. The susceptibilities to amphotericin B and azoles are variable, but remain in the normal range.

As far as we are aware, this is the first report on the prevalence, species distribution and resistance rate of yeasts causing bloodstream infections in Hungary.

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