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A one-year survey of candidemia in Belgium in 2002

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SUMMARY

A total of 211 episodes of bloodstream yeast infections in 207 patients, hospitalized in 28 Belgian hospitals participating in a National Surveillance Program, were evaluated. A total of 81% of the patients were more than 50 years of age. *Candida albicans* was the cause of infections in 55% of patients, 22% were due to *C. glabrata* and 13% to *C. parapsilosis*. The most common predisposing factors were antibacterial therapy (42%), residence in an intensive care unit (32·9%) and presence of an intravascular catheter (29·7%). Most patients had more than one predisposing factor. Fluconazole alone or in association with another antifungal agent was the treatment of choice for 89·7% of the cases. *In vitro* susceptibility testing of the isolates revealed that 99% were susceptible to amphotericin B, 95% to 5-fluorocytosine, 82% to fluconazole and 69% to itraconazole. Resistance to azoles was more common among *C. glabrata* isolates in the elderly. We conclude that the frequency of *C. albicans* infection is decreasing in Belgium and this is associated with the emergence of other species, most notably, *C. glabrata*.

INTRODUCTION

Yeasts are emerging as important aetiological agents of nosocomial infections, the most frequent being *Candida* spp. These are now the fourth most common cause of hospital-acquired bloodstream infection in the United States [1]. *Candida albicans* has historically been the most predominant *Candida* spp. associated with infections and remains the most common cause of candidemia today. However, the proportion of bloodstream infections due to other *Candida* spp. has increased in the last two decades [2]. The rise in the number of immunocompromised patients, linked to the use of prophylactic antifungal agents, are thought to have played a major role in these changes. Indeed, whereas *C. albicans* is generally susceptible to all antifungal drugs, other species, such as *C. glabrata*, may exhibit reduced susceptibility to antifungals [3]. Nevertheless, the reasons for the rise in non-*C. albicans* species remain unclear and are probably multifactorial [4].

Suetens et al. [5] performed a preliminary survey on bloodstream infections in Belgium in 2001 and found that 7·6% were due to *Candida* spp. with an associated mortality of 48·1%.

Owing to the lack of information on the incidence and epidemiology of candidemia in Belgium and sparse data on the susceptibility of clinical isolates to antifungal drugs, we organized the first mycological multicentre study in this country. The aims of the study were to document the distribution of species among bloodstream *Candida* isolates, to test

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Table 1. *Yeast strains isolated from blood cultures in Belgium in 2002*

Species	No. isolates (%)
Candida albicans	115 (55)
Candida glabrata	47 (22)
Candida parapsilosis	26 (13)
Candida tropicalis	6 (2·8)
Candida krusei	5 (2·3)
Saccharomyces cerevisiae	4 (1·8)
Pichia anomala	3 (1·4)
Candida lusitaniae	2 (0·9)
Candida guilliermondii	1 (0·4)
Candida norvegensis	1 (0·4)
Trichosporon mucoïdes	1 (0·4)
Total	211

Table 2. *Potential risk factors for 188 episodes of candidemia*

Potential risk factor	No. (%) of patients
Antimicrobial therapy	79 (42)
Hospitalization in ICU	62 (33)
Indwelling catheters	51 (27·1)
Underlying cancer	49 (25·9)
Major surgery	18 (9·6)
Steroid therapy	10 (5·3)
Parenteral nutrition	5 (2·6)
Pneumonia	4 (2·1)
Transplant patient	4 (2·1)
Cutaneous abscess	3 (1·5)
Alcoholism	1 (0·5)
Biotherapy	1 (0·5)
Colitis	1 (0·5)
Colon perforation	1 (0·5)
Down syndrome	1 (0·5)
Drug abuse	1 (0·5)
Mucoviscidosis	1 (0·5)
Osteomyelitis	1 (0·5)
Pancreatitis	1 (0·5)
Prematurity	1 (0·5)
Prostatitis	1 (0·5)
Renal failure	1 (0·5)
Traffic accident	1 (0·5)
Total	298

the susceptibilities of the isolates to antifungal agents, and to compile clinical and therapeutic data to inform patient management.

The 150 Belgian medical centres, registered with the Ministry of Public Health were asked to participate in the study on a voluntary basis. All bloodstream yeast isolates collected between 1 January and 31 December 2002 were requested and 28 centres contributed isolates and data to the study. Isolates were identified at the participating centre using the routine methodology in the local laboratory. On receipt, isolates were subcultured on Sabouraud's agar for 24 h at 35 °C and the identification confirmed using standard methods [6].

For susceptibility testing, isolates were assayed using the Sensititre YeastOne panels according to the manufacturer's instructions (Trek Diagnostics Systems Ltd, East Grinstead, UK). The inoculum concentration ranged from $1{\cdot}5 \times 10^3$ to 8×10^3 cells/ml and colorimetric minimum inhibitory concentration (MIC) end-points were read visually after incubation at 35 °C for 24 h. For amphotericin B, the MIC was recorded as the lowest drug concentration that prevented a discernible colour change whereas for itraconazole, fluconazole and flucytosine, the MIC was taken as the lowest concentration that showed a slight colour change compared to the positive growth control. Isolates were categorized as susceptible (S), susceptible-dose-dependent (SDD), intermediate susceptible (I), and fully resistant (R), according to the NCCLS breakpoints [7].

A total of 211 yeast isolates were collected from 207 patients hospitalized in 28 different medical centres. A single yeast species was isolated from 203 patients and four patients were infected by two different species. In total, 189 (95·6%) isolates were from patients >15 years, 30 patients (14·5%) were aged 16–49 years, 73 patients (35%) were aged 50–64 years, and 86 patients (41·5%) were ⩾65 years. Data on gender were available for 205 cases: 107 were male and 98 female (47·8%). Table 1 shows that more than half of the isolates were *C. albicans* and with *C. glabrata* and *C. parapsilosis* these species accounted for approximately 90% of all isolates. The remaining isolates were distributed among eight other species. The four mixed infections were due to two combinations of *C. albicans* and *C. glabrata* and two of *C. albicans* and *C. tropicalis*.

Information on risk factors for candidemia were provided for 188 cases and are listed in Table 2. Most patients had more than one risk factor but the most common single factors were previous antimicrobial therapy (79 patients, 42%), residence in an intensive care unit (62 patients, 33%), the presence of an indwelling catheter (51 patients, 27%) and an underlying cancer (49 patients, 26%). Steroid therapy, parenteral nutrition, pneumonia and organ transplantation were

Table 3. *Susceptibilities of 211 yeast isolates from blood cultures to antifungal agents*

Antifungal agents	Susceptible	Susceptible dose-dependent/ Intermediate	Resistant
Amphotericin B	209 (99%)	—	2
Flucytosine	201 (95%)	7	3
Itraconazole	145 (69%)	51 (24%)	15 (7%)
Fluconazole	172 (82%)	32 (15%)	7

the less common factors identified in a minority of patients.

Data on the initial antifungal treatment was available for 156 cases. Fluconazole monotherapy was prescribed for 132 (84·6%) patients; amphotericin B and itraconazole were used singly for 15 patients and one patient respectively. Eight patients received sequential monotherapies: six received amphotericin B followed by fluconazole, one fluconazole and itraconazole and the last amphotericin B, fluconazole and voriconazole.

The susceptibilities to antifungal agents by NCCLS criteria are presented in Table 3. All but two out of 211 isolates were fully susceptible to amphotericin B (MICs $<1\,\mu$g/ml). Only one isolate of *C. tropicalis* and one of *C. krusei* had higher MIC values for amphotericin B. A total of 201 isolates were susceptible to flucytosine (MICs $<4\,\mu$g/ml). Seven isolates (5 *C. krusei*, 1 *C. norvegensis* and 1 *Trichosporon mucoïdes*) showed intermediate susceptibilities to this agent with MICs between 8 and 16 μg/ml and three (1 *C. lusitaniae* and 2 *C. albicans*) were resistant (MICs $>32\,\mu$g/ml). In total, 145 isolates (69%) were susceptible to itraconazole (MICs $<0{\cdot}125\,\mu$g/ml), 51 (24%) were susceptible-dose-dependent with MICs between 0·25 and 0·5 μg/ml and 15 (13 *C. glabrata* and 2 *C. albicans*, 7%) were resistant (MICs $>1\,\mu$g/ml). One hundred and seventy-two isolates (82%) were susceptible to fluconazole (MICs $<8\,\mu$g/ml), 32 (15%) were susceptible-dose-dependent with MICs between 16 and 32 μg/ml and seven isolates (4 *C. glabrata*, 2 *C. krusei* and 1 *C. albicans*) were resistant (MICs $>64\,\mu$g/ml).

Four *C. glabrata* and one *C. albicans* isolates were resistant to both itraconazole and fluconazole. Three *C. glabrata* and one *C. albicans* isolate resistant to itraconazole were susceptible-dose-dependent to fluconazole.

The MIC_{50} and MIC_{90} of the four antifungal agents for the three most prevalent *Candida* spp. are presented in Table 4. For amphotericin B, similar MIC_{50} and MIC_{90} values were found for the three species. *C. glabrata* was the most susceptible of the species to flucytosine. The highest MIC_{90} for this agent were found with *C. albicans* and *C. parapsilosis*. Full susceptibility to itraconazole was shown by *C. albicans* ($MIC_{90}=0{\cdot}125\,\mu$g/ml) but *C. glabrata* isolates had the highest resistance to this agent. *C. glabrata* isolates were generally not susceptible to fluconazole ($MIC_{90}=32\,\mu$g/ml) followed by *C. parapsilosis*. Almost all *C. albicans* were susceptible.

The first objective was to document the distribution of yeast species responsible for candidemia in Belgium in 2002 and this was the first time that such a study had been performed in that country. The low rate (18·6%) of voluntary participation by registered medical centres was disappointing. Centres were asked to send all their bloodstream isolates over the test period but no checking occurred to establish that this was the case. Nevertheless we obtained sufficient samples to allow us to establish the relative frequency of species of yeasts causing bloodstream infections.

C. albicans remains the most common cause of candidemia accounting for 55% of the cases. This is less than the 64% reported from the National Surveillance in Belgian Hospitals from October 1992 to the end of June 2001 [5] and leads us to conclude that the proportion of non-*C. albicans* candidemia is also increasing in Belgium as observed elsewhere [2, 8, 9]. It is, however, difficult to compare our isolation rate for *C. albicans* with those reported from other European countries and which vary considerably between countries. For example, in Hungary a frequency of *C. albicans* of 73·5% over a 5-year period (1996–2000) was reported [10], in Norway, 66% (1991–1996) [11], in Iceland, 64·4% (1980–1999) [12], in France, 53% in 1995 [13]; the lowest rate (41·4%) was reported from Italy in a 10-year survey completed in 1997 [14]. The reasons for these differences are probably multifactorial but our rate of 55% is very close to that (58%) observed by Pfaller et al. [2] who gathered results from different European countries.

C. glabrata was the second most common species overall causing 22% of the bloodstream infections followed by *C. parapsilosis* in 13% of the cases. In most published studies *C. glabrata* has emerged as the second more frequent species responsible for bloodstream infections [11, 12, 14, 15]. This rise occurs with increasing patient age, an observation supported by our results where 81% of patients with this organism were older than 50 years. The higher incidence of this

Table 4. *Antifungal susceptibility profile of 188* Candida *bloodstream isolates*

Species (no.)	Antifungal agents	Range	MICs (μg/ml) MIC 50%	MIC 90%
C. albicans (115)	Amphotericin B	0·125–1·0	0·5	1·0
	Flucytosine	<0·03–>64	0·06	0·25
	Itraconazole	0·008–>16	0·06	0·125
	Fluconazole	0·06–>256	0·5	1·0
C. glabrata (47)	Amphotericin B	0·03–1·0	0·5	1·0
	Flucytosine	<0·03–16	0·03	0·03
	Itraconazole	0·06–>16	0·5	1·0
	Fluconazole	0·5–128	16	32
C. parapsilosis (26)	Amphotericin B	0·125–1·0	0·5	1·0
	Flucytosine	<0·03–1·0	0·12	0·25
	Itraconazole	0·03–0·25	0·125	0·25
	Fluconazole	0·5–16	2	4

endosaprophytic species may be the result of modification of the flora of the gastrointestinal and genital mucosa [16]. Further, as fluconazole alone or in association with another agent was the primary therapeutic choice in almost 90% of our patients, the rise of *C. glabrata* may be related to its decreased susceptibility to this drug [15, 17]. On the other hand, *C. parapsilosis*, which was isolated from 13% of the cases studied here, is an exogenously acquired yeast pathogen which is horizontally transmitted and has the propensity to adhere to implantable or semi-implantable synthetic materials, including indwelling central venous catheters [18]. Indeed, the rise of *C. parapsilosis* candidemia is known to be associated with invasive procedures involving the implantation of prosthetic devices [8, 18, 19]. *C. parapsilosis* also affects more frequently paediatric and neonatal patients [20, 21] as well as critically ill individuals with haematological malignancies [22]. In all those groups, *C. parapsilosis* can become the most common species after *C. albicans* and supplant *C. glabrata* with incidence rates as high as 36% [22].

The frequency of *C. parapsilosis* (13%) isolates in our study is low, considering that indwelling catheters were present in 27% of the patients. This may be explained by the age distribution of our population that is favourable to *C. glabrata*. If this hypothesis is verified, it suggests that the impact of age is more important than the presence of indwelling catheters.

All the other isolates belonged to species commonly found as aetiological agents of candidemia.

The other main objective of the study was to evaluate the susceptibilities of the isolates to commonly used antifungal drugs. Our results show that the susceptibility of different species to amphotericin B was excellent. Indeed, the two *C. lusitaniae* isolates, a species frequently resistant to amphotericin B, were both susceptible to the agent [23]. The responses to flucytosine were also favourable with only 1·5% of resistance and if only the 96 non-*C. albicans* isolates were considered, the frequency of resistant isolates diminishes to 1·1%. This is very low compared to previously published data, i.e. 7·3% in Italy [24], 6% in the United States [25] and 3% in Brazil [26]. In contrast, the profiles of susceptibilities to triazole-derived antifungal drugs were less favourable and *C. glabrata* was the most frequently encountered species exhibiting either low susceptibility or resistance to them: four were resistant to fluconazole (8·5%) and 13 to itraconazole (27·6%). The occurrence of cross-resistances between the two triazoles was also more frequently observed among *C. glabrata* isolates. Seven isolates, either susceptible-dose-dependent or resistant to fluconazole were also resistant to itraconazole.

In the SENTRY study mentioned above, Pfaller et al. [16] found that isolates of *Candida* spp. showed a trend of decreasing susceptibility to antifungal drugs and more especially to azoles with increasing patient age. In that study, none of the *C. glabrata* isolates from individuals $\leqslant$1 year old were resistant to fluconazole whereas they made up 9% of isolates from individuals aged $\geqslant$65 years. This may partly explain our results as 46% of our patients were elderly.

In conclusion, the frequency of occurrence of *C. albicans* as a cause of bloodstream infections is decreasing in Belgium. This is associated with the

emergence of other species, *C. glabrata* being the most frequent. Decreased susceptibilities to azoles was also observed and this is more prominent among *C. glabrata* isolates from individuals in the adult age group.

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