A single-centre 10-year experience with *Candida* bloodstream infections

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OBJECTIVE: To describe the clinical and microbiological features associated with *Candida* bloodstream infections observed at Hôpital Maisonneuve-Rosemont (Montreal, Quebec) between August 1996 and July 2006.

METHODS: Episodes were retrieved from the microbiology laboratory. Different patient episodes and different isolate episodes in the same patient were selected. Antifungal susceptibility was determined by the Clinical and Laboratory Standards Institute's (USA) M27A2 method.

RESULTS: A total of 190 different episodes of candidemia in 185 patients were identified. Eleven (6%) episodes occurred in outpatients. *Candida albicans* was identified in the majority of episodes (57%). Its frequency remained stable over the years. The proportion of *Candida krusei* candidemia episodes increased between 2003 and 2006, but this was not statistically significant. A central venous indwelling catheter or a peripherally inserted central catheter line was present in the majority of patients (167 [88%]). Of the indwelling catheters removed at the time of diagnosis, 39% were positive for *Candida* species on culture. Overall, voriconazole was the most active agent (the minimum inhibitory concentration required to inhibit the growth of 90% of organisms was 0.5 mg/L). Resistance to fluconazole was observed in 26 (14%) isolates (*C albicans*, 4%; versus non-*albicans Candida* species, 27%; P<0.001). Being on the hematology-oncology unit at the time of diagnosis (adjusted OR 7.8; 95% CI 2.3 to 27.1; P=0.001) and having received fluconazole or itraconazole within the past three months (adjusted OR 8.3; 95% CI 2.8 to 24.4; P<0.001) were significantly associated with resistance to fluconazole in multivariate analysis.

CONCLUSIONS: At Hôpital Maisonneuve-Rosemont, the frequency and species distribution of blood isolates of *Candida* remained stable over the past decade. In vitro resistance of *C albicans* to fluconazole and itraconazole remained minimal; resistance of non-*albicans Candida* species to fluconazole did not increase significantly. The new antifungal agents all had high in vitro activity against the bloodstream *Candida* isolates.

Key Words: *Antifungals; Bloodstream infections; Canada;* Candida *infections*

Dix ans d'expérience des infections sanguines à *Candida* dans un seul centre

OBJECTIF : Décrire les caractéristiques cliniques et microbiologiques associées aux infections sanguines à *Candida* observées à l'Hôpital Maisonneuve-Rosemont (de Montréal, au Québec) entre août 1996 et juillet 2006.

MÉTHODOLOGIE : Les chercheurs ont retracé les épisodes du laboratoire de microbiologie. Ils ont sélectionné divers épisodes et divers isolats chez un même patient. Ils ont déterminé la susceptibilité aux antifongiques d'après la méthode M27A2 du *Clinical and Laboratory Standards Institute* (États-Unis).

RÉSULTATS : Les chercheurs ont repéré 190 épisodes différents de septicémie à *Candida* chez 185 patients. Onze (6 %) épisodes se sont produits en consultations externes. On a dépisté le *Candida albicans* dans la majorité des épisodes (57 %). Sa fréquence est demeurée stable au fil des ans. La proportion d'épisodes de septicémie à *Candida kruset* a augmenté entre 2003 et 2006, sans être statistiquement significative. La majorité des patients avaient un cathéter veineux central à demeure ou un cathéter central périphérique (167 [88 %]). Des cathéters à demeure retirés au moment du diagnostic, 39 % étaient positifs aux espèces à *Candida* à la culture. Dans l'ensemble, le voriconazole était l'agent le plus actif (la concentration inhibitrice minimale nécessaire pour inhiber la croissance de 90 % des organismes était de 0,5 mg/L). Les chercheurs ont observé une résistance au fluconazole dans 26 (14 %) isolats (*C albicans*, 4 %, par rapport aux espèces non *C albicans*, 27 %; P<0,001). Selon l'analyse multivariée, le fait d'être à l'unité d'hématologie-oncologie au moment du diagnostic (RRR 7,8; 95 % IC 2,3 à 27,1; P=0,001) et l'administration de fluconazole ou d'itraconazole dans les trois mois précédents (RRR 8,3; 95 % IC 2,8 à 24,4; P<0,001) s'associaient de manière significative à une résistance au fluconazole.

CONCLUSIONS : Dans l'établissement, la fréquence et la répartition des espèces d'isolats sanguins de *Candida* sont demeurées stables depuis dix ans. La résistance *in vitro* du *C albicans* au fluconazole et à l'imaconazole est demeurée minime. La résistance des espèces non *C albicans* au fluconazole n'a pas tellement augmenté. Les nouveaux antifongiques avaient tous une activité *in vitro* élevée contre les isolats de *Candida* dans le sang.

Since the original observation by Krause et al (1) of the invasive potential of *Candida* species, a considerable number of studies have expanded our knowledge on *Candida* bloodstream infections (BSIs). In a large database of nosocomial BSIs in the United States, *Candida* species ranked fourth overall among etiological agents, and was the third most common cause of such infections in intensive care units (ICUs) (2). This reflects increasing numbers of immunocompromised patients, greater use of antibiotics, better diagnostic methods, frequent use of invasive procedures and improvements in life-support systems (3-8). Over the past 20 years, the epidemiology of candidemia has been considerably modified by the emergence of non-*albicans Candida* species, the occurrence of triazole-resistant species and the development of secondary resistance among previously sensitive isolates (4,5,9-11). Pfaller and Diekema (12) recently emphasized the importance of sustaining surveillance programs to

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TABLE 1
Distribution of *Candida* species isolates (by study period) in 190 episodes of candidemia

Species	1996–1999, n (%)	2000–2002, n (%)	2003–2006, n (%)	Total, n (%)
Candida albicans	34 (58)	42 (60)	33 (54)	109 (57)
Candida glabrata	7 (12)	10 (14)	11 (18)	28 (15)
Candida krusei	4 (7)	5 (7)	8 (13)	17 (9)
Candida parapsilosis	6 (10)	8 (11)	6 (10)	20 (11)
Candida tropicalis	4 (7)	5 (7)	3 (5)	12 (6)
Candida species*	4 (7)	0 (0)	0	4 (2)

*Candida lusitaniae *(2),* Candida lipolytica *(1),* Candida *species (1)*

maintain and improve the prevention and management of candidemia. Only a few Canadian studies (13-16) have reported on the epidemiology of *Candida* BSIs and the susceptibility of corresponding isolates. The present 10-year retrospective study was performed to capture the local epidemiology of *Candida* BSIs at Hôpital Maisonneuve-Rosemont (Montreal, Quebec), with an emphasis on secular trends, predisposing factors and in vitro susceptibility of the isolates.

METHODS

Hôpital Maisonneuve-Rosemont is a tertiary care academic hospital in Montreal, serving a population of approximately 500,000 inhabitants, with a large hematopoietic stem cell transplantation (HSCT) program since 1980, a kidney transplantation program, and all medical and surgical specialties. All candidemia episodes observed between August 1996 and July 2006, among patients 18 years of age or older, were retrieved from the microbiology laboratory records. Repetitive episodes, defined as the same species isolated from the same patient, were excluded. Different species isolates in the same patient were considered to be distinct episodes. Standardized forms were used to collect clinical data from hospital records. Patients' localization at the time of the diagnosis was categorized. Speciation and antifungal susceptibility testing of the recovered isolates were performed by means of germ tube, chlamydospore formation, Vitek YBC identification system (BioMérieux, Canada) and the Clinical and Laboratory Standards Institute (USA) broth microdilution susceptibility testing method (17).

Standard antifungal powders of amphotericin B, itraconazole (Sporanox, Janssen-Ortho Inc, Canada), ravuconazole (Bristol-Myers Squibb, Canada), fluconazole (Diflucan, Pfizer Canada), voriconazole (Vfend, Pfizer Canada Inc), posaconazole (Noxafil, Schering-Plough, USA), micafungin (Mycamine, Astellas Pharma Canada Inc), caspofungin (Cancidas, Merck Frosst Canada Ltd) and anidulafungin (Eraxis, Pfizer Canada Inc, Canada) were supplied from their respective manufacturers. Stock solutions were prepared according to manufacturers' specifications. Serial twofold dilutions were made in RPMI-1640 medium (Sigma Aldrich Canada) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid buffer (Sigma-Aldrich Canada) for all antibiotics. The final concentrations of the antifungal agents were 0.008 mg/L to 16 mg/L for amphotericin B, itraconazole, voriconazole, ravuconazole, posaconazole, micafungin, caspofungin and anidulafungin, and 0.25 mg/L to 256 mg/L for fluconazole. Drug-free and yeast-free controls were included. Trays were incubated in air at 35°C, and the minimum inhibitory concentration (MIC) end points were visually read after 48 h of incubation. The MICs of amphotericin B were defined as the lowest concentration resulting in a complete inhibition of growth, while the MICs of all the other compounds were defined as the lowest concentration that resulted in a substantial reduction of growth (80% inhibition) compared with that of growth-control wells. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used for quality control.

Data were analysed with Stata 8.0 (StataCorp LP, USA). Medians were compared using the Kruskal-Wallis test. Proportions were compared using the χ^2 test or Fisher's exact test when appropriate. Unconditional logistic regression was used for multivariate analysis. Models were built up sequentially, starting with the variable most strongly associated with the outcome and continuing until no other variable reached significance. When the final model was reached, each variable was dropped in turn to assess its effect. Different models were compared using the likelihood ratio test, keeping only variables significant at the P=0.05 level in the final model.

RESULTS

A total of 190 distinct episodes of candidemia in 185 patients were documented. Three patients were infected with two different *Candida* species on the same day, and two patients experienced two separate episodes of candidemia 10-day (*C parapsilosis* and *Candida glabrata*) and 11-day (*C albicans* and *C krusei*) intervals, respectively. The yearly number of episodes ranged from 11 to 26 (median of 18 episodes). The overall species distribution by study periods is described in Table 1. The proportion of *C albicans* over the 10-year observation period remained fairly stable (57% overall), and the higher proportion of *C krusei* candidemia episodes found between 2003 and 2006 were not statistically significant (13% compared with 8% between 1996 and 1999; OR 2.1; 95% CI 0.6 to 7.3). At least two different blood culture samples were drawn for 181 (95%) patients (median of four samples per patient; range two to 36 samples over one to 26 days). In 66 of 181 (36%) patients, only one sample was positive (transient candidemia). Overall, same episode blood cultures remained positive for two or more days in 82 (43%) episodes (median of five episodes; range two to 25 episodes).

Demographic characteristics, localization of patients and risk factors identified at the time of diagnosis are listed in Table 2. There were no significant differences between patients infected with *C albicans* strains and those infected with non-*albicans Candida* species. Among HSCT recipients, 24% were infected with *C albicans* and 14% with non-*albicans Candida* species. The overall median age increased over the study period (48 years of age between 1996 and 1999, 56 years of age between 2000 and 2002, and 64 years of age between 2003 and 2006; P=0.02). Episodes were evenly distributed among patients in the hematology-oncology unit (including hematopoietic stem cells recipients), those in the ICU and those in the medical/surgical wards, each contributing to approximately one-third of the episodes. However, the proportion of patients who were on the hematology-oncology unit at the time of their candidemia decreased from 41% between 1996 and 1999 to 19% between 2003 and 2006 (P=0.009). This trend was statistically significant for patients infected with *C albicans* (44% were on the

hematology-oncology unit at the time of their candidemia between 1996 and 1999, 29% between 2000 and 2002 and 15% between 2003 and 2006 [P=0.01]), but not for patients infected with other *Candida* species (40% were on the hematology-oncology unit at the time of their candidemia between 1996 and 1999, 36% between 2000 and 2002, and 21% between 2003 and 2006). The proportion of patients with a central venous catheter decreased, while there was a corresponding increase in the proportion of patients with a peripherally inserted central catheter (PICC) line. Eleven episodes (6%) occurred among 10 outpatients – four in hemodialyzed patients (three had tunnelled catheters and one had an arteriovenous fistula) and six in emergency room attendees (two of whom had an indwelling venous catheter for ambulatory antibiotics administration). Duration of hospital stay before the candidemia episode ranged from two to 308 days (median 23) for the 175 inpatients. A central venous indwelling catheter or PICC line was present in 161 patients (87%); 122 of these catheters were removed and cultured at the time of diagnosis and 47 (39%) were positive for *Candida* species. Patients infected by *C parapsilosis* were more likely to have a positive catheter culture than those infected with other *Candida* species (55% versus 23%; P=0.002). Forty-three (23%) patients had been exposed to at least one antifungal drug within three months of their candidemic episode. Amphotericin B, including deoxycholate and lipid-based formulations, had been used in 16 patients (9%), fluconazole in 26 patients (14%), itraconazole in two patients (1%) and caspofungin in one patient (1%). *C krusei* was recovered more often in patients previously exposed to fluconazole (28%) than in those unexposed (6%; OR 6.4, 95% CI 2.1 to 19.4; P<0.001).

Antifungal therapy was administered in 174 episodes (92%). Deoxycholate amphotericin B was used in 84 episodes (44%), a lipid-based amphotericin formulation in 26 (14%), fluconazole in 128 (67%), itraconazole in one (1%), caspofungin in 30 (16%) and anidulafungin in one (1%). Among the 16 patients who were not treated, nine died within two days before their blood culture positivity was known; in four others, the time to positivity of their blood cultures ranged from 20 h to 3.3 days; three patients had only one positive blood culture bottle (two with *C albicans* and one with *Candida tropicalis*), no known risk factor for *Candida* infection and survived. Death within 30 days of diagnosis occurred in 57 of 185 patients (17 of 58 [29%] between 1996 and 1999; 25 of 68 [37%] between 2000 and 2002; and 15 of 59 [25%] between 2003 and 2006), one of whom was infected with two different *Candida* species. Death was not significantly associated with age, sex, duration of hospital stay before the episode of candidemia, any of the risk factors listed in Table 2, infecting species (*C albicans* 34 of 109 [31%]; other species 24 of 81 [30%]) or antifungal resistance.

Comparative in vitro susceptibilities, along with the currently established Clinical and Laboratory Standards Institute (USA) interpretations criteria, are summarized in Table 3. All *C krusei* strains were considered resistant to fluconazole, irrespective of the MIC results. Among the other *Candida* species, nine (5%) isolates were resistant to fluconazole (MIC 64 mg/L or greater). Overall, the MICs required to inhibit the growth of 90% of organisms of fluconazole and voriconazole were lower than the breakpoint value for resistance. The MICs required to inhibit the growth of 90% of organisms of posaconazole and ravuconazole, for which breakpoints have not yet been established, were equally low at 1 mg/L. Itraconazole and fluconazole were less active against non-*albicans Candida* species taken together (n=81), than against *C albicans* species (only 26% of non-*albicans Candida* species isolates were susceptible to itraconazole [P<0.001] and 67% to fluconazole [P<0.001]). However, voriconazole activity against *C albicans* and non-*albicans Candida* species was identical (96%). For *C albicans*, fluconazole resistance remained fairly stable during the study period – 6% between 1996 and 1999, 2% between 2000 and 2002, and 3% between 2003 and 2006. For other species, the fluconazole resistance rate was higher between 2003 and 2006 (36%) compared with 1996 and 1999 (24%) and 2000 and 2002 (21%). This difference was not statistically significant and is likely due to a higher number of *C krusei* isolated between 2003 and 2006.

Of the 26 (14%) fluconazole-resistant isolates (MIC 64 mg/L or greater), 10 (38%) were also resistant to itraconazole; cross-resistance or reduced susceptibilities (MIC 4 mg/L or greater) for the newer azoles (voriconazole, ravuconazole and posaconazole) were seen in only nine isolates (two *C albicans*, six *C glabrata* and one *C tropicalis*). There was no obvious epidemiological link among any of these nine isolates. All four fluconazole-resistant *C albicans* were isolated in hematology-oncology patients.

High MICs (2 mg/L or greater) for echinocandins were observed mainly with *C parapsilosis*. A tendency for a class effect was observed among these isolates with reduced susceptibility to

TABLE 2
Characteristics of patients with candidemia distributed by study period (190 episodes in 185 patients)

Characteristics	1996–1999, n (%)	2000–2002, n (%)	2003–2006, n (%)	Total, n (%)
Age group (years)*				
18–44	26 (45)	14 (21)	10 (17)	50 (27)
45–64	19 (33)	33 (49)	20 (34)	72 (39)
≥65	13 (22)	21 (31)	29 (49)	63 (34)
Sex				
Male to female ratio	28:30 (48:52)	31:37 (46:54)	39:20 (66:34)	98:87 (53:47)
Localization at the time of diagnosis				
Outpatients	4 (7)	0 (0)	6 (10)	10 (5)
Medical or surgical units	17 (29)	21 (31)	24 (41)	62 (34)
Intensive care units†	13 (22)	26 (38)	18 (31)	57 (31)
Hematology-oncology units‡	24 (41)	21 (31)	11 (19)*	56 (30)
Risk factors				
Central venous catheter	43 (74)	32 (47)*	22 (37)§	97 (52)
Peripheral inserted central catheter	9 (16)	36 (53)§	34 (58)§	79 (43)
Solid organ malignancy	6 (10)	2 (3)	10 (17)	18 (10)
Hematological malignancy	25 (43)	33 (49)	14 (24)*	72 (39)
HSCT	17 (29)	15 (22)	4 (7)*	36 (19)
Steroid therapy	32 (55)	30 (44)	19 (32)*	81 (44)
Neutrophil count $<0.5\times10^9$/L	22 (38)	22 (32)	11 (19)*	55 (30)
Abdominal surgery (past 30 days)	10 (17)	11 (16)	7 (12)	28 (15)
Kidney transplantation	2 (3)	0 (0)	2 (3)	4 (2)
HIV	1 (2)	0 (0)	1 (2)	2 (1)

**P<0.05; †Combined medical-surgical intensive care units and coronary care unit; ‡Including the hematopoietic stem cell transplantation (HSCT) unit; §P<0.001*

TABLE 3
In vitro susceptibilities in 190 isolates of *Candida*

Organism (n)	Compound	MIC (mg/L)			Interpretation (%)*		
		Range	MIC_{50}	MIC_{90}	Sensitive	S-DD	Resistant
All organisms (190)	Amphotericin B	0.12–2	1.0	2.0	–	–	–
	Itraconazole	<0.008–≥32	0.12	2.0	60	23	17
	Fluconazole	<0.25–≥512	0.5	32.0	84	3	14†
	Voriconazole	<0.008–≥32	0.03	0.5	96	0	4
	Ravuconazole	<0.008–≥32	0.03	1.0	–	–	–
	Posaconazole	<0.008–≥32	0.06	1.0	–	–	–
	Caspofungin	0.03–2	0.12	1.0	–	–	–
	Micafungin	0.016–16	0.25	2.0	–	–	–
	Anidulafungin	0.016–4	0.06	1.0	–	–	–
Candida albicans (109)	Amphotericin B	0.12–2	1.0	1.0	–	–	–
	Itraconazole	<0.008–16	0.06	0.25	85	10	5
	Fluconazole	<0.25–≥512	0.5	2.0	96	0	4
	Voriconazole	<0.008–16	0.01	0.12	96	0	4
	Ravuconazole	<0.008–16	0.03	0.25	–	–	–
	Posaconazole	<0.008–16	0.06	0.12	–	–	–
	Caspofungin	0.03–0.5	0.12	0.25	–	–	–
	Micafungin	0.016–16	0.06	0.5	–	–	–
	Anidulafungin	0.016–4	0.03	0.06	–	–	–
Candida glabrata (28)	Amphotericin B	0.25–2	1.0	2.0	–	–	–
	Itraconazole	0.12–≥32	2.0	≥32.0	4	21	75
	Fluconazole	0.5–256	8.0	128.0	71	14	14
	Voriconazole	0.12–8	0.5	2.0	89	4	7
	Ravuconazole	<0.008–≥32	1.0	4.0	–	–	–
	Posaconazole	0.06–≥32	1.0	4.0	–	–	–
	Caspofungin	0.03–0.5	0.12	0.25	–	–	–
	Micafungin	0.016–0.5	0.06	0.25	–	–	–
	Anidulafungin	0.03–0.25	0.06	0.12	–	–	–
Candida krusei (17)	Amphotericin B	1–2	2.0	2.0	–	–	–
	Itraconazole	0.06–1	0.5	1.0	6	76	18
	Fluconazole	8–64	32.0	64.0	0	0	100†
	Voriconazole	<0.008–1	0.25	0.5	100	0	0
	Ravuconazole	0.03–2	0.25	1.0	–	–	–
	Posaconazole	0.03–1	0.5	1.0	–	–	–
	Caspofungin	0.12–1	0.5	1.0	–	–	–
	Micafungin	0.06–2	0.25	1.0	–	–	–
	Anidulafungin	0.06–0.25	0.12	0.25	–	–	–
Candida parapsilosis (20)	Amphotericin B	0.12–2	1.0	2.0	–	–	–
	Itraconazole	0.03–0.5	0.06	5.0	70	30	0
	Fluconazole	0.25–32	0.5	4.0	95	5	0
	Voriconazole	<0.008–0.12	0.01	0.12	100	0	0
	Ravuconazole	<0.008–0.12	0.03	0.12	–	–	–
	Posaconazole	<0.008–0.25	0.06	0.25	–	–	–
	Caspofungin	0.06–2	1.0	2.0	–	–	–
	Micafungin	0.03–16	8.0	16.0	–	–	–
	Anidulafungin	1–4	4.0	4.0	–	–	–
Candida tropicalis (12)	Amphotericin B	0.5–1	1.0	1.0	–	–	–
	Itraconazole	0.06–4	0.25	1.0	42	42	17
	Fluconazole	0.25–2	0.5	2.0	100	0	0
	Voriconazole	0.016–0.25	0.03	0.25	100	0	0
	Ravuconazole	0.016–0.25	0.06	0.25	–	–	–
	Posaconazole	0.016–0.25	0.06	0.25	–	–	–
	Caspofungin	0.06–0.25	0.12	0.25	–	–	–
	Micafungin	0.06–0.5	0.25	0.5	–	–	–
	Anidulafungin	0.03–0.5	0.06	0.5	–	–	–
Candida species‡ (4)	Amphotericin B	0.25–1	–	–	–	–	–
	Itraconazole	0.25–1	–	–	0	75	25
	Fluconazole	1.0–128	–	–	75	0	25
Candida species‡ (4)	Voriconazole	<0.008–1	–	–	100	0	0
	Ravuconazole	<0.008–0.12	–	–	–	–	–
	Posaconazole	0.03–1	–	–	–	–	–
	Caspofungin	1	–	–	–	–	–
	Micafungin	2–8	–	–	–	–	–
	Anidulafungin	0.016–1	–	–	–	–	–

**Interpretation according to the Clinical and Laboratory Standards Institute criteria; †*C krusei *is considered inherently resistant to fluconazole regardless of the minimum inhibitory concentration (MIC) obtained; ‡*Candida lusitaniae *(2),* Candida lipolytica *(1),* Candida *species (1). S-DD Susceptible dose-dependent (fluconazole MIC 16 mg/L to 32 mg/L; itraconazole MIC 0.25 mg/L to 0.5 mg/L; and voriconazole MIC 2 mg/L)*

echinocandins. Among triazoles-resistant isolates, 100% were inhibited by a maximum of 0.25 mg/L of anidulafungin, compared with 83% for caspofungin (*C albicans* seven of seven [100%]; other species 22 of 28 [79%]) and 80% for micafungin (*C albicans* five of seven [71%]; other species 23 of 28 [82%]).

Risk factors for infection caused by a resistant isolate are listed in Table 4. Age, hospital unit at the time of diagnosis and antifungal use within the past three months were significantly associated with fluconazole resistance. In multivariate analysis, being on the hematology-oncology unit at the time of diagnosis, compared with outpatients and patients on the medical or surgical wards (adjusted OR [AOR] 7.8; 95% CI 2.3 to 27.1; P=0.001) and having received fluconazole or itraconazole within the past three months (AOR 8.3; 95% CI 2.8 to 24.4; P<0.001) were significantly associated with resistance to fluconazole. Study period and duration of current hospital stay were not significantly associated with resistance to fluconazole.

TABLE 4
Risk factors for resistance to fluconazole in 190 episodes of candidemia

	Number of patients with a resistant* isolate/total (%)	OR (95% CI)	Adjusted† OR (95% CI)
Age group (years)			
18–44	9/50 (18)	1.0	1.0
45–64	14/75 (19)	1.1 (0.4–2.6)	1.1 (0.4–3.4)
≥65	3/65 (5)	0.2 (0.06–0.9)‡	0.4 (0.07–1.7)
Study periods			
1996–1999	8/59 (14)	1.0	1.0
2000–2002	7/70 (10)	0.7 (0.2–2.1)	0.8 (0.2–2.7)
2003–2006	11/61 (18)	1.4 (0.5–3.8)	2.1 (0.6–7.3)
Hospital unit at the time of diagnosis§			
Outpatients or medical/surgical wards	4/74 (5)	1.0	1.0
Intensive care	6/58 (10)	2.0 (0.5–7.5)	1.3 (0.3–5.3)
Hematology-oncology (including HSCT unit)	16/58 (28)	6.7 (2.1–21.3)¶	7.8 (2.3–27.1)¶
Duration of current hospital stay up to diagnosis (days)			
0–9	2/40 (5)	1.0	1.0
10–19	5/40 (13)	2.7 (0.5–14.9)	1.2 (0.2–7.7)
20–29	8/46 (17)	4.0 (0.8–20.1)	2.2 (0.4–13.4)
≥30	11/64 (18)	3.9 (0.8–18.8)	1.6 (0.3–9.4)
Previous antifungal use (within three months of diagnosis)			
None	13/144 (9)	1.0	1.0
Amphotericin B**	1/16 (6)	0.7 (0.08-5.5)	0.3 (0.03-2.4)
Fluconazole or itraconazole	12/29 (41)	7.1 (2.8-18.1)¶	8.3 (2.8-24.4)¶
Caspofungin	0/1	–	–

**Fluconazole minimum inhibitory concentration 64 mg/L or greater (n=26); †Adjusted for hospital unit at time of diagnosis and previous antifungal use; ‡P<0.05; §Eleven episodes occurred in outpatients (all infected with fluconazole-susceptible* Candida *species); medical units include cardiology, endocrinology, gastroenterology, geriatry, internal medicine, nephrology, neurology; surgical units include general surgery and gynecology; ¶P≤0.001; **Amphotericin B includes deoxycholate (n=13) and lipid-based formulations (n=3). HSCT Hematopoietic stem cell transplantation; NS Not significant*

DISCUSSION

The dominance of *C albicans*, which accounted for approximately 60% of our *Candida* BSI, is comparable with observations made in other Canadian centres (15,16,18,19). Although *C glabrata* was overall the second most frequent species (approximately 15%), variations were noted in the species ranking when analyzed by patients' localization. *C parapsilosis* was the second most common species in our outpatients' BSIs, reflecting the exogenous skin niche of this organism and its ability to form biofilms on indwelling intravascular catheters of patients managed at home (20-22). *C krusei* and *C tropicalis* ranked second (21%) and third (12%), respectively, in the hematology-oncology units patients, with *C glabrata* at a distant fourth place, with only 5% of the isolates. Each of these three species has been associated with hematological and solid tumours (23-25). Selective pressure from fluconazole prophylaxis has been incriminated in the emergence of *C krusei* and *C glabrata* among certain patient populations (11,23). In our institution, systematic prophylaxis has been used only in our HSCT recipients. Fluconazole has been our preferred prophylactic agent and was extensively used throughout the study period. The low prevalence of fluconazole-resistant *C glabrata* in our institution may explain why this species remained uncommon, although factors other than exposures to triazoles antifungals have been shown to select for these species (26). Approximately one-third of the episodes were noted in patients localized outside our ICU and hematology-oncology units at an increasing trend, which emphasizes the importance of maintaining a high level of clinical suspicion in the recognition of *Candida* BSIs in patient populations outside the traditional risk groups of compromised cancer and critical care patients.

During the latter years of our study, the frequency of candidemic patients with central venous catheters decreased, with a corresponding increase in the proportion of patients with a PICC line. This observation is likely attributable to a better accessibility in our centre to PICC line insertions. The insertion and nursing care conditions of these lines have been as rigorous as those applied to central venous lines.

Globally, the older triazoles (fluconazole and itraconazole) antifungals remain very active against the majority of our isolates. The echinocandins all had high in vitro activity against the various isolates, except for *C parapsilosis*. A class-effect tendency was observed with all three echinocandins, showing reduced activity against *C parapsilosis*, compared with their activity against the other *Candida* species. The clinical significance of this reduced in vitro activity appears negligible, with poor correlation between the MICs of echinocandin and treatment outcome, as reported by Kartsonis et al (27). However, correlation between clinical failure and rising in vitro caspofungin MICs was recently observed in patients with *C albicans* esophagitis (28,29).

Our study has several limitations due to its retrospective nature. The mean duration of five days of fungemia observed in 42% of our candidemic patients may be overestimated because it is possible that clinicians ordered additional blood cultures in sicker patients only.

Mixed fungemia was observed in only three (2%) of our patients. Jensen et al (30) recently reported a 3% rate over a 21-year period, while Al-Rawahi and Roscoe (19) observed a 6% rate over a six-year surveillance period. However, both groups used a differential subculture medium when yeasts were detected in positive blood culture bottles. Such an approach was not used in our study, which could underestimate this phenomenon.

The armamentarium of systemic antifungal antibiotics has expanded considerably over the past decade. Our study, as well as observations by others, confirms the high in vitro activity of fluconazole against the large majority of invasive *Candida* isolates. In patient populations not commonly exposed to azoles, such as medical or surgical unit patients, fluconazole is an appropriate initial therapeutic choice, particularly in clinically stable patients. In unstable patients, for whom organ dysfunction is frequent, and in patient populations in which previous exposure to azoles is common, such as hematology-oncology and HSCT patients, an echinocandin represents a preferred, less toxic alternative to amphotericin B pending the speciation and in vitro susceptibility results.

CONCLUSION

No significant changes were observed through this 10-year observational study in the frequency and species distribution of candidemic isolates in our institution. Over the years, *C albicans* has remained the main cause of fungemia. A tendency toward an increasing number of episodes of *Candida* BSI in older patients and in patients outside the traditional compromised cancer and critical care patients was noted. In vitro resistance to fluconazole and itraconazole of *C albicans* remained minimal**.** The new antifungal antibiotics all have high in vitro activity against our bloodstream *Candida* isolates.

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REFERENCES

1. Krause W, Matheis H, Wulf K. Fungaemia and funguria after oral administration of *Candida albicans*. Lancet 1969;1:598-9.
2. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309-17.
3. Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. Clin Infect Dis 2002;35:627-30.
4. Viscoli C, Girmenia C, Marinus A, et al. Candidemia in cancer patients: A prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). Clin Infect Dis 1999;28:1071-9.
5. Rodriguez D, Almirante B, Park BJ, et al. Candidemia in neonatal intensive care units: Barcelona, Spain. Pediatr Infect Dis J 2006;25:224-9.
6. Blumberg HM, Jarvis WR, Soucie JM, et al. Risk factors for Candidal bloodstream infections in surgical intensive care unit patients: The NEMIS prospective multicenter study. The National Epidemiology of Mycosis Survey. Clin Infect Dis 2001;33:177-86.
7. Tortorano AM, Peman J, Bernhardt H, et al. Epidemiology of Candidaemia in Europe: Results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur J Clin Microbiol Infect Dis 2004;23:317-22.
8. Hajjeh RA, Sofair AN, Harrison LH, et al. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J Clin Microbiol 2004;42:1519-27.
9. Diekema DJ, Messer SA, Brueggemann AB, et al. Epidemiology of candidemia: 3-year results from the emerging infections and the epidemiology of Iowa organisms study. J Clin Microbiol 2002;40:1298-302.
10. Marichal P, Koymans L, Willemsens S, et al. Contribution of mutations in the cytochrome P450 14alpha-demethylase (Erg11p, Cyp51p) to azole resistance in *Candida albicans*. Microbiology (Reading, England) 1999;145:2701-13.
11. Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N Engl J Med 1991;325:1274-7.
12. Pfaller MA, Diekema DJ. Role of sentinel surveillance of candidemia: Trends in species distribution and antifungal susceptibility. J Clin Microbiol 2002;40:3551-7.
13. Taylor GD, Buchanan-Chell M, Kirkland T, McKenzie M, Wiens R. Trends and sources of nosocomial fungaemia. Mycoses 1994;37:187-90.
14. Karlowsky JA, Zhanel GG, Klym KA, Hoban DJ, Kabani AM. Candidemia in a Canadian tertiary care hospital from 1976 to 1996. Diagn Microbiol Infect Dis 1997;29:5-9.
15. Macphail GL, Taylor GD, Buchanan-Chell M, Ross C, Wilson S, Kureishi A. Epidemiology, treatment and outcome of candidemia: A five-year review at three Canadian hospitals. Mycoses 2002;45:141-5.
16. St-Germain G PR, Laverdiere M. Epidemiology and antifungal susceptibility of bloodstream *Candida* isolates in Quebec, Canada: Report of 453 case from 2003-2005. Can J Infect Dis Med Microbiol 2008;19:55-62.
17. National Committee for Laboratory Standards. Reference method for broth dilution antifungal susceptibility of yeasts. Approved Standards M27-A 1997; National Committeee for Laboratory Standards, Wayne, PA.
18. Laupland KB, Gregson DB, Church DL, Ross T, Elsayed S. Invasive *Candida* species infections: A 5 year population-based assessment. J Antimicrob Chemother 2005;56:532-7.
19. Al-Rawahi G, Roscoe D. Candidemia: epidemiology, risk factors,and incidence of mixed candidemia in a Canadian tertiary health centre. Can J Infect Dis Med Microbiol 2008;19:113.
20. Kuhn DM, Mikherjee PK, Clark TA, et al. *Candida parapsilosis* characterization in an outbreak setting. Emerg Infect Dis 2004;10:1074-81.
21. Clark TA, Slavinski SA, Morgan J, et al. Epidemiologic and molecular characterization of an outbreak of *Candida parapsilosis* bloodstream infections in a community hospital. J Clin Microbiol 2004;42:4468-72.
22. Almirante B, Rodriguez D, Park BJ, et al. Epidemiology and predictors of mortality in cases of *Candida* bloodstream infection: Results from population-based surveillance, barcelona, Spain, from 2002 to 2003. J Clin Microbiol 2005;43:1829-35.
23. Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin Infect Dis 1997;24:1122-8.
24. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: Evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis 2000;181:309-16.
25. Wingard JR. Importance of *Candida* species other than *C albicans* as pathogens in oncology patients. Clin Infect Dis 1995;20:115-25.
26. Lin MY, Carmeli Y, Zumsteg J, et al. Prior antimicrobial therapy and risk for hospital-acquired *Candida glabrata* and *Candida krusei* fungemia: A case-case-control study. Antimicrob Agents Chemother 2005;49:4555-60.
27. Kartsonis N, Killar J, Mixson L, et al. Caspofungin susceptibility testing of isolates from patients with esophageal candidiasis or invasive candidiasis: Relationship of MIC to treatment outcome. Antimicrob Agents Chemother 2005;49:3616-23.
28. Hernandez S, Lopez-Ribot JL, Najvar LK, McCarthy DI, Bocanegra R, Graybill JR. Caspofungin resistance in *Candida albicans*: Correlating clinical outcome with laboratory susceptibility testing of three isogenic isolates serially obtained from a patient with progressive *Candida* esophagitis. Antimicrob Agents Chemother 2004;48:1382-3.
29. Laverdiere M, Lalonde RG, Baril JG, Sheppard DC, Park S, Perlin DS. Progressive loss of echinocandin activity following prolonged use for treatment of *Candida albicans* oesophagitis. J Antimicrob Chemother 2006;57:705-8.
30. Jensen J, Munoz P, Guinea J, Rodriguez-Creixems M, Pelaez T, Bouza E. Mixed fungemia: Incidence, risk factors, and mortality in a general hospital. Clin Infect Dis 2007;44:e109-14.