ORIGINAL ARTICLE

Epidemiology and antifungal susceptibility of bloodstream *Candida* isolates in Quebec: Report on 453 cases between 2003 and 2005

Guy St-Germain BSc[1], Michel Laverdière MD[2], René Pelletier MD[3], Pierre René MD[4], Anne-Marie Bourgault MD[5], Claude Lemieux MD[5], Michael Libman MD[4]

G St-Germain, M Laverdière, R Pelletier, et al. Epidemiology and antifungal susceptibility of bloodstream *Candida* isolates in Quebec: Report on 453 cases between 2003 and 2005. Can J Infect Dis Med Microbiol 2008;19(1):55-62.

BACKGROUND: Between May 2003 and April 2005, a population-based surveillance of *Candida* bloodstream infections was conducted in Quebec. A total of 453 episodes of candidemia (464 yeast isolates) from 54 participating hospitals were studied.

RESULTS: The annual incidence rate was three per 100,000 population. Global hospital mortality was 38%. The most common predisposing factors were the presence of an intravascular catheter (80%), use of antibacterial therapy (67%), stay in an intensive care unit (49%), use of parenteral nutrition (32%) and intra-abdominal surgery (31%). Fluconazole alone or in association with other antifungals was used for treatment in over 80% of cases. *Candida albicans* comprised 62% of isolates, followed by *Candida glabrata* (17%), *Candida parapsilosis* (9%), *Candida tropicalis* (5%), *Candida lusitaniae* (3%) and *Candida krusei* (3%). Of the 288 *C albicans* isolates, seven (2%) were resistant to flucytosine, one to fluconazole and none to itraconazole or voriconazole. Of the 75 non-*C albicans* species isolates with reduced susceptibility to fluconazole (minimum inhibitory concentration [MIC] 16 µg/mL or greater), none were susceptible to itraconazole (MIC 0.12 mg/L or lower), whereas 71 (95%) were susceptible to voriconazole (MIC 1 µg/mL or lower). However, only five of 12 (42%) fluconazole-resistant isolates were susceptible to voriconazole. Posaconazole, ravuconazole and caspofungin displayed a broad spectrum of activity against these isolates, with MICs of 1 mg/L or lower in 56%, 92% and 100% of isolates, respectively. Overall, a correlation (r^2>0.87) was observed among increasing fluconazole MICs and the geometric mean MICs of itraconazole, voriconazole, posaconazole and ravuconazole.

CONCLUSIONS: These surveillance results when compared with those of the 1993 to 1995 survey confirm little variation in the distribution of species causing invasive *Candida* infection over a 10-year period in Quebec, as well as the continuous excellent overall in vitro activity of fluconazole.

Key Words: *Antifungal resistance; Candidemia; Surveillance*

Épidémiologie des candidémies et leur sensibilité aux antifongiques : Rapport de 453 cas recensés entre 2003 et 2005 au Québec

HISTORIQUE : Entre mai 2003 et avril 2005, une surveillance des cas de candidémie dans la population a été réalisée au Québec. En tout, 453 épisodes de candidémie (464 isolats de levures) provenant de 54 hôpitaux participants ont été analysés.

RÉSULTATS : L'incidence annuelle a été évaluée à trois (3) cas par 100 000 de population. La mortalité hospitalière globale a été de 38 %. Les principaux facteurs prédisposants étaient la présence d'un cathéter intravasculaire (80 %), l'utilisation d'antibactériens (67 %), un séjour en unité de soins intensifs (49 %), la nutrition parentérale (32 %) et la chirurgie abdominale (31 %). Le fluconazole, en monothérapie ou associé à d'autres antifongiques, a été utilisé pour le traitement de plus de 80 % des cas. *Candida albicans* représentait 62 % des isolats, suivi de *Candida glabrata* (17%), *Candida parapsilosis* (9 %), *Candida tropicalis* (5 %), *Candida lusitaniæ* (3 %) et *Candida krusei* (3 %). Parmi les 288 isolats de *C. albicans*, sept (2 %) se sont révélés résistants à la flucytosine, un au fluconazole et aucun n'a été résistant à l'itraconazole ou au voriconazole. Parmi les 75 isolats non *albicans* moins sensibles au fluconazole (concentrations minimales inhibitrices [CMI] 16 µg/mL ou plus), aucun n'a été sensible à l'itraconazole (CMI 0,12 mg/L ou moins), tandis que 71 (95 %) ont été sensibles au voriconazole (CMI 1 µg/mL ou moins). Par contre, seulement cinq des isolats résistants au fluconazole sur 12 (42 %) se sont révélés sensibles au voriconazole. Le posaconazole, le ravuconazole et la capsofungine ont manifesté un large spectre d'activité contre ces isolats, avec des CMI de 1 mg/L ou moins chez 56 %, 92 % et 100 % des isolats, respectivement. Dans l'ensemble, on a pu observer une corrélation (r^2 > 0,87) entre les CMI croissantes du fluconazole et les moyennes géométriques des CMI de l'itraconazole, du voriconazole, du posaconazole et du ravuconazole.

CONCLUSIONS : Comparativement aux résultats de la surveillance exercée entre 1993 à 1995, cette analyse confirme que la distribution des espèces de *Candida* responsables d'infections invasives a peu varié au Québec en dix ans et que le fluconazole conserve une excellente activité globale *in vitro*.

The increasing incidence of nosocomial fungal infections observed over the past decades is mainly caused by the growing population of patients undergoing treatment for severe underlying medical conditions associated with concomitant use of antibiotics and intensive care support (1,2). A *Candida* bloodstream infection carries a significant risk of death, and strongly impacts on the length of hospital stay and the cost associated with treatment (3-5). Several surveillance programs have been set up worldwide to study the distribution of invasive *Candida* species and their susceptibility to antifungals (6-9). Population-based studies (6-8,10-13) in North America and Europe have reported annual incidence rates ranging between two to 10 per 100,000 population. In Canada, there are a paucity of surveillance data confounded by

[1]*Laboratoire de santé publique/Institut national de santé publique du Québec;* [2]*Hôpital Maisonneuve-Rosemont;* [3]*Centre hospitalier universitaire de Québec;* [4]*McGill University Health Center;* [5]*Centre hospitalier de l'Université de Montréal, Québec City, Québec*

Correspondence: Mr Guy St-Germain, Laboratoire de santé publique du Québec, 20045 chemin Sainte-Marie, Sainte-Anne-de-Bellevue, Québec H9X 3R5. Telephone 514-457-2070, fax 514-457-6346, e-mail guy.st-germain@inspq.qc.ca

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the fact that most studies have been performed in single centres (14-17). The present study follows in the footsteps of two previous studies (18,19) performed in 1985 and 1996 in the province of Quebec. The main objectives were to provide insight into the incidence, species distribution and resistance profiles of *Candida* species causing bloodstream infections in a large unselected population, and to detect variations over the years.

PATIENTS AND METHODS

Surveillance

The data were collected over the course of a two-year surveillance program between May 2003 and April 2005. Strains (one strain per species per patient) of *Candida* isolated from blood in hospital laboratories throughout Quebec were sent to the provincial reference laboratory. An episode was defined as a single positive blood culture with any *Candida* species; candidemias occurring in the same patient more than 30 days after an initial positive episode were considered to be a new episode. Demographic and clinical data were recorded on a standardized case report form and included age, sex, site of isolation, infectious diagnosis, underlying conditions, predisposing factors, history of exposure to antifungal agents pre- and postdetection of the isolates, central venous catheter withdrawal and culture, and clinical outcome.

Organism identification

Candida albicans and *Candida dubliniensis* isolates were recognized by germ tube analysis and growth characteristics on CHROMagar *Candida* medium (Dalynn Biologicals Inc, Canada). Differentiation of *C dubliniensis* from *C albicans* was initially performed on Staib agar, and was later confirmed by biochemical tests and sequencing of the D1/D2 region of large subunit 28S ribosomal RNA (20). Non-*C albicans-dubliniensis* isolates were identified by morphology evaluation on cornmeal Tween 80 agar, carbohydrate assimilation tests using API ID 32 C strips (bioMérieux Inc, USA), urease activity, sugar fermentation and supplemental tests when needed.

Drugs

Amphotericin B (Bristol-Myers Squibb, USA), flucytosine (Sigma-Aldrich Inc, USA), fluconazole (Pfizer Canada), itraconazole (Janssen Pharmaceutica, USA), voriconazole (Pfizer Canada), ravuconazole (Bristol-Myers Squibb, USA), posaconazole (Schering-Plough, USA) and caspofungin (Merck-Frosst Canada) were obtained from their respective manufacturers as standard powders. Diluted concentrations were distributed in flat-bottomed, 96-well microdilution plates and stored in plastic bags at –70°C until needed.

Susceptibility testing

Testing was performed at the Laboratoire de santé publique du Québec (Quebec City, Quebec), by a broth microdilution method as described in procedure M27-A2 of the Clinical and Laboratory Standards Institute (CLSI), with minor modifications (21). In each well, 50 µL of inoculum were added to 50 µL of broth containing the antifungals, for a total volume of 100 µL (CLSI method recommends 200 µL). The smaller volume was used to avoid any spillage or well-to-well contamination, while mechanically agitating the plates before reading. The culture media used were RPMI 1640 for flucytosine, the azoles and caspofungin, and M3 broth supplemented with 2% glucose for amphotericin B (21,22). Inhibitory concentrations were determined visually and spectrophotometrically, after both 24 h and 48 h of incubation in ambient air at 35°C (18). Before reading at both 24 h and 48 h, the plates were agitated for 2 min at 900 rpm with a shaker (SLT Lab Instruments, model EAS 2/4, Grödig, Austria). They were then examined with a reading mirror and the growth in each well was compared with the growth control well. Amphotericin B minimum inhibitory concentrations (MICs) were determined as 100% inhibition. For all other drugs, the MICs corresponded to the concentration at which a prominent decrease in growth relative to the growth control well was observed. Furthermore, the optical density of the growth in each well was determined with the use of an automatic plate reader set at 495 nm (Pasteur Diagnostic LP400, Adil Instruments, France). The MIC for amphotericin B was determined as the lowest drug concentration with an optical density corresponding to lower than 0.01 in turbidity or to a 50% decrease in turbidity compared with that of the growth control. In the present report, visually read MICs were given precedence over spectrophotometric results. Spectrophotometrically measured MICs were used to establish a cut-off point when trailing growth occurred. The MICs reported in the present study are those read at 48 h of incubation for all antifungals, with the exceptions of amphotericin B and caspofungin which were read at 24 h (22-24). Quality control was performed by testing *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 with each set of clinical isolates (21). *Candida lusitaniae*, ATCC 200950 (5W31), was also tested repeatedly as an indicator of amphotericin B resistance (22,25).

Analysis of results

The rate of incidence was based on a total population of 7.5 million, as determined for 2004 by Statistics Canada estimates. Interpretive breakpoints were applied only to flucytosine, itraconazole, fluconazole and voriconazole following CLSI recommendations (21,26). Guidelines for amphotericin B, ravuconazole, posaconazole and caspofungin have not yet been established. Relations between proportions were analyzed by χ^2 tests using Epi Calc 1.02 (Centers for Disease Control and Prevention and World Health Organization). A two-sided P value of less than 0.05 was used to determine statistical significance. The correlation coefficient, r^2, used to measure the relationship among MICs of fluconazole and geometric mean MICs of voriconazole, itraconazole, posaconazole and ravuconazole, was calculated with Excel (Microsoft Corporation, USA) functions, using the $\log_{10}$ of the MICs.

RESULTS

Patient population and clinical data

A total of 453 episodes of candidemia were reported through the participation of 54 hospital laboratories. These laboratories included all the institutions offering services in medical mycology in Quebec. Thirteen of these were university-affiliated and contributed 55% of all specimens. Overall, the number of isolates ranged from one to 56 per institution, with a median number of four isolates. The average annual incidence of candidemia for the province of Quebec was three cases per 100,000 population. Bloodstream infections were diagnosed in 240 male and 213 female patients, with ages ranging from one day to 97 years (mean of 57.5 years, median of 62.4 years). Clinical data questionnaires were available for 361 patients (Table 1). The crude mortality rate was 37.6% (115 of 306). Major predisposing factors were central venous catheter 79.9%

(266 of 333), antimicrobial therapy 67.3% (224 of 333) and stay in intensive care unit 49.2% (164 of 333). Overall, candidemias were treated with fluconazole alone or in combination in 80.1% of cases, while amphotericin B and caspofungin were used in 23.2% and 19.5% of patients, respectively. Among the 272 patients known to have received antifungal therapy, fluconazole monotherapy was used in 62.1% of cases. Fluconazole had been administered to 7.8% of patients within the 30 days preceding the onset of candidemia. Forty-seven patients were reported as not having been treated with any antifungal agent; the mortality rate in this group was 60.5%.

Etiology

A total of 464 *Candida* isolates were received for analysis and the overall distribution of species is shown in Table 2. The four most frequently isolated species were *C albicans* (62%), *Candida glabrata* (17%), *C parapsilosis* (9%) and *Candida tropicalis* (4.5%). Two different *Candida* species were isolated from the same patient in 11 of the 453 cases. Two patients had a recurrent episode. The frequency of isolation of *C parapsilosis* decreased with age, from 19.4% in infants younger than one year of age to 8.0% in patients 65 years of age or older. However, this difference did not reach statistical significance ($P>0.05$). In contrast, *C glabrata* was seen less often in neonates (3.2%) than in the elderly (21.6%) ($P<0.05$). Notably, nine of 13 isolates of *C lusitaniae* (69.2%) were from these two age groups. Among the *Candida* isolates recovered from the 25 patients treated with fluconazole before developing candidemia, nine (36%) were *C albicans*, six (24.0%) *C glabrata*, six (24.0%) *C krusei*, three (12.0%) *C parapsilosis* and one (4.0%) *C tropicalis*. Three (0.6%) isolates were identified as *C dubliniensis* in the course of the present study.

Susceptibility to antifungals

All 464 isolates were tested for susceptibility to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, ravuconazole, posaconazole and caspofungin (Table 2). Given the testing conditions used in the present study, resistance to amphotericin B appeared to be rare. MICs 2 mg/L or greater determined after 24 h of incubation were observed in one isolate each of *C albicans*, *C krusei* and *C lusitaniae*. Only one of 13 isolates of *C lusitaniae*, a recognized amphotericin B-resistant species, had a MIC of 2 mg/L or greater. Resistance to flucytosine was mainly observed in isolates of *C krusei* (8.3%) and *C tropicalis* (9.5%).

A very large proportion (98.9%) of *C albicans*, *C parapsilosis* and *C tropicalis* isolates were susceptible to fluconazole. All *C krusei* strains were considered resistant to this drug irrespective of MIC results (21). Seven (1.6%) isolates of *Candida* species, other than *C krusei*, were resistant to fluconazole (MICs 64 mg/L or greater). These included four isolates of *C glabrata* and one each of *C albicans*, *C tropicalis* and *Candida pelliculosa*. The *C albicans* strain originated from an AIDS patient previously treated with fluconazole, before the onset of candidemia. The *C tropicalis* strain was isolated from a patient during a second episode of candidemia, the first of which had been treated with fluconazole two months earlier. Another 58 *Candida* species isolates (one isolate each of *C albicans*, *C parapsilosis* and *Candida guilliermondii* and 55 *C glabrata*), other than *C krusei* exhibited fluconazole MICs of 16 mg/L to 32 mg/L. Both isolates of *C albicans* and *C parapsilosis* were from patients exposed to fluconazole before the diagnosis of candidemia. Excluding two isolates of *C tropicalis* exhibiting a 'low-high'

TABLE 1
Demographic and clinical characteristics of candidemia cases

Characteristic	n (%)
Age, years (n=453)	
<1	31 (6.8)
1–18	9 (2.0)
19–49	80 (17.7)
50–64	120 (26.5)
≥65	213 (47.0)
Fever/chills/hypotension (n=248)	224 (90.3)
Underlying disease and conditions (n=282)	
Diabetes mellitus	59 (20.9)
Cancer	52 (18.4)
Hematological malignancy	27 (9.6)
Neonate	13 (4.6)
Organ diseases	
Liver	11 (3.9)
Pulmonary	11 (3.9)
Cardiac	15 (5.3)
Renal	17 (6.0)
Gastrointestinal	33 (11.7)
Metabolic/autoimmune	13 (4.6)
HIV infection	5 (1.8)
Multiple trauma	3 (1.1)
Intravenous drug user	4 (1.4)
Risk factor and intervention (n=333)	
Central venous catheter	266 (79.9)
Antimicrobial therapy	224 (67.3)
Intensive care unit at time of diagnosis	164 (49.2)
Total parenteral nutrition	106 (31.8)
Intra-abdominal surgery	104 (31.8)
Corticosteroid therapy	61 (18.3)
Ablative chemotherapy	55 (16.5)
Recent surgery	45 (13.5)
Dialysis	6 (1.8)
Hematopoietic stem cell transplantation	6 (1.8)
Organ transplantation	4 (1.2)
Antifungal treatment before onset of candidemia (n=321)	
Fluconazole	25 (7.8)
Itraconazole	1 (0.3)
Antifungal treatment after onset of candidemia (n=272)	
Fluconazole	218 (80.1)
Amphotericin B (lipid formulation included)	63 (23.2)
Caspofungin	53 (19.5)
None	47 (17.3)
Central venous catheter (n=333)	
Present	266 (79.9)
Removed	221 (83.1)
Not removed	34 (12.8)
Cultured	198 (89.6)
Positive	95 (48.0)
Evolution	
Control blood cultures negative (n=224)	208 (92.9)
Resolution of signs and symptoms (n=257)	213 (82.9)
Mortality (n=306)	115 (37.6)

TABLE 2
Antifungal susceptibilities of 464 bloodstream *Candida* isolates

	Number of isolates with minimum inhibitory concentration (MIC; mg/L) of:																	
Antifungal agent	**0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**	**S-DD/I* (%)**	**R† (%)**
Candida albicans (n=288)																		
Amphotericin B	–	–	–	1	25	122	122	17	1	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	31	151	56	16	22	2	1	2	–	–	7	–	–	0.7	2.4
Fluconazole	–	–	–	–	–	268	13	1	1	2	1	–	1	1	–	–	0.3	0.3
Itraconazole	2	79	150	42	12	1	2	–	–	–	–	–	–	–	–	–	1.0	0.0
Voriconazole	246	27	11	2	–	2	–	–	–	–	–	–	–	–	–	–	0.0	0.0
Ravuconazole	270	10	–	5	–	2	–	–	–	–	–	–	–	–	–	–	–	–
Posaconazole	27	117	97	32	–	3	1	–	–	–	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	29	184	72	3	–	–	–	–	–	–	–	–	–	–	–
Candida glabrata (n=78)																		
Amphotericin B	–	–	–	–	2	7	35	34	–	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	54	23	–	1	–	–	–	–	–	–	–	–	–	0.0	0.0
Fluconazole	–	–	–	–	–	1	1	1	2	5	9	43	12	3	1	–	70.5	5.1
Itraconazole	–	–	–	–	2	1	10	33	17	9	6	–	–	–	–	–	14.1	83.3
Voriconazole	1	–	2	2	4	10	34	21	3	1	–	–	–	–	–	–	3.8	1.3
Ravuconazole	1	1	2	2	7	12	21	27	3	2	–	–	–	–	–	–	–	–
Posaconazole	–	–	–	–	5	2	9	24	33	2	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	–	33	35	10	–	–	–	–	–	–	–	–	–	–	–
Candida parapsilosis (n=43)																		
Amphotericin B	–	–	1	5	15	19	3	–	–	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	13	25	2	1	1	–	–	–	1	–	–	–	–	2.3	0.0
Fluconazole	–	–	–	–	–	1	8	14	17	1	1	–	1	–	–	–	2.3	0.0
Itraconazole	–	2	2	7	19	11	2	–	–	–	–	–	–	–	–	–	30.2	0.0
Voriconazole	3	7	11	17	3	1	1	–	–	–	–	–	–	–	–	–	0.0	0.0
Ravuconazole	6	12	17	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Posaconazole	2	1	3	20	16	1	–	–	–	–	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	–	2	11	29	–	–	–	–	–	–	–	–	–	–	–
Candida tropicalis (n=21)																		
Amphotericin B	–	–	–	–	7	8	5	1	–	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	5	4	3	4	1	2	–	–	–	1	1	–	–	0.0	9.5
Fluconazole	–	–	–	–	–	3	3‡	5‡	7	2	–	–	–	1	–	–	0.0	4.8
Itraconazole	–	–	2	5	8	1‡	2	3	–	–	–	–	–	–	–	–	14.3	14.3
Voriconazole	–	1	5‡	5	4	3	2	–	–	1	–	–	–	–	–	–	0.0	4.8
Ravuconazole	6	4	2‡	3	1	–	2	1	2	–	–	–	–	–	–	–	–	–
Posaconazole	–	1	4‡	8	1	3	2	1	–	–	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	2	8	10	1	–	–	–	–	–	–	–	–	–	–	–
Candida lusitaniae (n=13)																		
Amphotericin B	–	–	–	–	–	2	5	5	1	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	13	–	–	–	–	–	–	–	–	–	–	–	–	0.0	0.0
Fluconazole	–	–	–	–	–	1	2	7	3	–	–	–	–	–	–	–	0.0	0.0
Itraconazole	–	–	–	2	6	2	3	–	–	–	–	–	–	–	–	–	38.5	0.0
Voriconazole	8	3	1	1	–	–	–	–	–	–	–	–	–	–	–	–	0.0	0.0
Ravuconazole	2	5	5	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Posaconazole	–	–	4	8	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	–	–	4	9	–	–	–	–	–	–	–	–	–	–	–
Candida krusei (n=12)																		
Amphotericin B	–	–	–	–	–	–	4	7	1	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	–	–	–	–	–	–	–	4	7	1	–	–	–	91.7	8.3
Fluconazole	–	–	–	–	–	–	–	–	–	–	–	1	7	3	1	–	–	100.0§
Itraconazole	–	–	–	–	–	1	8	2	1	–	–	–	–	–	–	–	75.0	25.0
Voriconazole	–	–	–	–	–	4	7	1	–	–	–	–	–	–	–	–	0.0	0.0
Ravuconazole	–	–	1	1	2	5	3	–	–	–	–	–	–	–	–	–	–	–

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TABLE 2 – CONTINUED
Antifungal susceptibilities of 464 bloodstream *Candida* isolates

	Number of isolates with minimum inhibitory concentration (MIC; mg/L) of:																	
Antifungal agent	**0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**	**S-DD/I* (%)**	**R† (%)**
Candida krusei (n=12) – continued																		
Posaconazole	–	–	–	–	2	4	3	3	–	–	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	–	–	5	7	–	–	–	–	–	–	–	–	–	–	–
Candida dubliniensis (n=3)																		
Amphotericin B	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fluconazole	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–
Itraconazole	–	–	1	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Voriconazole	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ravuconazole	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Posaconazole	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	–	1	2	–	–	–	–	–	–	–	–	–	–	–	–
Other species¶ (n=6)																		
Amphotericin B	–	–	–	–	3	3	–	–	–	–	–	–	–	–	–	–	–	–
Flucytosine	–	–	–	4	2	–	–	–	–	–	–	–	–	–	–	–	–	–
Fluconazole	–	–	–	–	–	–	1	–	1	1	1	1	–	1	–	–	–	–
Itraconazole	–	–	–	–	1	1	2	2	–	–	–	–	–	–	–	–	–	–
Voriconazole	–	1	–	1	2	1	–	1	–	–	–	–	–	–	–	–	–	–
Ravuconazole	–	1	–	2	1	1	1	–	–	–	–	–	–	–	–	–	–	–
Posaconazole	–	–	–	1	1	2	1	1	–	–	–	–	–	–	–	–	–	–
Caspofungin	–	–	–	–	1	2	3	–	–	–	–	–	–	–	–	–	–	–

**Susceptible dose-dependant/intermediate (S-DD/I) breakpoints – fluconazole MIC of 16 mg/L to 32 mg/L, itraconazole 0.25 mg/L to 0.5 mg/L, flucytosine 8 mg/L to 16 mg/L, voriconazole 2 mg/L; †Resistance (R) breakpoint – fluconazole MIC of 64 mg/L or greater, itraconazole 1 mg/L or greater, flucytosine 32 mg/L or greater, voriconazole 4 mg/L or greater; ‡'Low-high' phenomenon observed in one isolate which was categorized as susceptible; §*C krusei *is considered inherently resistant to fluconazole regardless of the MIC obtained; ¶Includes* Candida guilliermondii *(three isolates),* Candida pelliculosa *(two isolates) and* Candida kefyr *(one isolate)*

phenomenon with azoles, a total of 75 isolates (16.6%, including C *krusei*) presented fluconazole MICs of 16 mg/L or greater; of these isolates, 75 (100%) and five (6.7%) exhibited reduced susceptibility to itraconazole (MIC 0.25 mg/L or greater) and voriconazole (MIC 2 mg/L or greater), respectively. Ravuconazole and posaconazole MICs of 2 mg/L or greater were observed for seven (9.3%) and 35 (46.7%) of the 75 isolates, respectively; for posaconazole all 35 isolates were C *glabrata*. Taking into account all 464 isolates, a correlation (r^2>0.87) was observed between increasing fluconazole MICs and the geometric mean MICs for itraconazole, voriconazole, posaconazole and ravuconazole (Figure 1). None of the isolates had a caspofungin MIC exceeding 1 mg/L. Discrepancies in interpretation, when comparing the 24 h and the 48 h MICs for flucytosine, itraconazole, fluconazole and voriconazole, are shown in Table 3.

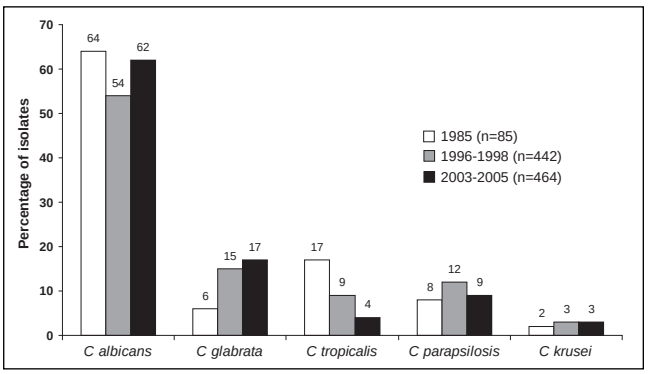

Figure 1) *Variation in the distribution of the five most frequently isolated species of* Candida *according to three Quebec surveillance studies in 1985, 1996 to 1998, and 2003 to 2005*

DISCUSSION

The average annual incidence of candidemia in Quebec during our two-year study period was three per 100,000 population. This rate is comparable with a previous 1992 to 1994 Canadian study (17) that reported incidence rates varying from 1.2 to 5.1 in metropolitan Hamilton, Burlington and Ottawa in the province of Ontario, and in the province of Manitoba. In a more recent population-based study between 1999 and 2004 in Alberta, Laupland et al (15) reported an incidence rate of 2.8 per 100,000 population for central nervous system and bloodstream infections. Overall, rates reported in Canadian studies are lower than those from the United States which are, on average, two to three times higher (7,8,11). The reasons for these discrepancies remain unclear, but could be related to variations in patient population, patient management or differences in study methodologies (7).

In North America and in most countries worldwide, C *albicans* continues to be the single most common species causing candidemia (7,27-29). Compared with the findings of our 1996 to 1998, and 1985 studies, the present results indicate little variation in the frequency of C *albicans* isolates and a slight, but gradual, increase in number of C *glabrata* isolates with a commensurate decrease in C *tropicalis* (Figure 1) (18,19). With the decline in frequency of C *tropicalis*, C *parapsilosis* has become the third most prevalent invasive *Candida* species observed in Quebec. These variations are congruent with the

TABLE 3
Discrepancies in interpretation when comparing 24 h to 48 h minimum inhibitory concentrations for four antifungal agents

Candida species	Flucytosine		Itraconazole		Fluconazole		Voriconazole	
(number of isolates)	Minor*	Very major†	Minor	Very major	Minor	Very major	Minor	Very major
C albicans (n=288)	2	1	1	–	1	–	–	–
C glabrata (n=78)	–	–	5	1	50	–	–	–
C parapsilosis (n=43)	1	–	10	–	–	–	–	–
C tropicalis (n=21)	–	–	4	–	1	2‡	–	2‡
C lusitaniae (n=13)	–	–	2	–	–	–	–	–
C krusei (n=12)	1	–	3	–	6	–	–	–
C dubliniensis (n=3)	–	–	–	–	–	–	–	–
C guilliermondii (n=3)	–	–	1	–	1	–	–	–
C pelliculosa (n=2)	–	–	–	–	–	–	–	–
C kefyr (n=1)	–	–	–	–	–	–	–	–
Total (n=464)	4	1	26	1	59	2	–	2

**Minor error – intermediate or susceptible dose-dependant isolate interpreted as susceptible or, resistant isolate interpreted as intermediate or susceptible dose-dependant; †Very major error – resistant isolate interpreted as susceptible; ‡Low-high isolates – errors attributed to 48 h and not 24 h readings*

observations made in some longitudinal studies (9,29,30,31). *C dubliniensis* is a newly reported species in candidemia surveillance studies. The rate of isolation of 0.6% observed during our study period is similar to those of 0.8% and 0.2% reported by Hajjeh et al (7) and Pfaller et al (31), respectively. It has been suggested that the higher prevalence of *C glabrata* reported in older patients may be institution dependant (7). In our study, *C glabrata* accounted for 21.6% of isolates recovered in elderly patients (65 years of age or older) compared with 3.2% in infants (one year of age or younger); the difference was statistically significant. The 47 bloodstream isolates recovered in older patients originated from 22 different hospitals with diversified patient populations, suggesting that the high prevalence of *C glabrata* in the elderly is likely independent of hospital settings. Our observations on the high prevalence of *C parapsilosis* in the neonatal and infant population concur with those of previous studies (7,8,29).

At the present time, concerns over the inability of the CLSI method M27-A to adequately detect amphotericin B resistance remain, and the clinical significance of in vitro resistance to amphotericin B has yet to be established (21,22,25,32). As reported in other studies, our MIC data indicate little in vitro resistance to this drug (7,33). Decreased susceptibility to amphotericin B was mainly seen in *C glabrata*, *C krusei* and *C lusitaniae* with 90% of the isolates tested inhibited at a MIC of 1 mg/L. These species were previously observed to be innately less susceptible in vitro to amphotericin B, and Goldman et al (34) have reported that a significantly better response to *C krusei* infections was obtained when patients were treated with amphotericin B dosages greater than 1 mg/kg/day compared with patients receiving lower dosages (8,35,36). The proportion of *C albicans* isolates resistant to flucytosine was 2.4% and is essentially the same as that previously observed in our 1996 to 1998 survey (3%) (18). Similar results have been reported in other studies (7,28).

Of the 272 patients evaluable for antifungal treatment after the onset of their candidemia, 80% were treated with fluconazole. The species distribution and susceptibility profiles of the isolates indicate that the widespread use of this antifungal agent has not resulted in the selection of *Candida* species known to be less susceptible to fluconazole, such as *C krusei* and *C glabrata* (Table 2), nor in the increase of azole resistance in other species. This observation is essentially similar to our 1996 to 1998 survey, and agrees with many other reports (18,27-30). Three *C tropicalis* isolates were found to have 48 h MICs exceeding 64 mg/L. One of these exhibited a fluconazole MIC of 32 mg/L at 24 h and was from a solid organ transplant patient exposed to fluconazole before the diagnosis of candidemia. However, the other two isolates, which were from patients who were not exposed previously to azoles, exhibited low MICs of 0.5 mg/L and 1.0 mg/L at 24 h. These two isolates exhibiting 'low-high' MICs at 24 h and 48 h of incubation were reported as susceptible in accordance with recommendations from two in vivo investigations of this phenomenon (37,38). Interestingly, for one of these isolates, the same phenomenon was observed with all four other azoles tested in our survey. Although only four (5.1%) isolates of *C glabrata* were resistant to fluconazole, 59 (75.6%) had reduced susceptibility to this antifungal. Similar results have been reported and support the hypothesis that fluconazole should not be the initial treatment of choice for *C glabrata* candidemia (7). It has been suggested that previous exposure to fluconazole may select for *C krusei* or *C glabrata* candidemia (27). Our results are supportive of this observation. Six *C krusei* strains and six *C glabrata* strains (24.0% each) were isolated from 12 of 25 patients previously exposed to fluconazole, compared with 50 isolates of *C glabrata* (16.9%) and six of *C krusei* (2.0%) isolated from 56 of 296 nonexposed patients.

With regard to itraconazole, none of the 75 *Candida* isolates with reduced susceptibility to fluconazole (MIC 16 mg/L or greater) were susceptible to this drug (MIC 0.12 mg/L or lower). However, Pfaller et al (39) recently suggested that MICs of 1 mg/L or lower may better reflect 'susceptibility' in invasive candidiasis, due to the higher serum concentrations achievable with the new nanocrystal intravenous formulation of itraconazole. Given this new threshold, 58.0% of our isolates with reduced susceptibility to fluconazole would be considered susceptible to itraconazole. Furthermore, our observations are similar to those of Pfaller et al, with all four of our *C glabrata* isolates resistant to fluconazole also resistant to itraconazole, and only three of 12 (25%) isolates of *C krusei* showing complete cross-resistance. Overall, 92.7% of all isolates in our study were inhibited by 1 mg/L of itraconazole or lower.

Our study includes susceptibility testing with new antifungal agents. To date, there have been few population-based studies (15,32) reporting on the activity of these agents against *Candida* bloodstream isolates. As observed in these previous

studies, voriconazole, posaconazole and ravuconazole all displayed a broad spectrum of activity against *Candida* species recovered in our survey. Of the 75 isolates with reduced susceptibility to fluconazole (MIC 16 mg/L or greater), 93% were susceptible to voriconazole. However, as observed also by Pfaller et al (40), decreasing susceptibility to fluconazole was most often associated with decreasing susceptibility to voriconazole and only five of 12 (42%) fluconazole-resistant isolates were susceptible to voriconazole. All C *krusei* isolates were inhibited by 1 mg/L of voriconazole or lower, ravuconazole and posaconazole. Also, 94.9%, 93.6% and 53.3% of isolates of C *glabrata* were inhibited by 1 mg/L or lower of each of these drugs respectively. Among the 11 isolates with fluconazole MICs 64 mg/L or greater, two were resistant to voriconazole (MIC 4 mg/L or greater) and three exhibited MICs 2 mg/L or greater for both ravuconazole and posaconazole. Strong correlations (P>0.95) were observed when the geometric mean MICs of all 464 isolates for voriconazole, itraconazole posaconazole and ravuconazole are categorized according to fluconazole MICs (Figure 2). This does not necessarily indicate a trend toward full cross-resistance, but shows that the mechanisms responsible for increasing fluconazole MICs almost invariably have some impact on other azoles. Caspofungin was active in vitro against all *Candida* species tested in this study. All MICs, including those of isolates resistant to fluconazole, were 1 mg/L or lower. These results concur with those of the population-based studies of Laupland et al (15) and Cuenca-Estrella et al (33), as well as those of a global surveillance study (24,32,41). Our MIC data also shows a shift toward higher values for C *parapsilosis* relative to C *albicans*.

Our survey also provides information on the discrepancies in MICs noted with triazoles when comparing 24 h and 48 h readings. Reading discrepancies between 24 h and 48 h had little impact on the final categorical interpretation for C *albicans* and C *tropicalis*, but resulted in minor errors, mainly with C *glabrata*, C *parapsilosis* and C *krusei*. Overall, 59 minor errors were observed with fluconazole, but this number would be reduced to 11 with 24 h breakpoints set at one dilution lower than the actual 48 h breakpoints. These observations concur with those of Espinel-Ingroff et al (42) who have recently suggested that the reporting of 24 h MICs for azoles should be considered in the next revised version of the CLSIs M27 reference method. The only very major errors observed were with the two 'low-high' C *tropicalis* isolates resistant to fluconazole at 48 h and susceptible at 24 h. Murine models of invasive candidiasis have shown that isolates with such behaviour should be considered susceptible rather than resistant (37,38).

CONCLUSION

The present surveillance program provides a population-based description of candidemia in Quebec, and shows little variation in species distribution and antifungal resistance profiles compared with results from our 1996 to 1998 study. Resistance to fluconazole in C *albicans* remains rare and the frequency of isolation of intrinsically azole-resistant C *krusei* remains low. At the present time, resistance of C *albicans* to fluconazole appears to be essentially transitory and associated with long-term exposure to this drug. In Quebec, the systematic susceptibility testing of *Candida* bloodstream isolates appears to be warranted only in patients previously exposed or undergoing long-term azole treatment. Also, through prompt identification, over 20% of our candidemic isolates would have had been identified as species with known intrinsic resistance or lesser susceptibility to fluconazole.

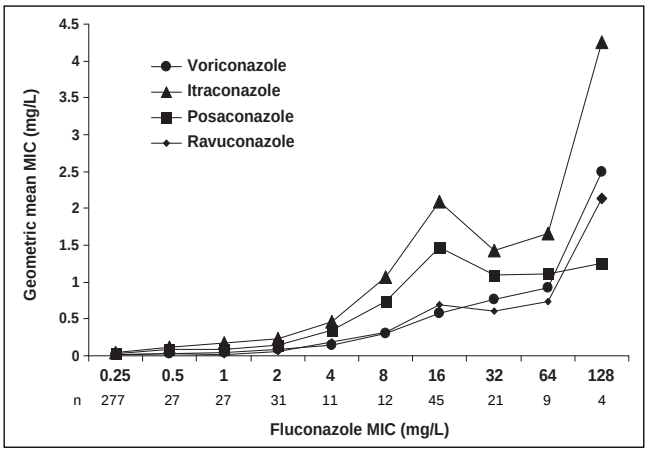

Figure 2) *Geometric mean minimum inhibitory concentrations (MICs) of 464* Candida *species isolates for four azole antifungal agents categorized according to fluconazole MICs*

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