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Species distribution and antifungal susceptibility of invasive *Candida* isolates from Canadian hospitals: results of the CANWARD 2011–16 study

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Objectives: Understanding the epidemiology of invasive *Candida* infections is essential to patient management decisions and antifungal stewardship practices. This study characterized the species distribution and antifungal susceptibilities of prospectively collected isolates of *Candida* species causing bloodstream infections (BSIs) in patients admitted to tertiary care hospitals located in 14 cities across 8 of the 10 Canadian provinces between 2011 and 2016.

Methods: Antifungal susceptibility testing was performed by broth microdilution using CLSI methods, breakpoints and epidemiological cut-off values. DNA sequencing of *fks* loci was performed on all echinocandin-non-susceptible isolates.

Results: *Candida albicans* (49.6%), *Candida glabrata* (20.8%) and *Candida parapsilosis* complex (12.0%) were the most common species out of 1882 isolates associated with BSIs. *Candida tropicalis* (5.2%), *Candida krusei* (4.3%), *Candida dubliniensis* (4.1%), *Candida lusitaniae* (1.4%) and *Candida guilliermondii* (1.1%) were less frequently isolated. Between 2011 and 2016, the proportion of *C. albicans* significantly decreased from 60.9% to 42.1% ($P<0.0001$) while that of *C. glabrata* significantly increased from 16.4% to 22.4% ($P=0.023$). *C. albicans* ($n=934$), *C. glabrata* ($n=392$) and *C. parapsilosis* complex ($n=225$) exhibited 0.6%, 1.0% and 4.9% resistance to fluconazole and 0.1%, 2.5% and 0% resistance to micafungin, respectively. Mutations in *fks* hot-spot regions were confirmed in all nine micafungin non-susceptible *C. glabrata*.

Conclusions: Antifungal resistance in contemporary isolates of *Candida* causing BSIs in Canada is uncommon. However, the proportion of *C. glabrata* isolates has increased and echinocandin resistance in this species has emerged. Ongoing surveillance of local hospital epidemiology and appropriate antifungal stewardship practices are necessary to preserve the utility of available antifungal agents.

Introduction

Understanding the species distribution and antifungal resistance risks associated with invasive *Candida* infections in healthcare institutions is integral to directing appropriate empirical antifungal therapy.[1–3] *Candida* species are the most common fungal pathogens causing nosocomial bloodstream infections (BSIs) and are associated with significant mortality.[4–6] The distribution of *Candida* species causing BSIs is led by *Candida albicans* in most studies, while *Candida glabrata*, *Candida parapsilosis*

complex, *Candida tropicalis* and *Candida krusei* comprise the remaining majority of cases.[6–9] *C. glabrata* is the second most common cause of candidaemia in many regions and is intrinsically less susceptible to fluconazole and other azole agents. Recent reports from Europe, Australia and the USA indicate that candidaemia caused by *C. glabrata* has increased.[4,8,10] Further, reports of echinocandin resistance in *Candida* BSIs in association with poorer patient outcome have been increasingly reported and found almost exclusively in *C. glabrata*.[11–14]

In Canada, the incidence of invasive candidiasis is estimated to be 5.8/100000 population, with *C. albicans*, *C. glabrata* and *C. parapsilosis* as the most common species causing candidaemia.[9,15–17] Previous Canadian surveillance studies of candidaemia isolates from 1999 to 2007 describe very few antifungal resistance concerns, using current breakpoints to interpret their findings,[16–18] and there have been limited Canadian data published since then to aid antifungal therapy management decisions.[19] As part of the CANWARD surveillance programme, this study describes the epidemiology and antifungal susceptibility of a contemporary collection of *Candida* species causing BSIs over a 6 year period in patients admitted to Canadian hospitals.

Materials and methods

Candida isolates

In conjunction with the bacterial objectives of the CANWARD study, isolates of *Candida* species causing BSIs in patients admitted to Canadian tertiary care centres were prospectively collected by clinical microbiology laboratories in 14 cities across 8 of the 10 Canadian provinces from 2011 to 2016 and shipped to a central reference laboratory (Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta) for antifungal susceptibility testing and confirmation of identification using MALDI-TOF. From January to October of each year, participating centres collected up to 10 bloodstream isolates of *Candida* species per month from patients with incident candidaemia (1 per patient). Isolate subcultures were prepared and shipped at room temperature in Amies transport medium. Patient age, gender and hospital admission location (general medicine, ICU, surgical unit, emergency care or clinic) were submitted with each isolate. In total, 1882 isolates of *Candida* species were received and available for antifungal susceptibility testing.

Antifungal susceptibility testing

In vitro antifungal susceptibility testing of *Candida* isolates to polyenes (amphotericin B), azoles (fluconazole, voriconazole, itraconazole and posaconazole) and echinocandins (caspofungin and micafungin) was performed by the reference broth microdilution (BMD) method described in the CLSI standard M27.[20] MICs for all antifungal agents were determined after 24 h of incubation. Weekly quality control of all BMD testing was verified using *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 strains according to M27.[20] MIC breakpoints and interpretive categories [susceptible, intermediate, susceptible-dose dependent (S-DD) and resistant] were applied according to the CLSI M60 document,[21] with the exception of *C. glabrata* and caspofungin, which is known to experience significant *in vitro* test variability.[22,23] Epidemiological cut-off values (ECVs) and interpretations of WT and non-WT, as described in the CLSI M59 document, were used for *Candida* species lacking CLSI-defined breakpoints.[24]

Molecular analysis

PCR and sequencing of the *fks* loci, which encode the 1,3-β-D-glucan synthase inhibitor subunits, were performed on *Candida* species with echinocandin MICs that were not susceptible. This was accomplished using a previously described method and panel of oligonucleotide primers designed to target *fks* hot-spot regions.[25] Briefly, DNA was extracted using rapid lysis, PCR was performed for each *fks* hot spot and locus, and amplicons were sequenced using the ABI 3500. The sequences were translated using https://web.expasy.org/translate/ and compared with reference sequences in the UniProt Knowledge Database. Molecular analysis of azole resistance was beyond the scope of this study.

Statistical analysis

The Cochran–Armitage test was used to determine significant trends in the proportional distribution of *Candida* species across the duration of the study ($P<0.05$ was considered significant). All analyses were performed using XLSTAT software version 2016.02.28540 (Addinsoft).

Ethics

The CANWARD study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Results

Surveillance and patient characteristics

Candida bloodstream isolates were collected from a total of 18 hospital clinical microbiology laboratories located in 14 cities across 8 of the 10 Canadian provinces. Eight sites participated in all six surveillance years (73.5% of isolates) and three sites participated in five surveillance years (17.0% of isolates). Of the 1882 *Candida* isolates received, annual isolate volumes were 238, 277, 347, 337, 333 and 350 isolates in 2011, 2012, 2013, 2014, 2015 and 2016, respectively. The overall proportion (and annual range) of isolates from the provinces of British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, New Brunswick and Nova Scotia was 12.0% (10.5%–13.8%), 13.9% (11.3%–16.9%), 6.6% (5.8%–7.2%), 12.3% (10.4%–16.3%), 34.1% (25.2%–40.0%), 11.3% (3.3%–21.0%), 2.4% (1.4%–3.8%) and 7.4% (4.2%–9.7%), respectively. In hospital, the total proportion (and annual range) of *Candida* BSIs associated with patients admitted to medicine wards, ICUs, surgical wards, emergency care units and clinics was 42.8% (38.1%–46.6%), 31.7% (27.7%–35.7%), 13.0% (11.4%–16.8%), 8.2% (7.4%–9.7%) and 3.6% (2.9%–5.2%), respectively (data not provided for 15 cases). The proportion of *Candida* BSIs in adults (>16 years) was 45.1% female ($n=803$) to 54.9% male ($n=976$) while the proportion in paediatric patients was 53.5% female ($n=53$) to 46.5% male ($n=46$); gender was not provided for three adult patients and one paediatric patient. The median age (all patients) was 58 (no age provided for 26 cases) with 56.8% patients aged 17–64 years ($n=1055$) and 37.9% ($n=705$) aged ≥65. The median age of 100 paediatric patients (4 days to 16 years) was 3 years.

Species distribution

Overall, *C. albicans* (49.6%, $n=934$), *C. glabrata* (20.8%, $n=392$) and *C. parapsilosis* complex (12.0%, $n=225$) were the most common species of the 16 species identified between 2011 and 2016 (Figure 1). *C. tropicalis* (5.2%), *C. krusei* (4.3%), *Candida dubliniensis* (4.1%), *Candida lusitaniae* (1.4%) and *Candida guilliermondii* (1.1%) were less frequently isolated, while *Candida inconspicua*, *Candida kefyr*, *Candida norvagensis*, *Candida pelliculosa*, *Candida*

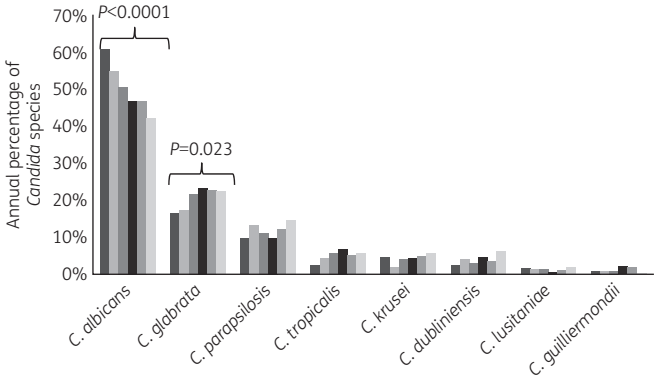

Figure 1. Temporal distribution of *Candida* species causing BSIs in patients admitted to Canadian hospitals between 2011 and 2016. Shaded bars represent the proportion (%) of each species submitted relative to the total number of isolates for each surveillance year, which are ordered from 2011 to 2016, left to right, for each species. Statistically significant trends in the proportional distribution of *C. albicans* and *C. glabrata* across the study period are indicated with respective *P* values (Cochran–Armitage test).

sake, *Candida sphaerica* and *Candida utilis* as agents of BSIs were rare (1.4%). Over the 6 year study period, the annual proportion of *C. albicans* significantly decreased from 60.9% to 42.1% ($P<0.0001$) while that of *C. glabrata* significantly increased from 16.4% to 22.4% ($P=0.023$), as shown in Figure 1. These temporal changes were not affected when only the eight sites that consistently participated in six surveillance years were considered. Significant trends were not identified for any of the other *Candida* species. As shown in Table 1, the rank order of *C. albicans*, *C. glabrata* and *C. parapsilosis* complex did not change based on patient ward location. The rank order of these common species was also observed for the 11 individual hospital sites that submitted isolates for at least five of the surveillance years. However, between medicine, ICU and surgical patients, representing 87.5% of *Candida* isolates, there was an increased proportion of *C. glabrata* and *C. parapsilosis* complex recovered from surgical and medicine patients, respectively.

MIC distribution and antifungal activity

The majority of *C. albicans* isolates were susceptible to fluconazole, with <1% resistance detected (6/934 isolates) and a normal MIC distribution (modal MIC, 0.12 mg/L) well below the susceptible MIC breakpoint. Similarly, 99% of *C. glabrata* were S-DD (MIC, ≤32 mg/L) to fluconazole and 4/392 were fluconazole resistant. Within the S-DD population, 87.8% of *C. glabrata* had fluconazole MICs ≤4 mg/L; annual modal MICs ranged between 1 and 4 mg/L with no indication of temporal changes. Voriconazole showed good activity against *C. glabrata* (no CLSI breakpoint) using the established ECV (≤0.25 mg/L), which identified 27/392 (6.9%) isolates as non-WT; 4/27 were fluconazole resistant and 26/27 had fluconazole MICs ≥4 mg/L. Eleven of 225 *C. parapsilosis* isolates (4.9%) were resistant to fluconazole, three of which were intermediate to voriconazole. All other *C. parapsilosis* were susceptible to voriconazole (Table 2). *C. krusei* isolates ($n=81$) were poorly inhibited by fluconazole (modal MIC, 8 mg/L; MIC_{90}, 16 mg/L), as expected, but were completely susceptible to voriconazole (MIC_{90}, 0.25 mg/L).

The echinocandins were also highly active against most *Candida* species. *C. albicans* was 98.8% and 99.9% susceptible to caspofungin and micafungin, respectively; all non-susceptible isolates tested in the intermediate category and no resistant isolates were identified. Echinocandin non-susceptibility (intermediate or resistant) was detected in nine *C. glabrata* isolates (2.3%) using micafungin as a surrogate agent for the class. Five isolates were resistant, with MIC values of 0.5–4 mg/L, and four isolate MICs were interpreted as intermediate (Table 2). These *C. glabrata* isolates were recovered from adult patients, except for a single paediatric case, in four of the surveillance years from four different healthcare centres. Molecular testing of *fks* loci identified mutations in the conserved hot-spot regions of all nine *C. glabrata* (Table 3). Three of these *C. glabrata* showed reduced susceptibility to fluconazole, exhibiting MIC values of 64 mg/L (resistant), 32 mg/L (S-DD) and 16 mg/L (S-DD). Echinocandin resistance was not detected in *C. parapsilosis*, *C. tropicalis*, *C. krusei* or *C. guilliermondii*, and CLSI breakpoints have not been established for other *Candida* species.

Using the amphotericin B ECV (≤2 mg/L) published for *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*, all isolates for these species were considered WT (Table 2). Similarly, WT MIC values were recorded for fluconazole and micafungin against all isolates of *C. dubliniensis* ($n=78$), *C. lusitaniae* ($n=26$) and *C. guilliermondii* ($n=21$). In the absence of published breakpoints or ECVs, antifungal MIC values for isolates of the rare species could not be interpreted. However, abnormally high MICs were not observed and none of these species is known to express intrinsic resistance.

Discussion

The findings from this prospective laboratory-based surveillance study confirm that the epidemiology of *Candida* species causing BSIs in Canadian hospitals is relatively consistent with the results of other previous Canadian surveillance investigations.[9,18,19] *C. albicans* was the most commonly detected species in each year of surveillance, followed by *C. glabrata* and *C. parapsilosis*, comprising >80% of all isolates. There was a significant shift in the proportion of *C. glabrata* over time, increasing from 16.4% to 22.0% from 2011 to 2016, respectively, and a concomitant decrease in *C. albicans* from 60.9% to 42.1%. The emergence of *C. glabrata* combined with its propensity for resistance to fluconazole and other azole agents is a notable clinical concern. In Australia, the proportion of *C. glabrata* in candidaemic patients almost doubled between 2004 and 2014 (26.7%).[8] In the USA, several large surveillance programmes have independently noted increases in *C. glabrata*-associated candidaemia over the past two decades and collectively verify contemporary BSI isolation rates of 25%–27%.[6,26,27] These same studies report *C. albicans* identification rates of 36%–44.4%. Although we can only speculate on what may be driving this apparent species shift, *C. glabrata* BSIs are associated with prior azole exposure and older patient age, while patients with *C. albicans* BSIs are younger and less likely to have prior antifungal exposure.[6,26,28] The median age of candidaemic patients infected with *C. albicans* and *C. glabrata* in this study was 58 and 59 years, respectively, and remained constant across the study period.

Reported rates of antifungal resistance in *C. albicans* are extremely low[29,30] and our Canadian cohort was no different.

Table 1. Distribution of *Candida* species causing BSIs in hospitalized patients based on ward location, 2011–16

	% Isolates per location				
Species	medicine (*n*=805)	ICU (*n*=596)	surgery (*n*=224)	emergency care (*n*=154)	clinic (*n*=68)
C. albicans	46.7	55.2	53.7	40.9	38.2
C. glabrata	19.3	19.3	25.8	26.0	26.5
C. parapsilosis	13.7	8.7	9.4	18.2	13.2
C. tropicalis	6.7	4.2	3.3	3.3	7.4
C. krusei	5.1	4.9	1.6	2.0	5.9
C. dubliniensis	3.9	4.7	4.1	3.9	4.4
C. lusitaniae	1.6	1.5	0.4	0.7	1.5
C. guilliermondii	1.5	0.5	0.0	3.3	1.5
Other *Candida* species	1.6	1.0	1.7	1.7	1.4

Table 2. Antifungal MIC distributions and susceptibility interpretations of common *Candida* species causing candidaemia in patients admitted to Canadian hospitals between 2011 and 2016

Organism (no. tested)	Antifungal agent	Modal MIC (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	% S (% S-DD)[a]	% R	% WT
C. albicans (934)	amphotericin B	0.25	0.5	≤0.06–1	–	–	100
	fluconazole	0.12	0.25	≤0.06 to >64	99.4	0.6	
	voriconazole	≤0.015	≤0.015	≤0.015 to >16	99.6	0.4	
	posaconazole	≤0.015	0.03	≤0.015 to >16	–	–	99.0
	caspofungin	≤0.008	0.12	≤0.008–0.5	98.8	0	
	micafungin	≤0.008	≤0.008	≤0.008–0.5	99.9	0	
C. glabrata (392)	amphotericin B	0.5	0.5	≤0.06–1	–	–	100
	fluconazole	2	8	≤0.06–64	(99.0)	1.0	
	voriconazole	0.06	0.25	≤0.015–2	–	–	93.1
	posaconazole	0.12	0.5	≤0.015–4	–	–	99.5
	micafungin	≤0.008	0.015	≤0.008–4	97.5	2.5	
C. parapsilosis (225)	amphotericin B	0.5	1	0.12–1	–	–	100
	fluconazole	0.5	1	≤0.06–16	95.1	4.9	
	voriconazole	≤0.015	0.03	≤0.015–0.5	98.7	0	
	posaconazole	≤0.015	0.03	≤0.015–0.5	–	–	99.1
	caspofungin	0.5	1	0.015–2	100	0	
	micafungin	0.5	1	≤0.008–2	100	0	
C. tropicalis (98)	amphotericin B	0.5	1	0.25–1	–	–	100
	fluconazole	0.25	0.5	≤0.06–8	96.9	3.1	
	voriconazole	≤0.015	0.06	≤0.015–2	96.9	2.1	
	posaconazole	≤0.015	0.06	≤0.015–0.5	–	–	98.0
	caspofungin	0.12	0.25	≤0.008–0.5	99.0	0	
	micafungin	≤0.008	0.015	≤0.008–0.12	100	0	

[a]–, no CLSI-published breakpoint.

C. albicans were quite susceptible to the azoles (fluconazole MIC_{90}, 0.25 mg/L) and echinocandins (micafungin MIC_{90}, ≤0.008 mg/L), with only six fluconazole-resistant isolates and a single isolate with intermediate susceptibility to the echinocandins.

The reduced utility of fluconazole against *C. glabrata*, associated with the up-regulation of drug transporter mechanisms,[31,32] and the high rates of resistance consistently identified by ongoing global surveillance studies[29,33–35] have, in part, influenced the role of echinocandins as first-line treatment options for various patient populations with candidaemia.[1,36] Azoles have a role in managing patients that are not critically ill and infected with an azole-susceptible *Candida* isolate.[3] For *C. glabrata* infections, higher doses of fluconazole and voriconazole are recommended, based on low-quality evidence, for azole therapy when isolates are susceptible.[1] Clinicians must be aware, however, that there is no susceptible interpretation available for *C. glabrata* against fluconazole or voriconazole. Rather, all fluconazole MICs ≤32 mg/L are considered S-DD and voriconazole MICs do not correlate with clinical outcome and have no predictive value.[21] Evidence informing clearer guidelines for the appropriate interpretation and use of azoles

Table 3. Molecular analysis of *fks* mutations in *C. glabrata* isolates with reduced susceptibility to echinocandin agents

Year	Province	Micafungin MIC (mg/L)	Micafungin interpretation	*fks1* HS1	*fks1* HS2	*fks2* HS1	*fks2* HS2
2011	Alberta	0.12	intermediate	WT	WT	F659Y	WT
2013	Manitoba	0.12	intermediate	WT	WT	F659-DEL	WT
2013	Ontario	0.12	intermediate	S629P	WT	WT	WT
2013	British Columbia	4	resistant	WT	WT	S663P	WT
2013	British Columbia	0.5	resistant	WT	WT	S663P	WT
2014	Manitoba	1	resistant	S629P	WT	WT	WT
2014	Alberta	2	resistant	S629P	WT	S663P	WT
2016	Alberta	0.12	intermediate	S629P	I1376V	WT	WT
2016	Alberta	0.5	resistant	WT	WT	S663P	WT

HS1, hot spot 1; HS2, hot spot 2.

against *C. glabrata* is truly warranted to optimize azole utility and limit the selection of echinocandin resistance.

In this surveillance, we observed that echinocandin resistance in invasive *C. glabrata* is emerging in Canadian hospitals, from an otherwise susceptible population (micafungin MIC_{90}, 0.015 mg/L), which had not been described in previous Canadian studies.[16–18] In the USA, several studies have recently highlighted significant increases in echinocandin-resistant *C. glabrata* in patients with invasive candidiasis, with rates ranging between 7.8% and 12.3%.[11,12,37] Of equal or greater concern is the discovery that large proportions of echinocandin-resistant *C. glabrata* are also resistant to azoles.[11–13] Co-resistance in this study was only identified in a single *C. glabrata* isolate and echinocandin resistance was infrequent and intermittently distributed across study years and participating centres. The amino acid substitutions conferred by the *fks* mutations detected in our resistant isolates, namely S629P in FKS1 and S663P in FKS2, have been associated with a marked reduction in the activity of the *C. glabrata* 1,3-β-D-glucan synthase (echinocandin target) and resistant MIC values.[38,39] Further, a recent case report of an invasive candidiasis patient infected with one of the *fks* mutant *C. glabrata* strains described in the present study concurs with predisposing risk factors and echinocandin resistance development associated with clinical treatment failure.[40] The evidence to date clearly demonstrates that the relationship of *fks* mutations, resistant MICs and clinical treatment failure in *C. glabrata* candidaemia patients with previous or prolonged exposure to echinocandin therapy is very strong and underscores the importance of appropriate antifungal stewardship measures, in alignment with treatment guidelines, to mitigate further resistance selection.[1,11,41–43]

The level of fluconazole resistance detected in *C. parapsilosis* was comparable to rates recently reported elsewhere.[35] Less is understood about the mechanisms of azole resistance in *C. parapsilosis* but a recent study established a correlation with an alteration in the 14-α-demethylase target and overexpression of an efflux pump.[44] Due to a naturally encoded FKS polymorphism, the potency of echinocandins is reduced against *C. parapsilosis* and, although there are no reports of emerging resistance, their role in patient management is secondary to azoles in various patient groups.[1]

Current evidence highlights that resistance to azoles and echinocandins is more common in *Candida* species other than *C. albicans*, which is attributed, in part, to inherent mechanisms of resistance encoded in these species. Risk factors associated with invasive infections caused by non-*albicans Candida* species and the concomitant selection of resistance are well described but strategies to mitigate these epidemiological changes are limited by the few antifungal class options available for patient management.

In conclusion, our findings demonstrate that the activity of current antifungal agents against *Candida* species causing BSIs in Canadian hospitals is excellent. However, laboratory-based surveillance of BSIs cannot fully capture the extent of *de novo* resistance emergence in the broader context of invasive candidiasis. Ongoing surveillance of the local epidemiology of invasive *Candida* infections must also be integrated with appropriate risk-based susceptibility testing practices and antifungal stewardship programmes.

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Members of CARA

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