J Antimicrob Chemother 2013; **68**: 2847–2851
doi:10.1093/jac/dkt283 Advance Access publication 18 July 2013

Journal of Antimicrobial Chemotherapy

Species distribution and antifungal susceptibility of bloodstream fungal isolates in paediatric patients in Mexico: a nationwide surveillance study

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Received 12 March 2013; returned 28 May 2013; revised 10 June 2013; accepted 13 June 2013

Objectives: To establish the species distribution and *in vitro* susceptibilities of 358 bloodstream fungal isolates from paediatric patients in Mexico.

Methods: Isolates were collected during a 2 year surveillance programme in 14 medical centres in 10 Mexican states. A molecular approach was used to determine the *Candida parapsilosis* species complex. *In vitro* susceptibility to amphotericin B, fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, anidulafungin and micafungin was determined according to CLSI procedures. Species-specific clinical breakpoints for fluconazole, voriconazole and echinocandins were applied.

Results: *Candida* spp. accounted for 98.33% of fungaemias, including 127 *Candida albicans* isolates, 127 *C. parapsilosis* complex isolates (121 *C. parapsilosis sensu stricto*, 4 *Candida orthopsilosis* and 2 *Candida metapsilosis* strains) and 72 *Candida tropicalis* isolates. *C. albicans* and *C. parapsilosis* complex were the species predominant in neonates (48 cases each; 41.02%). *C. parapsilosis* complex was also the predominant species in patients 1 month to <2 years of age ($P=0.007$). In contrast, *C. albicans* was the most frequent species in patients aged 2 to <12 years ($P=0.003$). Antifungal resistance was rare among the subset of isolates. *Candida glabrata* showed the highest resistance rate to amphotericin B (1/9 isolates), fluconazole (1/9 isolates) and itraconazole (2/9 isolates).

Conclusions: The species distribution differed with the age of the patients, with *C. albicans* and *C. parapsilosis* complex being the most commonly isolated species. *C. glabrata* showed the highest resistance rate to amphotericin B, fluconazole and itraconazole. This is the first study of fungaemia episodes in Mexican children.

Keywords: fungaemia, *Candida albicans*, *Candida parapsilosis* complex, *Candida tropicalis*, antifungal *in vitro* susceptibility

Introduction

Fungal bloodstream infections (BSIs) are severe diseases that lengthen hospital stay, have elevated morbidity and mortality and increase medical care costs. Their incidence has recently increased, likely due to an increase in the number of susceptible hosts.[1,2] There are few surveillance studies on the species distribution and susceptibility of causative agents of fungaemia in paediatric patients. *Candida* spp. represent the principal aetiology of fungal BSIs and even though *Candida albicans* was reported as the most common species causing candidaemia, an epidemiological shift towards non-*albicans Candida* species has been reported in various geographical areas.[3,4]

A local surveillance programme in Monterrey, Mexico, showed that the species distribution varied according to the age of the patients.[5] Mexican national programmes studying BSIs in the

paediatric population due to fungi have been limited. As a result, the causative fungal aetiology and antifungal susceptibility patterns of isolates involved in BSIs in tertiary care hospitals in Mexico remain unknown. The recognition of causative agents of BSIs is important, because treatment strategies are often guided by species identification.

Methods

Data collection

The data were collected during a 2 year surveillance programme from January 2010 through December 2011. The study included 14 tertiary-level hospitals in 10 cities in Mexico. Neonates were defined as <1 month of age, infants and toddlers were 1 month to <2 years old, children were aged 2 to <12 years old and adolescents were between 12 and 18 years old. A case was defined as the isolation of a yeast or mould from blood culture in a hospitalized patient. If multiple episodes of fungaemia occurred in the same patient during the study period, the patient was included as a study participant using only the first episode of fungaemia.

Identification of strains

Organisms were identified at the medical institutions by routine procedures and immediately submitted to our laboratory. The isolates were subcultured onto Sabouraud dextrose agar (SDA; Difco, Detroit, MI, USA) for further corroboration of species identification and susceptibility testing. Confirmation of species identification was performed with API 20C AUX strips (bioMérieux, Mexico) and by standard morphological methods such as germ tube assays and microscopic evaluation on corn meal-Tween 80 agar. The molecular identification of *Candida parapsilosis* species complex was performed according to Tavanti *et al*.[6] Type strains ATCC 22019, ATCC 96139 and ATCC 96144 were used as controls for *C. parapsilosis sensu stricto*, *Candida orthopsilosis* and *Candida metapsilosis*, respectively.

All isolates were stored as suspensions in water at room temperature and on agar slants at −20°C until needed. Prior to testing, each isolate was passaged at least twice on SDA plates to check purity and viability.

Antifungals and susceptibility testing

Fluconazole and voriconazole (Pfizer, Inc., New York, NY, USA), itraconazole (Janssen Pharmaceutica, Beerse, Belgium), amphotericin B (Bristol-Myers Squibb, Princeton, NJ, USA) and posaconazole (Merck, Rahway, NJ, USA) were obtained in reagent-grade powder form from their respective manufacturers. Caspofungin, anidulafungin and micafungin were purchased as Cancidas, Eraxis and Mycamine.

Serial 2-fold dilutions of each antifungal agent were prepared according to document M27-A3 of the CLSI.[7] Final dilutions were made in RPMI 1640 with L-glutamine and buffered with 165 mM MOPS (Hardy Diagnostics) for all antifungals. The final concentrations of the drugs ranged from 0.03 to 16 mg/L for amphotericin B, itraconazole, voriconazole and posaconazole; from 0.125 to 64 mg/L for fluconazole; and from 0.015 to 8 mg/L for the three echinocandins. *C. parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as quality control organisms.

The interpretative MIC breakpoints of the assayed antifungals were those suggested by the CLSI M27-A3 document[7] as well as the species-specific clinical breakpoints (SSCBs) of fluconazole, voriconazole and echinocandins.[8–10]

Statistics

The χ^2 test was used for comparison of changes in the species distribution. *P* values of <0.05 were considered significant.

Table 1. Patient characteristics and distribution of fungal aetiology involved in BSIs in 2 years of surveillance in 14 medical centres in Mexico

	Total (%)	*C. albicans*	*C. parapsilosis* complex	*C. tropicalis*	*C. guilliermondii*	*C. glabrata*	*C. rugosa*	*C. utilis*	*C. lusitaniae*	*C. krusei*	*C. pelliculosa*	*C. famata*	*S. cerevisiae*	*T. mucoides*
Age category														
<1 month	117 (32.68)	48 (41.02)	48 (41.02)	9 (7.69)	5 (4.27)	4 (3.40)	1 (0.85)	0	0	0	0	0	1 (0.85)	1 (0.85)
1 month to <2 years	105 (29.32)	32 (30.47)	43 (40.95)	22 (20.95)	2 (1.90)	2 (1.90)	0	1 (0.95)	1 (0.95)	1 (0.95)	0	0	0	1 (0.95)
2 years to <12 years	113 (31.56)	41 (36.28)	29 (25.66)	33 (29.20)	0	3 (2.65)	0	1 (0.88)	1 (0.88)	0	2 (1.76)	0	0	3 (2.65)
12 years to 18 years	23 (6.42)	6 (26.08)	7 (34.43)	8 (34.78)	0	0	0	0	1 (4.34)	0	0	1 (4.34)	0	0
	358	127	127	72	7	9	1	2	3	1	2	1	1	5
Gender														
female	160 (44.69)	58 (45.66)	53 (41.73)	29 (40.27)	4 (2.59)	5 (3.24)	0	2 (1.29)	2 (1.29)	1 (0.65)	2 (1.29)	1 (0.65)	1 (0.65)	2 (1.29)
male	198 (55.30)	69 (54.33)	74 (58.26)	43 (59.72)	3 (1.56)	4 (2.08)	1 (0.52)	0	1 (0.52)	0	0	0	0	3 (1.56)

Results and discussion

The species distribution of fungi involved in BSIs is shown in Table 1. *Candida* species accounted for 98.32% of the fungal isolates. *C. albicans* accounted for 36.08% (127 isolates), whereas frequently isolated non-*C. albicans* species accounted for 56.53% (199 isolates), represented mainly by *C. parapsilosis* (36.08%, 127 isolates) and *Candida tropicalis* (20.45%, 72 isolates). Sporadically, yeast non-*Candida* species were identified: *Trichosporon mucoides* (1.4%, five isolates) and *Saccharomyces cerevisiae* (0.28%, one isolate).

The aetiological agent of fungaemia varied according the age of the patient. *C. albicans* and *C. parapsilosis* complex were the predominant species in neonates (48 cases each; 41.02%).

Table 2. *In vitro* susceptibility to eight antifungal agents of the most frequent fungaemia isolates from a surveillance programme in Mexico, 2010 and 2011

		MIC (mg/L)			Percentage resistant	
Species (no. of isolates)	Antifungal	range	50%	90%	M27-A3	SSCBs
C. albicans (127)	AMB	0.06–0.5	0.125	0.5	0	ND
	FLC	0.125–2	1	2	0	0
	ITC	0.06–0.125	0.06	0.125	0	ND
	VRC	0.03–0.125	0.06	0.125	0	0
	POS	0.03–1	0.06	0.125	ND	ND
	CAS	0.015–0.25	0.03	0.06	0	0
	ANF	0.015–0.25	0.03	0.06	0	0
	MCF	0.015–0.25	0.03	0.06	0	0
C. parapsilosis complex (127)	AMB	0.125–1	0.5	1	0	ND
	FLC	0.125–4	1	2	0	0
	ITC	0.03–0.125	0.06	0.125	0	ND
	VRC	0.015–0.25	0.03	0.06	0	ND
	POS	0.015–0.5	0.06	0.06	0	ND
	CAS	0.015–4	0.25	1	0.82	0
	ANF	0.03–4	1	2	2.47	0
	MCF	0.015–4	1	2	3.30	0
C. tropicalis (72)	AMB	0.125–2	0.25	0.5	1.4	ND
	FLC	0.125–64	0.5	2	1.4	1.4
	ITC	0.03–2	0.06	0.125	1.4	ND
	VRC	0.03–1	0.03	1	0	1.4
	POS	0.03–1	0.06	1	ND	ND
	CAS	0.015–0.5	0.06	0.25	0	0
	ANF	0.015–0.5	0.125	0.25	0	0
	MCF	0.015–1	0.06	0.125	0	0
C. glabrata (9)	AMB	0.125–2	0.25		11.11	ND
	FLC	1 to >64	4		11.11	11.11
	ITC	0.125–2	0.125		22.22	ND
	VRC	0.06–4	0.125		11.11	ND
	POS	0.06–4	0.25		ND	ND
	CAS	0.06–1	0.125		0	11.11
	ANF	0.125–2	0.25		0	11.11
	MCF	0.06–2	0.125		0	11.11
C. guilliermondii (7)	AMB	0.125–0.5	0.125		0	ND
	FLC	0.25–4	0.25		0	ND
	ITC	0.03–0.5	0.25		0	ND
	VRC	0.015–0.125	0.03		0	ND
	POS	0.015–0.25	0.03		ND	ND
	CAS	0.015–0.25	0.125		0	0
	ANF	0.25–1	1		0	0
	MCF	0.25–1	0.5		0	0

AMB, amphotericin B; FLC, fluconazole; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; CAS, caspofungin; ANF, anidulafungin; MCF, micafungin; ND, not defined.

C. parapsilosis complex was also the predominant species in patients 1 month to <2 years of age ($P=0.007$). In contrast, *C. albicans* was the most frequent species found in patients aged 2 to <12 years ($P=0.003$). The last age range included in this study showed a similar distribution among *C. albicans*, *C. parapsilosis* and *C. tropicalis* (26.08%, 34.43% and 34.78%, respectively). Compared with candidaemia due to *C. albicans* and *C. parapsilosis*, candidaemia due to *C. tropicalis* was less likely to occur among patients <1 month old (41.02% versus 7.69%; $P<0.0001$). The species distribution also varied substantially between hospitals.

Of the 127 *C. parapsilosis* isolates, 121 (95.3%) were identified as *C. parapsilosis sensu stricto*, while 4 (3.15%) and 2 (1.6%) corresponded to *C. orthopsilosis* and *C. metapsilosis*, respectively. *C. parapsilosis sensu stricto* was found in all age categories and in all medical centres included in this study. Owing to the small number of *C. orthopsilosis* and *C. metapsilosis* isolates obtained in this study, we did not find any association either with geographic area or age range.

The results of *in vitro* susceptibility testing of the five most frequent *Candida* species (342, 95.53%) from BSIs to the assayed antifungals are summarized in Table 2. The MICs for the control strains were within the acceptable range for the drugs tested. Overall, the resistance rates were similar when applying the SSCBs of fluconazole, voriconazole and echinocandins.

In our study, there are several notable findings in isolates from BSIs in paediatric patients. First, the predominant fungal species isolated were *C. albicans* and *C. parapsilosis* (127 isolates each), followed by *C. tropicalis* (72 isolates); this contrasts with other studies.[11–13] It has been suggested that the higher prevalence of *C. parapsilosis* in some medical centres might be related to poor catheter care or infection control practices.[14] *C. tropicalis* is associated with cancer and neutropenia.[15,16] We report a low proportion of *C. tropicalis* (7.69%) candidaemia in neonates. Several series have consistently reported this fact, with 2%–10% in Europe and 10%–12% in the USA and Canada.[16,17]

The rank order of species in this study was distinctive of each institution, possibly being related to the different geographical regions, antifungal prophylaxis, treatment practices and individual patient characteristics.[18] It is noteworthy that just one episode of candidaemia due to *C. krusei* was obtained in this study. Clinically, this microorganism is of particular importance due to its well-known resistance to fluconazole and its decreased susceptibility to amphotericin B.

Second, our study clearly shows the low occurrence of *Candida glabrata* as an aetiological species of candidaemia in neonates, infants, toddlers and children (9 cases, 2.56%) and the absence of this species in adolescents. Lin *et al*.[19] suggested that the higher prevalence of nosocomial *C. glabrata* and *C. krusei* fungaemia in some medical centres might be related to exposure to piperacillin/tazobactam and vancomycin. Since in this study *C. krusei* and *C. glabrata* were infrequent, fluconazole could be still a reasonable choice for the treatment of candidaemia before species identification.

Third, yeast species were identified according to standard procedures. However, identification of the *C. parapsilosis* species complex required molecular approaches. We identified yeast species recently defined as *C. orthopsilosis* and *C. metapsilosis* isolated from blood cultures, agreeing with Treviño-Rangel *et al*.,[20] who recently conducted a local survey in Monterrey, Mexico, and established that *C. parapsilosis sensu stricto* was the most frequent species of the complex, mainly recovered from blood. Resistance was found in one to four *C. parapsilosis* complex isolates depending on the echinocandin, but these same are considered to be echinocandin-susceptible strains according to SSCBs. Although resistance rates change moderately when applying the new breakpoints, the variation observed could possibly be due to the use of clinical commercial presentations of these drugs for the assays and not pure substances as established by CLSI.

Fourth, this study reflects that antifungal resistance was rare among isolates of *C. albicans*, *C. parapsilosis* complex, *C. tropicalis* and *Candida guilliermondii*. However, our data confirm the importance of *C. glabrata* as a problem in hospitals, even in the small number of isolates obtained from paediatric patients in this study; *C. glabrata* exhibited the highest resistance to amphotericin B and triazoles.

In conclusion, these findings should promote the establishment of a permanent nationwide surveillance programme. This paper is the first to provide national-level information about the species distribution and antifungal susceptibility profiles of *Candida* BSI isolates in paediatric patients from Mexico.

Acknowledgements

We thank Sergio Lozano of the Dr Jose Eleuterio González University Hospital (Monterrey, Mexico) for his review of the manuscript prior to submission.

Funding

This study was funded by a research grant from Merck Sharp & Dohme (30600).

Transparency declarations

None to declare.

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