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Species distribution and antifungal susceptibility profile of *Candida* isolates from bloodstream infections in Lima, Peru

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Yeast identification and *in vitro* susceptibility testing provide helpful information for appropriate administration of antifungal treatments; however, few reports from the Latin American region have been published. The aim of this study was to identify the species present in isolates from bloodstream infections diagnosed in nine hospitals in Lima, Peru and to determine their *in vitro* susceptibility to four antifungal drugs. We tested and identified 153 isolates collected between October 2009 and August 2011 using standard methods. PCR and PCR-RFLP assays were performed to distinguish *Candida albicans* from *Candida dubliniensis* and to identify species of the *Candida parapsilosis* and *Candida glabrata* complexes. Antifungal susceptibility testing for fluconazole, anidulafungin and voriconazole was performed using the CSLI M27-A3 method, and amphotericin B susceptibility was determined using the Etest method. The most frequently isolated species were: *C. albicans* (61; 39.9 %), *C. parapsilosis* (43; 28.1 %), *C. tropicalis* (36; 23.5%) and *C. glabrata* (8; 5.2 %). The overall susceptibility rates were 98.0 %, 98.7 %, 98.0 % and 97.4 % for amphotericin B, fluconazole, voriconazole and anidulafungin, respectively. No isolate was resistant to more than one drug. These results showed that the rate of resistance to four antifungal drugs was low among *Candida* bloodstream isolates in Lima, Peru.

INTRODUCTION

Candidaemia is one of the most frequently occurring nosocomial infections, and is associated with mortality rates of up to 40 % (Erdem *et al.*, 2010; Pfaller & Diekema, 2007; Sampaio Camargo *et al.*, 2010). Yeast identification and *in vitro* susceptibility testing provide valuable information for antifungal treatment selection. The current Infectious Disease Society of America (IDSA) guidelines for the management of candidiasis recommend fluconazole or any echinocandin as a first-choice therapy for candidaemia in non-neutropenic patients, while echinocandins are recommended as a first-line therapy in neutropenic patients. Amphotericin B (as liposomal formulation or deoxycholate) or voriconazole is used as an alternative treatment (Pappas *et al.*, 2009). In resource-constrained settings, echinocandins, voriconazole and liposomal formulations of amphotericin B are neither available nor affordable. Currently, fluconazole and amphotericin B deoxycholate are the drugs most commonly used in Peru.

Abbreviation: BSI, bloodstream infection.

Several studies showed that *Candida* species distribution and *in vitro* drug susceptibility vary among countries and even in different regions of a single country (Cleveland *et al.*, 2012; Nishikaku *et al.*, 2010), although most of these studies were conducted in the USA or in Europe. These studies have shown that there is a consistently decreasing level of susceptibility to fluconazole of both *Candida albicans* and non-*C. albicans* species (Arendrup *et al.*, 2013; Pfaller *et al.*, 2006). Limited information is available from the Latin American region, and, until now, there were no large studies in Peru that evaluated species distribution and antifungal susceptibility of yeast isolates from bloodstream infections (BSIs).

The limited information available suggests that non-*C. albicans* species are more frequent aetiological agents of BSIs in Peru than previously thought (Becerra *et al.*, 2010; Paz Rojas *et al.*, 2008). These data may have implications for the treatment of infections because *Candida parapsilosis* may display high MIC values for echinocandins (Ostrosky-Zeichner *et al.*, 2003). *Candida glabrata* is also considered a fluconazole acquired-resistant species, and *C. krusei* is considered to be resistant to fluconazole irrespective of its MIC (Pfaller *et al.*, 2012a).

Due to the lack of information on *Candida* species distribution and drug susceptibility patterns for yeast isolates in Peru, there is an urgent need for research into this topic, followed by the implementation of a surveillance program. The aim of this study was to identify the *Candida* species obtained from blood cultures in Peruvian sentinel hospitals and to determine their *in vitro* susceptibilities to four antifungal drugs.

METHODS

Isolates. The baseline bloodstream *Candida* isolate from each episode of candidaemia was recovered from 153 patients in nine hospitals of Lima, Peru, during the period October 2009 to August 2011. The isolates were transported to the Mycology Laboratory of the Instituto de Medicina Tropical Alexander von Humboldt-Universidad Peruana Cayetano Heredia. CHROMagar *Candida* medium (Difco) was used to evaluate colony purity and viability and for the presumptive identification of *Candida* species.

Isolates were preserved using both yeast extract-peptone-glucose broth (YEPD) and distilled water at −20 °C and skimmed milk at −70 °C.

Identification. Strain identification was determined using standard methods such as microscopic morphology on cornmeal agar + Tween 80, growth at 37 °C and 42 °C, growth in hypertonic sabouraud dextrose broth supplemented with 6.5% sodium chloride, and using an API 20 C AUX system (bioMérieux).

PCR and RFLP assays were performed following procedures described elsewhere (Luo & Mitchell, 2002; Mannarelli & Kurtzman, 1998; Tavanti *et al.*, 2005) to discriminate *C. albicans* from *Candida dubliniensis* and to identify the *C. parapsilosis* and *C. glabrata* complexes. Control strains, obtained from the American Type Culture Collection (ATCC) included *C. albicans* ATCC 90028, *C. dubliniensis* ATCC MYA-646, *C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019, *Candida metapsilosis* ATCC 96143 and *Candida orthopsilosis* ATCC 96141.

***In vitro* susceptibility testing.** Fluconazole, voriconazole and anidulafungin (Pfizer) were used for susceptibility testing.

Susceptibility testing for azoles and for anidulafungin was performed using the broth microdilution method following Clinical Laboratory Standards Institute (CLSI) standard procedures (CLSI, 2008a). *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019, obtained from the ATCC, were used as quality control strains. Briefly, stock solutions were prepared by dissolving anidulafungin or voriconazole in DMSO and by dissolving fluconazole in sterile distilled water. Final drug dilutions were prepared in standard RPMI 1640 medium (HiMedia Lab. Pvt.) buffered to pH 7.0 with 0.165 mol MOPS l^{-1}; microtitre plates containing the entire range of dilutions for a particular antifungal were prepared and kept at −70 °C until use. Concentration ranges for the assays were 0.125–64 μg ml^{-1} for fluconazole, 0.0313–16 μg ml^{-1} for voriconazole and 0.015–8 μg ml^{-1} for anidulafungin.

After inoculation, the microtitre plates were incubated at 35 °C for 24 h. MICs were determined visually using a mirror and were compared with drug-free controls. MIC breakpoints were interpreted according to the CLSI M27-S3 and M27-S4 documents, as well as the new CLSI species-specific clinical breakpoints for fluconazole, voriconazole and echinocandins for the six most common species (CLSI, 2008b, 2012; Pfaller & Diekema, 2012b). Isolates of *C. albicans*, *Candida tropicalis* and *C. parapsilosis* with MICs of $\geqslant$8 μg ml^{-1} and isolates of *C. glabrata* with MICs of $\geqslant$64 μg ml^{-1} were considered resistant to fluconazole; *C. krusei* was considered intrinsically resistant to fluconazole; isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis* with MICs of $\geqslant$1 μg ml^{-1} and *C. krusei* isolates with MICs of $\geqslant$2 μg ml^{-1} were considered resistant to voriconazole; isolates of *C. guillermondii* and *C. famata* with MICs of $\leqslant$1 μg ml^{-1} were considered susceptible to voriconazole; isolates of *C. albicans*, *C. tropicalis* and *C. krusei* with MICs of $\geqslant$1 μg ml^{-1}, isolates of *C. parapsilosis* and *C. guillermondii* with MICs of $\geqslant$8 μg ml^{-1} and isolates of *C. glabrata* with MICs of $\geqslant$0.5 μg ml^{-1} were considered resistant to anidulafungin. Isolates of *C. famata* with MIC of $>$2 μg ml^{-1} were considered non-susceptible to anidulafungin. Other *Candida* species were considered susceptible to fluconazole with MICs of $\leqslant$8 μg ml^{-1}, susceptible-dose dependent with MICs of 16–32 μg ml^{-1} and resistant with MICs of $\geqslant$64 μg ml^{-1}.

Amphotericin B susceptibility testing was performed using the agar-based Etest method (bioMérieux) according to the manufacturer's instructions. The test was read after 24 h of incubation at 35 °C. The Etest was used because this methodology has been cited as more sensitive and reliable than the CLSI reference method for the detection of isolates with decreased susceptibility to amphotericin B (Wanger *et al.*, 1995). As the Etest strips contain a continuous amphotericin B gradient, the Etest MICs of this drug were increased to the concentration of the next twofold dilution that matched the drug dilution scale used for the CLSI procedures. The breakpoints used for amphotericin B were: susceptible $\leqslant$1 μg ml^{-1}; resistant $\geqslant$2 μg ml^{-1} (Park *et al.*, 2006).

MICs from 50 % and 90 % of the total population were defined as MIC_{50} and MIC_{90}. The chi-squared test was used to compare proportions, and $P<0.05$ was considered statistically significant.

RESULTS

Among 153 *Candida* isolates evaluated, non-*C. albicans* species (92; 60.1 %) were more frequent than *C. albicans* (61; 39.9 %). The non-*C. albicans* species included *C. parapsilosis sensu stricto* (43; 28.1 %), *C. tropicalis* (36; 23.5 %), *C. glabrata* (8; 5.2 %), *Candida guillermondii* (3; 2 %), *C. krusei* (1; 0.7 %) and *Candida famata* (1; 0.7 %). No isolates of *C. dubliniensis*, *C. metapsilosis*, *C. orthopsilosis*, *Candida bracarensis* or *Candida nivariensis* were found.

The susceptibility results of the *Candida* isolates for the four antifungal drugs are shown in Tables 1 and 2. Overall, 98.0 % of the isolates were susceptible to amphotericin B, 98.7 % to fluconazole, 98.0 % to voriconazole, and 97.4 % to anidulafungin. In total, 141 (92.2 %) isolates were fully susceptible, while 12 (7.8 %) isolates were resistant to one of the tested antifungal drugs. None of the isolates were resistant to more than one drug, and resistance tended to be more frequent among the non-*C. albicans* isolates (8; 8.7 %) than the *C. albicans* isolates (4; 6.6 %); however, this difference was not statistically significant ($P=0.86$).

Resistance to amphotericin B was observed in *C. parapsilosis sensu stricto* isolates (3; 2.0 %), and resistance to voriconazole was observed in *C. albicans* isolates (3; 2.0 %). Resistance to anidulafungin (2.6 %) was found in one *C. albicans* isolate, two *C. tropicalis* isolates, and one *C. glabrata* isolate, and resistance to fluconazole was observed in one *C. parapsilosis sensu stricto* isolate and the only *C. krusei* (1.3 %) isolate, which is considered intrinsically resistant to this drug.

Table 1. MIC distributions of antifungal drugs against 153 isolates

Values in bold indicate resistance.

Antifungal agent	Species	No. of isolates tested	No. of isolates at MIC (μg ml^{-1})								
			⩽0.03	0.06	0.12	0.25	0.5	1	2	4	⩾8
Amphotericin B	*C. albicans*	61		20	14	17	10				
	C. parapsilosis	43	7	4	9	17	3		**3**		
	C. tropicalis	36		4	15	13	4				
	C. glabrata	8	1		2	4	1				
	C. guilliermondii	3	1		1	1					
	C. krusei	1					1				
	C. famata	1			1						
Anidulafungin	*C. albicans*	61	60					**1**			
	C. parapsilosis	43	12	2		4	10	11	4		
	C. tropicalis	36	31	2	1			**2**			
	C. glabrata	8	5	1	1		**1**				
	C. guilliermondii	3	1					2			
	C. krusei	1	1								
	C. famata	1				1					
Fluconazole	*C. albicans*	61			7	5	34	11	4		
	C. parapsilosis	43				12	22	4	2	2	**1**
	C. tropicalis	36				5	19	10	1	1	
	C. glabrata	8				1	1	1		4	1*
	C. guilliermondii	3					1		1	1	
	C. krusei	1				**1**					
	C. famata	1									1†
Voriconazole	*C. albicans*	61	52		4	2					**3**
	C. parapsilosis	43	37	1	4	1					
	C. tropicalis	36	7	15	12	2					
	C. glabrata	8	3		4	1					
	C. guilliermondii	3	1	1	1						
	C. krusei	1	1								
	C. famata	1	1								

*MIC=8 μg ml^{-1}.
†MIC=16 μg ml^{-1}.

DISCUSSION

This study showed that non-*C. albicans* (n=92, 60.1 %) isolates were the main species causing candidaemia in Peruvian hospitals, consistent with reports from other countries, including Spain (55.4 %), South Korea (62 %) and the USA (59 % in Atlanta and 66 % in Baltimore), and with recent reports from Latin American countries (62.4 %) (Bonfietti *et al.*, 2012; Córdoba *et al.*, 2012; Cleveland *et al.*, 2012; Cruz & Piontelli, 2011; Jung *et al.*, 2010; Nucci *et al.*, 2013; Pemán *et al.*, 2012; Pereira *et al.*, 2010; Porte *et al.*, 2012; Rodero *et al.*, 2005). Most cases were caused by infection with *C. albicans*, *C. parapsilosis* or *C. tropicalis*, in agreement with the data found in the present study.

Disseminated candidiasis due to *C. parapsilosis* is generally observed in neonates with very low birth weight, in patients with an indwelling central venous catheter, and in recipients of parenteral alimentation (Pfaller & Diekema, 2002; Levy *et al.*, 1998). Although this study was carried out in tertiary care hospitals, for both adults and paediatrics patients, we did not have clinical and epidemiological information to determine the populations affected by the *Candida* species.

C. parapsilosis sensu stricto was the most frequent strain among the non-*C. albicans* isolates. *C. metapsilosis* and *C. orthopsilosis* were not found in our samples. The absence of these cryptic species in bloodstream isolates was also observed in countries such as Finland, the UK and Spain, but the reason for this is not known (Lockhart *et al.*, 2008; Miranda-Zapico *et al.*, 2011).

We found a high amphotericin B resistance rate of 7 % (3 out of 43 isolates) among the *C. parapsilosis* isolates in comparison with previous studies, which reported rates of 2–3 % (Kovacicova *et al.*, 2001; Ostrosky-Zeichner *et al.*, 2003). Invasive candidiasis due to amphotericin B-resistant *Candida* isolates has been increasingly associated with the use of this antifungal agent (Kovacicova *et al.*, 2001). One explanation for this higher rate could be the wide use of

Table 2. Susceptibility of 153 bloodstream isolates

NC, not calculated.

Species (*n*)	Antifungal agents	Range (mg ml^{-1})	MIC_{50} (mg ml^{-1})	MIC_{90} (mg ml^{-1})	Resistance (%)
C. albicans (61)	Amphotericin B	0.06–0.38	0.125	0.38	0
	Fluconazole	⩽0.125–2	0.5	1	0
	Voriconazole	0.03–>16	0.03	0.125	5
	Anidulafungin	⩽0.03–1	0.03	0.03	1.6
C. parapsilosis (43)	Amphotericin B	0.002–2	0.19	0.38	7
	Fluconazole	0.25–8	0.5	2	2.3
	Voriconazole	0.03–0.25	0.03	0.125	0
	Anidulafungin	⩽0.03–2	0.5	1	0
C. tropicalis (36)	Amphotericin B	0.06–0.5	0.125	0.5	0
	Fluconazole	0.25–4	0.5	1	0
	Voriconazole	0.03–1	0.06	0.125	0
	Anidulafungin	⩽0.03–1	0.03	0.06	5.5
C. glabrata (8)	Amphotericin B	0.03–0.5	0.19	0.5	0
	Fluconazole	0.25–8	4	8	0
	Voriconazole	0.03–0.25	0.125	0.25	WT*
	Anidulafungin	⩽0.03–0.5	0.03	0.5	12.5
C. guilliermondii (3)	Amphotericin B	0.03–0.25	NC	NC	0
	Fluconazole	0.5–4	NC	NC	0
	Voriconazole	0.03–0.125	NC	NC	0
	Anidulafungin	⩽0.03–1	NC	NC	0
Other yeasts* (2)	Amphotericin B	0.15–0.38	NC	NC	NC
	Fluconazole‡	0.25–16	NC	NC	NC
	Voriconazole	⩽0.03	NC	NC	NC
	Anidulafungin	⩽0.03–0.25	NC	NC	NC

*All *C. glabrata* isolates were considered as wild-type (WT) strains for voriconazole.
†*C. krusei* (1); *C. famata* (1).
‡*C. krusei* isolate was considered resistant to fluconazole irrespective of MIC.

this polyene in Peru due to its low cost compared with newer antifungal drugs.

Although fluconazole is widely used in Peru, the levels of fluconazole and voriconazole resistance among the isolates tested were relatively low, similar to published data from Korea, Malaysia and Canada (Montreal and Quebec) (Amran *et al.*, 2011; Jang *et al.*, 2013; Labbé *et al.*, 2009).

Notably, all *C. glabrata* isolates were susceptible to fluconazole. However, in the USA, 8.8 % of samples showed resistance to this triazole, justifying continuous and close surveillance (Pfaller *et al.*, 2013). According to Lee *et al.* (2009), previous fluconazole and linezolid use are independent risk factors for fluconazole-resistant *C. glabrata* BSIs. However, the number (n=8) of *C. glabrata* isolates in this study was too low to estimate the real resistance to fluconazole in the Peruvian population.

Only *C. albicans* isolates (4.9 %) were resistant to voriconazole, but none of these isolates showed cross-resistance with fluconazole. Cuenca-Estrella *et al.* (2011) reported identical (5 %) *in vitro* resistance to voriconazole among clinical isolates of *Candida* spp. collected in a Spanish reference laboratory. Otherwise, most of their isolates were obtained from oropharyngeal infections and showed cross-resistance to other azole agents.

Although *Candida* isolates are highly sensitive to voriconazole in most countries, it is necessary to undertake national surveillance to detect emerging resistance or shifts in MIC distribution in Peruvian clinical *Candida* isolates (Pfaller *et al.*, 2010). Globally, the tested azole agents (fluconazole and voriconazole) remain very active against the majority of isolates tested.

The only echinocandin available for testing was anidulafungin. In general, this antifungal drug showed good activity against the 153 bloodstream isolates. Only one isolate of *C. albicans* (1.6 %) and two isolates of *C. tropicalis* (5.6 %) were resistant to anidulafungin. Interestingly, we found that one out of the eight (12.5 %) *C. glabrata* isolates was resistant to anidulafungin. This isolate did not show cross-resistance with fluconazole. The emergence of co-resistance to both fluconazole and echinocandins in clinical isolates of *C. glabrata* has been documented previously and represents a growing concern (Pfaller *et al.*, 2012a). In the last few years, an increase in the proportion of *C. glabrata*

isolates from BSIs was recognized in the USA, with 3 % resistance to anidulafungin among 318 *C. glabrata* isolates in Atlanta (Lockhart *et al.*, 2012). It will be essential to monitor the emergence of echinocandin resistance closely because these drugs are increasingly being used in Lima. None of the *C. parapsilosis sensu stricto* strains were resistant to anidulafungin. This species is known for its reduced *in vitro* susceptibility to this echinocandin. Although Lockhart *et al.* (2012) found some MIC differences for caspofungin, anidulafungin, micafungin and fluconazole among *C. parapsilosis* complex species, an evaluation into whether this leads to differences in clinical outcomes has not yet been conducted.

There are some limitations to this study. Although it was carried out in nine centres of the largest Peruvian city, which contains one-third of the total population of Peru, the results cannot be generalized to the whole Peruvian population. The second limitation is the lack of epidemiological and clinical data from the patients infected by the isolates tested.

Conclusion

This is the largest candidaemia study conducted in Peru and shows that non-*C. albicans* isolates are the most common candidaemia agents. Additionally, we found that 7.8 % of the strains were resistant to the most frequent antifungal drugs used in Peru. As *C. tropicalis* and *C. parapsilosis* are the most frequent non-*C. albicans* species and because they are associated with high mortality and high MICs to echinocandins (Costa *et al.*, 2000; Ma *et al.*, 2013), it will be important to monitor trends for the emergence of resistant strains. A future Peruvian surveillance program could help with the selection of effective antifungal therapies.

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