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Epidemiology of *Candida* species from sterile specimens in Germany and susceptibility to antifungal agents in vitro using the EUCAST method

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Introduction and Purpose

Candida species have emerged as major pathogens of invasive infections in hospitalised patients. They rank fourth among the organisms causing bloodstream infections and represent a major cause of morbidity and mortality in critically ill patients. *C. albicans* is the predominant species, but a shift to increasing rates of non-*albicans* *Candida* spp. with reduced susceptibility to commonly used antifungal agents has been observed during the last two decades [1, 2].

The purpose of this surveillance study was to assess the distribution and antifungal susceptibility patterns of *Candida* species isolated from blood and other sterile body sites.

Methods

Candida isolates

Between October 2010 and September 2011, 24 laboratories from Austria (n=1), Switzerland (n=3) and all regions of Germany (n=20) were requested each to consecutively collect 20 non-duplicate isolates from sterile specimen.

Identification and susceptibility testing

Preliminary identification of the yeasts was performed by local laboratory methods. At the end of the collection period isolates were shipped to a coordinating laboratory (Antiinfectives Intelligence) for species confirmation and susceptibility testing. Identification of the species was performed using MALDI-TOF (MALDI-Biotyper, Bruker Daltonic GmbH, Bremen, Germany).

Susceptibility to amphotericin B, anidulafungin, caspofungin, fluconazole, 5-fluorocytosine, itraconazole, micafungin, posaconazole and voriconazole was determined by the microdilution method described in the EUCAST document EDef 7.1 [3] using industrially prepared ready-to-use microdilution panels purchased from Merlin Diagnostika (Bornheim, Germany). Plates were read with a plate reader (Micronaut Skan, Labsystems, Helsinki, Finland). Two wavelengths (405 and 450 nm) were used for measuring the absorbance. MICs were read after 24 h and interpreted by EUCAST species-related or non-species-related (for uncommon *Candida* species only) clinical breakpoints (version 6.1) if available [4].

Results

A total of 542 *Candida* isolates were included in the study. Most common species were *C. albicans* (62.5%), *C. glabrata* (21.4%), *C. parapsilosis* and *C. tropicalis* (5% each), and *C. krusei* (2.4%). Patients ranged in age from <1 to 94 years (median 65 years); the majority of isolates were recovered from male patients (57.6%). Two hundred and seventy-seven (51.1%) isolates were obtained from ICU-patients and 253 (46.7%) from patients on general wards. 70.3% of all isolates derived from blood specimens.

In general, MICs tended to be higher at 450 nm than at 405 nm resulting in higher resistance rates for three antifungals (amphotericin B, fluconazole, micafungin) at 450 nm (**Table 1; Figure 1**). Nevertheless, the overall level of drug resistance was low, with 16 and 25 strains categorized as resistant at 405 nm and 450 nm, respectively. Notably, MICs for most resistant strains were one dilution step above the breakpoint resulting from the fact that the breakpoint divided the wild-type MIC distribution of the organism. An example is presented in **Figure 2**. MICs for the nine strains that were susceptible at 405 nm but resistant at 450 nm are listed in **Table 2**.

One strain of *C. albicans* exhibited high-level resistance to fluconazole (MIC 128 mg/L) due to overexpression of the CDR1 efflux pump [5], while one strain of *C. glabrata* was resistant to anidulafungin (0.125 mg/L) and micafungin (0.25 mg/L). This strain showed also reduced susceptibility to caspofungin (0.5 mg/L). All isolates of *C. albicans, C. parapsilosis* and *C. tropicalis* were susceptible to posaconazole and voriconazole. Of the 27 *C. tropicalis* strains, 70.4% showed reduced susceptibility to 5-fluorocytosine. MICs of micafungin and fluconazole for *C. parapsilosis* fell into the intermediate category. Concerning the isolates of infrequent *Candida* species, one strain of *C. lusitaniae* exhibited raised MICs for 5-fluorocytosine and fluconazole (16 mg/L each) at both wavelengths, suggesting a mutation in the gene *fcy1* or *fcy2* encoding a cytosine deaminase and purine-cytosine permease, respectively [6]

Table 1: Summary of resistant strains

Species (no. of strains tested)	No. (%) of resistant strains at 405 nm and 450 nm											
	Amphotericin B		Fluconazole		Posaconazole		Voriconazole		Anidulafungin		Micafungin	
	405 nm	450 nm	405 nm	450 nm	405 nm	450 nm	405 nm	450 nm	405 nm	450 nm	405 nm	450 nm
C. albicans (339)	1 (0.29)	2 (0.59)	1 (0.29)	1 (0.29)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.88)	5 (1.47)
C. glabrata (116)	0 (0)	1 (0.86)	7 (6.0)	8 (6.9)	n.a.	n.a.	n.a.	n.a.	1 (0.86)	1 (0.86)	1 (0.86)	1 (0.86)
C. parapsilosis (27)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C. tropicalis (27)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	n.a.	n.a.
C. krusei (13)	1 (7.7)	4 (30.8)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0 (0)	0 (0)	n.a.	n.a.
Other (20)*	n.a.	n.a.	1 (5.0)	2 (10.0)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total no. of resistant strains / total no. of evaluable strains (%)	2/522 (0.38)	7/522 (1.34)	9/529 (1.7)	11/529 (2.1)	0/393 (0)	0/393 (0)	0/393 (0)	0/393 (0)	1/522 (0.19)	1/522 (0.19)	4/482 (0.83)	6/482 (1.24)

n.a., no EUCAST breakpoints available; *Non-species related breakpoints were applied to uncommon *Candida* species (*C. lusitaniae, C. dubliniensis, C. guilliermondii, C. kefyr, C. norvegensis, C. orthopsilosis, Candida* sp.);
Cells in blue indicate higher resistance rates at 450 nm than at 405 nm.

Table 2: Strains categorised as susceptible or intermediate at 405 nm but resistant at 450 nm (n=9)

Strain	Antifungal agent	MIC (mg/L)		Category	
		405 nm	450 nm	405 nm	450 nm
C. albicans 103-6	Amphotericin B	0.5	2	S	R
C. albicans 91-71	Micafungin	0.016	0.031	S	R
C. albicans 102-81	Micafungin	0.016	0.031	S	R
C. glabrata 61-81	Amphotericin B	1	2	S	R
C. glabrata 41-65	Fluconazole	32	64	I	R
C. krusei 34-18	Amphotericin B	1	2	S	R
C. krusei 53-35	Amphotericin B	1	2	S	R*
C. krusei 109-71	Amphotericin B	1	2	S	R
C. norvegensis 109-69	Fluconazole	4	8	I	R

S, susceptible; I, intermediate; R, resistant; *The MIC was 1 mg/L (susceptible) in a repeat test.

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Conclusions

- ***C. albicans* was the predominant *Candida* species isolated from blood and other sterile body sites as has been shown in previous studies from Germany [7, 8].**
- **If a microdilution plate reader is used for MIC determination, as recommended by EUCAST, reading at a wavelength of 450 nm will confer higher MIC values for many strains and may thus result in elevated resistance rates as compared to reading at 405 nm.**
- **Overall, the level of resistance to first-line antifungal agents among invasive *Candida* isolates seems to be favourable in Germany and the central European area.**

Figure 1: Overall percentage of resistant strains among evaluable isolates at 405 nm and 450 nm

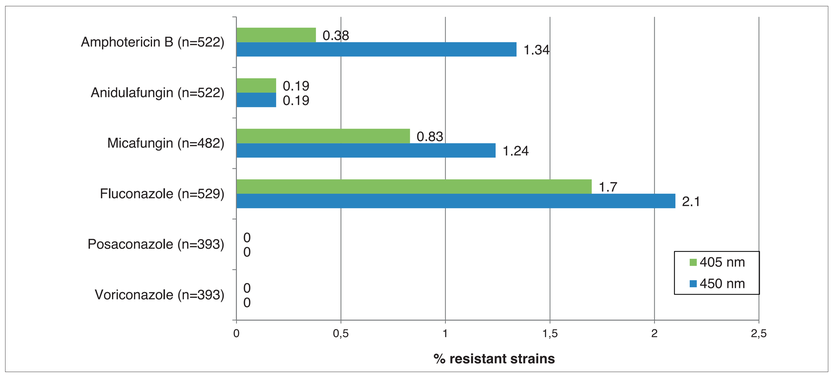
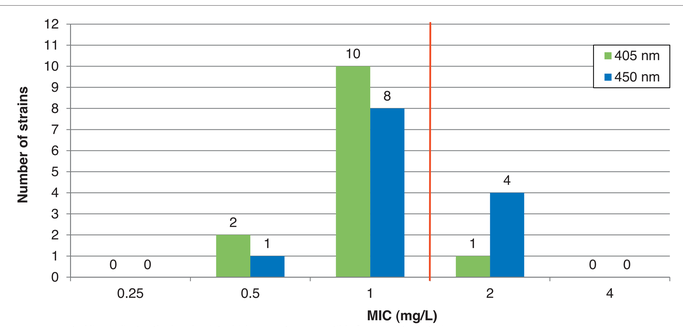


Figure 2: MIC distributions for amphotericin B and *C. krusei* obtained at 405 and 450 nm



Red line indicates the breakpoint which seperates susceptible from resistant strains