ORIGINAL ARTICLE

Prevalence of *Candida* species in different hospital wards and their susceptibility to antifungal agents: results of a three year survey

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Key words

Candida • Antifungal agents

Summary

Over a three years period, 472 Candida isolates were obtained from specimens of patients hospitalized either in "at risk", Bone Marrow Transplant Unit and Intensive Care Unit, or in conventional wards, Pneumological Divisions of the "Binaghi" Hospital of Cagliari (Italy). Antifungal susceptibility profile to amphotericin B, voriconazole, fluconazole and ketoconazole was determined. Candida albicans was the predominant species while Candida krusei was the most frequent non-albicans species. C. krusei was significantly more common among Bone Marrow Transplant Unit and Intensive Care Unit than Pneumological Divisions patients (17.9% and 14.1% vs. 6.0%; p < 0.05). No significant differences were observed when the same distribution was analysed with regard to the other Candida species or when Bone Marrow Transplant Unit and Intensive Care Unit were compared. The profiles of susceptibility to the antifungal drugs among isolates from the different hospital wards showed no significant differences, even though most of MIC values were higher for Intensive Care Unit isolates compared to those for Bone Marrow Transplant Unit and Pneumological Divisions. For C. albicans isolates, amphotericin B was the more efficient antifungal (97.7% S), while fluconazole (6.1% R [Resistant] and 2.6% SDD [Susceptible Dose Dependent]) and ketoconazole (4.1% R and 3.2% SDD) showed the lowest activity. Voriconazole was the more efficient antimycotic for C. krusei (96.7% S) and Candida glabrata (100% S [Sensible]) isolates.
This study has shown a significantly higher presence of non-albicans Candida in at risk wards as well as a decreased susceptibility to the older azoles (ketoconazole and fluconazole) among C. albicans isolates.

Introduction

In recent years, the genus *Candida* has became the principal cause of human yeast infections, with *Candida albicans* being the most commonly isolated species, presumably for its capacity to reside in the natural flora of healthy individuals as a commensal organism.
An increasing incidence of infections, caused by species other than *C. albicans*, has been recently reported by several investigators [1-3]. The contribution of non-*albicans* species to fungemia has been summarized by Krcmery and Barnes [4] and Pfaller and Diekema [2] who showed, among other things, that there are clear geographical differences in the distribution of *Candida* spp., other than *C. albicans*, causing invasive disease.
Sandven [5] reviewed that, in the United States, from 1972 to 1998 the proportion of the most important non-*albicans* species has varied: in fact *Candida tropicalis* was prevalent in earlier studies while *C. glabrata* increased in the latter years.
More recent epidemiological data, coming from a study conducted in seven European countries by the European Confederation of Medical Mycology (ECMM), reported that *C. glabrata* was the second most common species in all the countries except in Italy and Spain where *C. parapsilosis* was isolated with higher frequency [3].
Many are the suggested risk factors for infections with *Candida* species: other than impaired immunity for *C. albicans*, prophylaxis with azole compounds for *C. krusei* and *C. glabrata* [6], neutropenia and bone marrow transplantation for *C. tropicalis* [7], low age, parenteral alimentation and intravascular device for *C. parapsilosis* [8, 9]. Recently, mucous membrane colonization has been demonstrated as a significant risk factor for candidemia in several biomolecular studies [10, 11].
A knowledge of the distribution and antifungal susceptibility of *Candida* species in patients hospitalized can be helpful in detecting epidemics, deciding empirical treatment regimes and selecting the right antimycotics [12].
This study was conducted to evaluate the distribution of *Candida* species and their antifungal susceptibility in patients hospitalized in three different types of ward of a single institution during a three years period.

Methods

Organisms and media

Four hundred and seventy two *Candida* isolates, obtained from clinical specimens of patients hospitalized in Pneumological Divisions (PD), Bone Marrow Transplant Unit (BMTU) and Intensive Care Unit (ICU) of the "Binaghi" Hospital of Cagliari (Sardinia, Italy) between April 2003 and April 2006, were included in the study. More than one *Candida* isolate from a single patient was included if the specimen was obtained from separate body sites and/or at different time intervals, or if multiple *Candida* species were identified.

Surveillance cultures were performed twice weekly in ICU and once in pre-transplant period in BMTU. Specimens included five biological samples in ICU (bronchus fluid, pharingeal and nasal swabs, faeces or rettal swab and urine) and four in BMTU (pharingeal and nasal swabs, faeces or rettal swab and urine), respectively. Sputum was analyzed once a week on each patient admitted to PD until discharge. In addition, biological samples were performed each time a candidiasis was suspected by physicians.

Isolates were identified, in the hospital laboratory, by biochemical tests using API ID 32 C (bioMerieux, St Louis, MO) as recommended by the manufacturer, and maintained at -20 °C in skim milk (Oxoid, Basingstoke, UK) until the transfer to the university laboratory.

Prior to antifungal susceptibility testing, each isolate was subcultured on Sabouraud dextrose agar (SDA) (Microbiol, Cagliari, Italy) for 24 h at 35 °C to ensure purity and viability and to confirm identification.

Antifungal susceptibility testing

The susceptibility to antifungal drugs of *Candida* isolates was determined by using the broth microdilution method described by the Clinical and Laboratory Standards Institute (CLSI), document M27-A2 [13]. The following antifungal drugs, as pure standard compounds, were tested: amphotericin B (Sigma, S. Louis, Missouri, USA), voriconazole and fluconazole (Pfizer Pharmaceuticals Group, New York, USA), ketoconazole (Sigma).

Amphotericin B and ketoconazole final concentrations ranged from 0.003 to 16 μg/ml, voriconazole from 0.015 to 16 μg/ml and fluconazole from 0.125 to 128 μg/ml. MICs (Minimum Inhibbitory Con-

Tab. I. Distribution of 472 *Candida* spp. grouped according to the biological site and the hospital ward. PD (Pneumological Division), BMTU (Bone Marrow Transplant Unit), ICU (Intensive Care Unit).

Biological	*C. albicans*			*C. krusei*			*C. glabrata*			*C. parapsilosis*			*C. tropicalis*			Other species			Total
sample	PD	BMTU	ICU	PD	BMTU	ICU	PD	BMTU	ICU	PD	BMTU	ICU	PD	BMTU	ICU	PD	BMTU	ICU	
Sputum	64	12	5	3		1		4			1		4				1		95
Bronchus fluid	23	1	48	3		15	1					3	1		5			1	101
Pharingeal swab	6	23	50		10	8		2	1		2	10		1			4		117
Tongue swab		13			3						1			1					18
Nasal swab			13			3						1							17
Faeces		16	14		6	3			2						1		1		43
Urine	4		35	1		1	2	1	7			1	2					1	55
Blood		1	3							1		1				1		1	8
Vascular catheter			8			4			2										14
Other sites	1	2	1																4
Total strains	343			61			22			21			15			10			472

Other species: *C. sake* (4 isolates), *C. dubliniensis* (3 isolates), *C. kefir*, *C. pelliculosa* and *C. pseudotropicalis* (each 1 isolate). Other sites: Vaginal and skin swabs

centrations) were determined visually at 48 h by comparison with the drug-free growth control well. MIC interpretative criteria for fluconazole and voriconazole were those proposed by the CLSI and recently published by Pfaller et al. [14, 15]: fluconazole (S: MIC ≤ 8 μg/ml, SDD: MIC 16 to 32 μg/ml, R: MIC ≥ 64 μg/ml), voriconazole (S ≤ 1 μg/ml, SDD 2 μg/ml, R ≥ 4 μg/ml). Due to a lack of interpretative criteria for amphotericin B and ketoconazole, values were established according to previous studies [16-18] and were as follows: S ≤ 1 μg/ml and R ≥ 2 μg/ml for amphotericin B, S ≤ 0.125 μg/ml, SDD 0.25 and 0.50 μg/ml, R ≥ 1 μg/ml for ketoconazole.
C. krusei (ATCC 6258) and *C. parapsilosis* (ATCC 22019) were included as quality control strains and MICs were within the recommended range for each test [19].

Statistical analysis

The statistical significance of the differences between the study groups was determined by the chi-square test. All comparisons were considered statistically significant for P values of 0.05 or less.

Results

During the 3-year study period, 472 *Candida* spp. isolates were recovered from the cultures of 255 patients submitted to the hospital laboratory and distributed as follows: 117 strains from PD, 106 from BMTU and 249 from ICU patients. The strains investigated included isolates from patients receiving antifungal

Tab. II. Susceptibility to antimycotics of prevalent *Candida* species (MIC_{50} and MIC_{90} values of isolates grouped according to the hospital ward).

Species (n. of isolates)	Antifungal agents	MIC (μg/ml)								
		PD		BMTU		ICU				
		n. of isolates	$MIC_{50}/_{90}$* mean value	n. of isolates	$MIC_{50}/_{90}$ mean value	n. of isolates	$MIC_{50}/_{90}$ mean value	% S	% S-DD	% R
C. albicans (343)	amphotericin B	98	0.25/0.5	68	0.5/1	177	0.5/1	97.7	/	2.3
	voriconazole		0.015/0.06		0.125/1		0.25/1	94.8	5.2	0
	fluconazole		0.25/8		0.25/4		0.25/32	91.3	2.6	6.1
	ketoconazole		0.03/0.125		0.03/0.06		0.03/0.125	92.7	3.2	4.1
C. krusei (61)	amphotericin B	7	0.25/1	19	1/2	35	1/4	86.9	/	13.1
	voriconazole		0.03/0.06		0.03/0.06		0.06/0.125	96.7	/	3.3
	fluconazole		32/ > 64		16/32		16/ > 64	8.2	62.3	29.5
	ketoconazole		0.25/1		0.5/2		0.5/2	18.0	65.6	16.4
C. glabrata (22)	amphotericin B	3	1/-	7	0.5/1	12	0.5/1	95.5	/	4.5
	voriconazole		0.5/-		0.5/0.5		0.5/0.5	100	/	0
	fluconazole		2/-		0.25/4		4/32	77.3	13.6	9.1
	ketoconazole		0.03/-		0.06/0.125		0.06/0.5	68.2	18.2	13.6
C. parapsilosis (21)	amphotericin B	1	0.25/-	4	0.25/1	16	0.06/1	100	/	0
	voriconazole		0.06/-		0.06/0.125		0.06/0.125	100	/	0
	fluconazole		1/-		1/2		1/2	100	0	0
	ketoconazole		0.06/-		0.03/0.06		0.03/0.06	100	0	0
C. tropicalis (15)	amphotericin B	7	0.06/0.25	2	0.25/-	6	0.25/1	100	/	0
	voriconazole		0.06/0.125		0.125/-		0.125/1	100	/	0
	fluconazole		1/2		1/-		1/8	100	0	0
	ketoconazole		0.03/0.03		0.03/-		0.03/0.125	100	0	0

* = the MIC_{90} value was not included when the number of strains was ≤ 3

prophylaxis and patients without prophylaxis as well as isolates from infection and colonization.

The distribution of *Candida* species in each hospital ward and in the biological cultures is summarized in Table I. Overall, 343 (72.7%) isolates were identified as *C. albicans*. Among other *Candida* species (27.3%), the most common was *C. krusei* (12.9%), followed by *C. glabrata* (4.7%), *C. parapsilosis* (4.4%) and *C. tropicalis* (3.2%). Other *Candida* species, sporadically recovered (2.1%) included 4 *C. sake*, 3 *C. dubliniensis* and one each of *C. pelliculosa*, *C. pseudotropicalis* and *C. kefir* strains.

Non-*albicans Candida* presence was significantly higher in BMTU (35.8%; $p = 0.005$) and ICU (28.9%; $p = 0.01$) compared to PD (16.23%) patients. *C. krusei* was significantly more common among BMTU and ICU than PD patients (17.9% and 14.1% *vs.* 6.0%; $p < 0.05$).

No significant differences were observed when the same distribution was analysed with regard to the other *Candida* species or when comparing BMTU and ICU patients.

The profiles of susceptibility to amphotericin B, voriconazole, fluconazole and ketoconazole among the prevalent *Candida* species isolates, grouped according to the hospital ward, are compared in Table II. MICs (μg/ml) at which 50% (MIC_{50}) and 90% (MIC_{90}) of the strains were inhibited as well as the percentages of susceptible, susceptible-dose dependent (for voriconazole, fluconazole, ketoconazole) and resistant strains were reported.

C. parapsilosis and *C. tropicalis* isolates were susceptible to all agents examined. The profiles of susceptibility to the antifungal drugs among isolates from the different hospital wards showed no significant differences, even though higher MIC values for ICU isolates compared to those for BMTU and PD were obtained.

For *C. albicans* isolates, amphotericin B was the most efficient antifungal (97.7% S), while fluconazole (6.1% R and 2.6% SDD) and ketoconazole (4.1% R and 3.2% SDD) showed the lowest activity. No *C. albicans* strains resistant to voriconazole were observed but 18 (5.2%) strains showed lowered susceptibility to this drug and the highest values of MIC_{50} and MIC_{90} were detected in ICU and BMTU isolates.

Voriconazole was the more efficient antimycotic for *C. krusei* (96.7% S) and *C. glabrata* (100% S) isolates. *C. krusei* showed a high level of resistance to fluconazole and ketoconazole (91.8% and 82% between SDD and R strains, respectively), and decreased susceptibility to fluconazole and ketoconazole was demonstrated in *C. glabrata* isolates, with 36.4% and 40.9% of SDD and 31.8% and 27.2% of R strains, respectively.

Discussion

Hospital acquired infections due to fungi still present formidable problems in terms of their diagnosis and even more so in terms of therapy. Therefore, prevention is a very important strategy in controlling these diseases. Among the preventive measures, epidemiological surveys are needed to better characterize the species distribution and the antifungal susceptibility profile of isolates because it is known that the epidemiology of infections in one institution may differ greatly from the epidemiology in another.

We focused our study on the number of isolates of *Candida* spp., during a period of three years, in patients hospitalized either in "at risk" (BMTU and ICU) or conventional (PD) wards. Although many studies were conducted to provide epidemiological information on the frequency and susceptibility profile of different species of *Candida* isolates in bloodstream infections [3, 20, 21], few data concerning the distribution of yeast species isolated from various body sites and in different areas of the same hospital are available in the literature.

In our study the total number of strains isolated in the hospital laboratory was taken into account without distinction between colonization and infection. In fact, several studies indicate colonization by *Candida* species as a prerequisite for the development of candidiasis both in immunosuppressed and non-neutropenic patients [22, 23].

In the present study, it is not surprising that *C. albicans* was the most common species isolated in all the wards, but we found troubling the significantly higher presence of non-*albicans Candida* species, and in particular *C. krusei* and *C. glabrata*, in "at risk" wards compared to conventional ward, although the role of these species as emergent pathogens in ICU and BMTU has recently been pointed out. Trick et al. [21] described a significant increase in incidence of bloodstream infections associated with *C. glabrata* among ICU patients in the United States during 1989-1999. *C. albicans*, followed by *C. glabrata* and *C. krusei*, was the most frequently isolated species in urine specimens of patients with indwelling urinary catheters in the ICU of "Sao Paulo" hospital, Brasil [24]. Safdar et al. [25] reported *C. glabrata* and *C. krusei*, as the most common yeast species responsible for hematogenous candidiasis in patients undergoing allogenic BMT during 1997-1999.

The high presence of *C. krusei* obtained in our study differs from data published in other studies. Even if the estimates of *C. krusei* isolation vary between institutions and over different years, it has been rarely encountered as responsible for infections. A recent ECMM multi-istitutional survey of *Candida* bloodstream infections performed in Europe, reported a limited role of *C. krusei* with an isolation average of 1.9% [3]. The same rate was reported by SENTRY Antimicrobial Surveillance Program in North America, Europe and Latin America medical centres [26]. However, the rates of *C. krusei* are higher in studies concerning units with neutropenic or highly immunocompromised patients. Safdar [25] reported, among

15 episodes of candidemia after high-risk allogenic marrow transplantion, *C. glabrata* as the most common species (53.3%), followed by *C. krusei* (33.3%) and *C. parapsilosis* (13.3%) while *C. albicans* was absent. Krcmery and Barnes [4] reviewed that *C. krusei* occurred more frequently in leukaemic patients and bone marrow transplant recipients. Moreover, *C. krusei* was associated with a high mortality rate in several types of patients [27-29].

The particularly high incidence of *C. krusei* in this study, mainly in "high risk" wards, may be due to its presence among the species colonizing the upper and lower respiratory and orointestinal tracts [30]. At the same time, an exogenous infection related to invasive procedure or a selection of less susceptible species by an overuse of antifungal agents could be hypothesized.

Antimicrobial resistances are generally more common in at risk than in conventional wards and our results support, in part, this fact as ICU isolates showed the highest MICs, even if no significant difference was observed between the rate of resistant strains among the ICU and BMTU compared to PD.

Amphotericin B was the most active antifungal drug against *C. albicans*, although the overall resistance rate (2.3%) is higher than that reported in Italy [31] and in other countries [32, 33].

In our study a decreased susceptibility to fluconazole and ketoconazole was observed in *C. albicans* isolates and, among non-*albicans Candida*, *C. krusei* and *C. glabrata* had the highest MICs to these drugs. Moreover, higher MICs for voriconazole were detected in strains of *C. albicans* with decreased susceptibility to fluconazole, while no correspondence between resistance to fluconazole and voriconazole has been found in *C. glabrata* and *C. krusei* strains. These data support, in part, the possibility of cross-resistance between fluconazole and voriconazole, as hypothesized by other Authors [32, 34], and certainly they confirm the previously reported favorable in vitro activity of voriconazole against isolates with decreased susceptibility to fluconazole [19].

Conclusions

This study has shown a significantly higher presence of non-*albicans Candida* species, in particular *C. krusei* and *C. glabrata*, in at risk wards as well as a relatively decreased susceptibility to the older azole drugs (ketoconazole and fluconazole) among *C. albicans* isolates.

Despite the limitations of this study, that include the lack of data about antifungal use in the hospital wards and of detailed clinical information for individual patients, we retain that the routine surveillance cultures provide a prevention of invasive candidiasis mainly guiding initiation of preemptive antifungal therapy in critically ill patients.

Further studies are in progress in order to identify possible changes in the species distribution, to assay the real rate of *Candida* infections and to better establish the correlation between the type of resistance and the patterns of usage of antifungal agents.

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