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ARTICLE

A first Portuguese epidemiological survey of fungaemia in a university hospital

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Abstract A prospective, observational study was conducted at the biggest Portuguese hospital, aiming to evaluate the epidemiology of bloodstream fungal infection. During a period of 12 months (2004), all yeasts isolated from the blood cultures of patients with fungaemia admitted at a university hospital of Porto were collected. Demographic and clinical data, as well as haematological and biochemical profiles, were registered. Antifungal susceptibility was evaluated. The incidence of fungaemia and nosocomial fungaemia were 2.7 and 2 per 1,000 hospital admissions, respectively. Blood strains from 117 patients were identified. Thirty-five percent of yeast isolates were *Candida albicans*, followed by *C. parapsilosis* (25.6%). The mortality rate associated with fungaemia was 39.3%; the highest values were found in patients with *C. glabrata* and *C. tropicalis* infection. Seventy-five percent of the fungaemia episodes were nosocomial, with 48% mortality; the main predisposing factors were parenteral nutrition, gastric protection with omeprazole, surgical drainage and the presence of central venous catheters (CVCs). Thrombocytopaenia, urinary catheter, gastrointestinal pathology and nosocomial fungaemia were independently associated with a poor outcome. Antifungal susceptibility testing showed high fluconazole resistance (15%), mostly in *C. tropicalis*. We observed a high incidence of nosocomial fungaemia with high mortality rates. Important predisposing factors were identified, deserving further investigation. Local surveillance is warranted to monitor the incidence of in vitro antifungal resistance.

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Introduction

The dramatic increase of nosocomial invasive fungal infection over the past two decades has led to increased interest in this area. Yeasts represent the fourth most common group of isolates from bloodstream infections in the United States, and reports also suggest an increase in candidaemia in Europe [1–3]. Factors contributing to this trend include a growing population of immunocompromised patients with acquired immune deficiency syndrome (AIDS) or non-immunocompromised critically ill patients submitted to aggressive and invasive therapy [2, 4]. During the last decade, *Candida* was the eighth cause of invasive infection in Europe [5]. Risk factors such as the use of aggressive medical and surgical strategies are some aspects that need a regular and local re-evaluation.

Antifungal prophylaxis with fluconazole seems to have favoured the increase of resistance in yeasts, as well as the

replacement of some species by resistant ones, such as *C. glabrata* and *C. krusei* [6, 7]. With the increase of clinical and/or microbiological resistance, susceptibility tests play an ever-increasing role in the selection of antifungal drugs [8]. The approved Clinical and Laboratory Standards Institute (CLSI) document M27-A2 2002 (formerly National Clinical Collaborative Laboratory Standards [NCCLS]) supports the standardisation of testing, but it still has considerable limitations [9]. It does not provide interpretative breakpoints for all antifungals, raises problems of trailing endpoints, is labour-intensive and gives late results. For these reasons, most clinical laboratories do not undertake such a procedure. Particularly in life-threatening situations like sepsis, this topic is of crucial relevance.

This is the first epidemiological survey performed in Portugal. The incidence of fungaemia and its main risk factors were established, as well as the distribution between the fungal species and susceptibility profiles.

Patients and methods

Study population

Hospital São João is the biggest university hospital located in the northern region of Portugal, with 1,350 beds, including 40 intensive care unit (ICU) beds. All yeast isolates were collected from the blood cultures of patients admitted to the hospital during a 12-month period (starting from 1st January until 31st December of 2004). Isolates from outpatients and patients without clinical signs of fungaemia were excluded.

The first bloodstream fungal isolate from each patient was considered for this study. The proportion of yeasts relative to other microorganisms isolated from the blood culture during the same observational period was also calculated.

Definitions

An episode of fungaemia was defined as the first isolation of a yeast species from blood culture in a patient showing related clinical signs and symptoms.

Nosocomial fungaemia was defined whenever a patient showed at least one positive blood culture for fungi after the initial 48 h of hospital admission, with concomitant signs and symptoms of sepsis [10].

Primary fungaemia was defined according Garner et al. [10]. The positive culture of catheters helped to define fungaemia associated with catheters [11].

Secondary fungaemia was defined whenever an infection by the same fungal agent and with the same susceptibility pattern was isolated from another site before the first positive blood culture [10].

Polimicrobial fungaemia was defined whenever more than a single fungal strain was isolated from a blood culture in a period of 48 h [12].

For each patient, the outcome of fungaemia was evaluated 30 days after the first episode of fungaemia. Death related to fungaemia was defined as death within 30 days after the first positive blood culture for fungi (30 d unfavourable outcome), without signs of intracerebral or gastrointestinal bleeding or pulmonary embolism. In the case of death occurring 30 days after the first episode, a subsequent episode was considered [12].

Underlying disease was classified using the international classification of diseases—"International Statistical Classification of Diseases and Related Health Problems" [13].

Neutropaenia and leukopaenia were defined, respectively, as $<500/mm^3$ neutrophils or a total of white blood cells (WBC) $<1{,}000/mm^3$. Severe and very severe thrombocytopaenias were considered when the platelet count was below 50,000 or $10{,}000/mm^3$, respectively.

Clinical data

Date of admission, sex, age, hospital department and associated diseases were registered for each patient. At the collection date of the first positive fungal blood culture, haematological and biochemical parameters, use of catheter lines, antimicrobial therapy prescribed (prophylactic or therapeutic), previous immunosuppression (chemotherapy/radiotherapy), corticotherapy, use of vasoactive/inotropic amines, gastric protection, as well as the use of albumin, parenteral nutrition and mechanical ventilation for more than 24 h before the episode of fungaemia were registered in a database.

Underlying diseases, presence of gastrointestinal disease, dialysis (during the three previous months), type of surgery or admission to an ICU for more than 24 h were also registered.

Fungal characterisation

Yeasts isolated from blood cultures incubated on the BACTEC 9240 System (Becton Dickinson, Sparks, MD) and concomitant fungal isolates from other biological products of the same patient, as well as fungi from subsequent blood cultures were stored and identified using Vitek YBC identification cards (bioMérieux, Paris, France).

Antifungal susceptibility testing

Antifungal susceptibility testing to amphotericin B (Bristol-Myers Squibb, New York), fluconazole (Pfizer, Groton, CT), itraconazole (Janssen, Beerse, Belgium) and vorico-

nazole (Pfizer) were performed as recommended by the CLSI protocol M27-A2 [9]. Minimal inhibitory concentration (MIC) was registered after 24 and 48 h. Strains were classified as susceptible (S), susceptible-dose dependent (S-DD) and resistant (R) to fluconazole and itraconazole according to breakpoints defined by the CLSI [9]. For voriconazole, MICs≤1 μg/ml were considered as S, 2 μg/ml as S-DD and ≥4 μg/ml as R (newly established breakpoints) [14]. Although susceptibility breakpoints have not yet been established for amphotericin B, strains that were inhibited by ≤1 μg/ml of each were considered to be susceptible [15]. Regarding caspofungin (Merck, Rahway, NJ), the drug was prepared in sterilised water and stored at −70°C until use. M27-A2 [9] and the Etest (AB Biodisk, Solna, Sweden) were performed and the results were obtained after 48 h for *Candida* spp. and 72 h for *Cryptococcus* spp. and *Rhodotorulla* spp. MICs were defined as the minimal concentration of the drug that showed 100% inhibition.

Strains *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included in susceptibility tests as quality control (QC) strains. The MICs of antifungal agents for both QC strains were established within the MIC limits [9, 15].

Data analysis and establishment of correlations

The chi-square test was used to compare proportions and analyse differences in the species distribution. Multivariable logistic regression was used to evaluate factors associated with nosocomial, primary fungaemia and 30 d unfavourable outcome. Crude mortality rates were calculated. Alpha was set at 0.05 and all reported *p*-values were two-tailed.

The SPSS v13.0 program for Windows was used to perform the statistical analysis.

Results

Epidemiology

A total of 117 patients with the diagnosis of fungaemia were enrolled in the study during the established time period. The incidence of fungaemia and nosocomial fungaemia were 2.7 and 2.0 per 1,000 hospital admissions, respectively. Yeasts were the fourth most common agent isolated from blood cultures, corresponding to 3.5% of all of the microorganisms (*Staphylococcus* spp.: 58.6%, *Escherichia coli*: 7%, *Enterococcus faecalis*: 4.2%); *C. albicans* was isolated in 35% of the cases, *C. parapsilosis* in 25.6%, *C. tropicalis* in 12.8%, *C. neoformans* in 10.3% and *C. glabrata* in 7.7%. *Rhodotorulla* spp. was isolated in three neoplasic patients with central venous catheters (CVC). *C. guilliermondii* and *C. lusitaniae* were isolated in two patients. Single cases of *C. krusei*, of *Pichia anomala* and of *Saccharomyces cerevisiae* were found.

Demographic characteristics and risk factors

The results regarding the patient demographics and clinical characteristics are found in Table 1. The mean duration of hospitalisation was 49 days (median=39 days); the duration between admission and the collection of the first positive blood culture had a mean value of 24 days (median=19).

Seventy-five percent of fungaemia episodes were of nosocomial origin (Table 1); nosocomial fungaemia and primary fungaemia were more frequent in neoplasic patients than non-nosocomial or secondary fungaemia, respectively; secondary fungaemia was more frequent than primary fungaemia in patients with underlying infectious diseases.

Primary fungaemia was nosocomial in 73% of the cases and in 9% of the patients related with CVCs. The secondary fungaemia was nosocomial in 26% of the cases. *C. albicans* was isolated in 46% of patients with secondary fungaemia (Table 1). Polimicrobian fungaemia occurred in only two patients, with *C. tropicalis* and *C. guilliermondii* being isolated in a blood strain.

Half of the patients had positive blood cultures for bacteria previously to fungaemia (Table 1).

Twenty-nine percent of fungaemia patients had been submitted to gastrointestinal surgery (59.6% of the total surgical patients).

Sixty-five percent of patients showed thrombocytopaenia (Table 1), being severe in 25% of the cases and very severe in 12% of the cases.

The distribution of yeast isolates changed according to the underlying disease. *C. albicans* and *C. parapsilosis* were the most frequent yeasts encountered in neoplasic patients (Table 1). All of the 18 patients belonging to the Infectious Diseases Department were HIV-positive and 41% of them had AIDS; in this group, *C. neoformans* was the most frequent isolate.

Statistically significant differences in the *Candida* species distribution were found for the presence of a urinary catheter (p=0.009), nasogastric tube (p=0.012), over 24 h of admission in an ICU (p=0.008) and chemotherapy/radiotherapy (p=0.016).

Concomitant therapy/antifungal treatment

C. albicans was the most common isolate from patients receiving corticosteroids, inotropic/vasoactive amines, albumin infusion, total parentheral nutrition and gastric protection before the first episode (Table 2). *C. parapsilosis* was the most common isolate from patients receiving renal

Table 1 Demographics and clinical characteristics of patients with fungaemia and the distribution of species isolated

Characteristics	No. of isolates (%)						
	C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. neoformans*	Other fungi	Total
Gender							
Female	20 (44)	7 (16)	8 (18)	3 (7)	3 (7)	4 (9)	45 (39)
Male	21 (29)	23 (32)	7 (10)	6 (8)	9 (13)	6 (8)	72 (61)
Age (years)							
<20[a]	6 (35)	10(59)	0	0	1 (6)	0	17 (14)
20–40	7 (27)	4 (15)	4 (15)	0	7 (27)	4 (15)	26 (22)
41–60	8 (29)	6 (21)	4 (14)	4 (14)	3 (11)	3 (11)	28 (24)
61–70	8 (53)	1 (7)	3 (20)	2 (13)	0	1(7)	15 (13)
>70	12 (39)	9 (29)	4 (13)	3 (10)	1 (3)	2 (7)	31 (27)
Length of hospitalisation (days)[b]							
≤7	9 (28)	11 (34)	1 (3)	1 (3)	9 (28)	1 (3)	32 (27)
8–18	10 (42)	3 (13)	5 (21)	2 (8)	0	4 (17)	24 (20)
19–29	11 (37)	8 (27)	3 (10)	4 (13)	2 (7)	2 (7)	30 (26)
>30	11 (36)	8 (26)	6 (19)	2 (7)	1 (3)	3 (10)	31 (27)
Hospital department[c]							
ICU	16 (46)	6 (17)	7 (20)	3 (9)	2 (6)	1 (3)	35 (30)
Surgery	10 (36)	9 (32)	4 (14)	4 (14)	1 (4)	0	28 (24)
Haematology	5 (21)	10 (42)	2 (8)	1 (4)	0	6 (25)	24 (21)
Infectious Diseases	2 (15)	0	1 (8)	0	9 (69)	1 (8)	13 (11)
Other	8 (47)	5 (29)	1 (6)	1 (6)	0	2 (12)	17 (14)
Hospitalisation >24 h ICU*	23 (54)	9 (21)	7 (16)	2 (5)	1 (2)	1 (2)	43 (37)
Previous hospitalisation[d]	23 (33)	20 (29)	7 (10)	5 (7)	8 (11)	7 (10)	70 (60)
Nosocomial fungaemia	35 (40)	22 (25)	12 (14)	8 (9)	3 (3)	8 (9)	88 (75)
Primary fungaemia	24 (30)	28 (35)	10 (13)	7 (9)	4 (5)	6 (8)	79 (68)
Secondary fungaemia	17 (46)	2 (5)	5 (14)	2 (5)	8 (22)	3 (8)	37 (32)
Positive bacterial blood cultures*	23 (38)	19 (32)	6 (10)	6 (10)	1 (2)	5 (8)	60 (51)
Gastrointestinal pathology	21 (40)	14 (26)	7 (13)	7 (13)	2 (4)	2 (4)	53 (45)
Neoplasic patients	16 (30)	16 (30)	6 (11)	7 (13)	1 (1)	8 (15)	54 (46)
Chemotherapy/radiotherapy	8 (24)	11 (34)	3 (9)	3 (9)	0	8 (24)	33 (28)
Previous surgery	20 (35)	16 (28)	11 (19)	5 (9)	2 (4)	3 (5)	57 (49)
CVC at diagnostic**	37 (38)	27 (28)	15 (15)	8 (8)	2 (2)	9 (9)	98 (84)
Urinary catheter	32 (47)	14 (21)	12 (18)	5 (7)	3 (4)	2 (3)	68 (58)
Surgical drainage	13 (35)	9 (24)	9 (24)	3 (8)	2 (5)	1 (3)	37 (31)
Mechanical ventilation	14 (44)	9 (28)	5 (16)	1 (3)	2 (6)	1 (3)	32 (27)
Nasogastric tube	27 (47)	14 (25)	10 (18)	4 (7)	1 (2)	1 (2)	57 (50)
Other devices***	9 (33)	6 (22)	4 (15)	5 (19)	1 (4)	2 (7)	27 (23)
Neutropaenia/leucopaenia [e]	2 (11)	4 (22)	3 (17)	1 (6)	3 (17)	5 (28)	18 (15)
Low haemoglobin level [f]	34 (34)	25 (25)	14 (14)	9 (9)	10 (10)	9 (9)	101(88)
Low platelet count [f]	30 (41)	13 (18)	7 (10)	7 (10)	11 (15)	5 (7)	73 (65)
High glucose level [f]	22 (38)	10 (17)	10 (17)	7 (12)	4 (7)	5 (9)	58 (52)
Low total proteins level [f]	30 (40)	19 (25)	9 (12)	8 (11)	3 (4)	6 (8)	75 (67)
Low albumin level [f]	34 (40)	19 (22)	10 (12)	9 (11)	9 (11)	4 (5)	85 (77)

[a] 4%<1 yr
[b] Until collection day of the first positive blood culture from the first episode of fungaemia
[c] Hospital department where the first episode of fungaemia occurred
[d] Hospitalisation three months prior to the first fungaemia episode
[e] Neutropaenia at diagnosis
[f] When compared to reference values
*In the week prior to the first fungaemia episode
**Mean days with central vascular catheter until the first fungaemia episode: 25.3
***Medical implants, ileostomy bags

Table 2 Concomitant therapeutic and antifungal treatment of fungaemia patients. The distribution of the most common species were isolated

	No. of isolates (%)						
	C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. neoformans*	Other fungi	Total
Concomitant therapy							
Corticosteroid	12 (41)	5 (17)	5 (17)	3 (10)	1 (3)	3 (10)	29 (25)
Renal support	6 (35)	7 (41)	3 (18)	1 (6)	0	0	17 (15)
Inotropic/vasoactive support	15 (56)	5 (19)	5 (19)	1 (4)	0	1 (4)	27 (23)
Albumin infusion	19 (44)	11 (26)	7 (16)	4 (9)	1 (2)	1 (2)	43 (37)
Total parenteral nutrition	18 (50)	7 (19)	4 (11)	6 (17)	0	1 (3)	36 (31)
Gastric protection	23 (37)	15 (24)	8 (13)	6 (10)	2 (3)	8 (13)	62 (53)
Antibiotic therapy							
One antibiotic	8 (28)	11 (38)	1 (3)	0	6 (21)	3 (10)	29 (25)
More than one antibiotic	32 (41)	17 (22)	13 (17)	8 (10)	4 (5)	5 (6)	79 (68)
Antifungal treatment							
Azoles	17 (39)	12 (27)	7 (16)	0	1 (2)	7 (16)	44 (38)
Amphotericin B	4 (57)	2 (29)	0	1 (14)	0	0	7 (6)
Caspofungin	0	0	1 (50)	0	0	1 (50)	2 (2)
Azoles+amphotericin B	4 (20)	4 (20)	2 (10)	0	10 (50)	0	20 (17)
Other combinations	2 (22)	2 (22)	2 (22)	3 (33)	0	0	9 (8)

support (haemodialysis/peritoneal dialysis) (Table 2). Ninety-three percent of the patients had received antibacterial drugs and in 68% of the cases, a combination of two or more antibacterial drugs were administered simultaneously (Table 2).

Twenty-seven percent of the patients (mainly neoplasic) had been submitted to antifungal prophylaxis, with fluconazole being the most common drug administered. Seventy-one percent of the patients had already been started on antifungal therapy, with fluconazole being the most used drug; one patient had received voriconazole and two patients received itraconazole. Only 10 patients were treated with caspofungin, unaccompanied in two cases (Table 2) or associated with azoles or amphotericin B. Fungaemia persisted, despite treatment for 26 days (mean value).

Outcome of fungaemia

The crude mortality rate associated to fungaemia was 39.3%. The 30 d unfavourable outcome differs significantly for patients aged 60 years or older ($p<0.001$). The differences in overall mortality due to fungaemia by *C. glabrata* and *C. albicans* were statistically significant ($p<0.001$). Neoplasic disease (44%), nosocomial fungaemia (48%), primary (39%) and secondary fungaemia (41%) were associated with unfavourable outcome (death) (Table 3).

Predisposing factors

Patients with parenteral nutrition, gastric protection with omeprazole, presence of a CVC and surgical drainage were more prone to develop nosocomial fungaemia (Table 4).

Low platelet count, urinary catheter, gastrointestinal pathology (particularly inflammatory bowel disease) and nosocomial fungaemia showed an independent risk of unfavourable outcome (Table 5).

Table 3 Outcome of fungaemia at day 30 according to aetiological agent, underlying disease, age group and nosocomial, primary and secondary fungaemia

Characteristics	No. of deaths	Crude mortality (%)
Aetiological agent		
C. albicans (*n*=41)	19	46
C. parapsilosis (*n*=30)	9	30
C. tropicalis (*n*=15)	8	53
C. glabrata (*n*=9)	7	78
C. neoformans (*n*=12)	2	17
Other fungi (*n*=10)	1	10
Underlying disease		
Infections and parasites diseases A00–B99 (*n*=18)	4	22
Malignancies C00–D48 (*n*=54)	24	44
Endocrine disease E00–E90 (*n*=11)	2	18
Others (*n*=30)	14	46
Age group (years)		
<20 (*n*=17)	1	6
20–40 (*n*=26)	5	19
41–60 (*n*=28)	10	36
61–70 (*n*=15)	10	67
>70 (*n*=31)	20	63
Nosocomial fungaemia (*n*=88)	42	48
Primary fungaemia (*n*=79)	31	39
Secondary fungaemia (*n*=37)	15	41

Table 4 Predisposing factors associated with nosocomial fungaemia. Univariable and multivariable analysis (logistic regression) was considered

Variables	Nosocomial fungaemia no. (%)	Univariable analysis			Multivariable analysis		
		OR*	CI 95%**	*p*	$OR^*_{adjusted}$	CI 95%**	*p*
Albumin support	41 (95)	12.03	2.69–53.75	<0.001	–	–	ns
Parenteral nutrition	35 (97)	18.49	2.40–142.17	<0.001	13.46	1.65–109.90	0.015
ICU>24 h	40 (93)	7.22	2.03–25.63	0.001	–	–	ns
Gastric protection	55 (88)	5.40	2.07–14.04	<0.001	3.20	1.04–9.84	0.042
Positive bacterial blood cultures	50 (83)	2.50	1.04–5.99	0.037	–	–	ns
CVC	81 (82)	8.16	2.80–23.70	<0.001	6.06	1.47–25.01	0.013
Surgical drainage	36 (97)	19.38	2.52–149.9	<0.001	14.35	1.626–126.74	0.017
Other devices	25 (93)	5.35	1.18–24.23	0.017	–	–	ns
Urinary catheter	58 (85)	3.67	1.51–8.88	0.003	–	–	ns
Nasogastric tube	51 (90)	5.58	2.06–15.12	<0.001	–	–	ns
Gastrointestinal pathology	48 (91)	5.76	2.01–16.47	<0.001	–	–	ns

ns=not significant
*OR=odds ratio
**CI 95%=confidence interval at 95%

The analysis of factors associated with primary fungaemia disclosed that patients with a CVC showed a higher risk for primary fungaemia (odds ratio adjusted [OR_{adj}] 6.66, 95% confidence interval (CI) 2.12–20.95; p=0.001). In contrast, therapy with inotropic/vasoactive amines and female gender were significantly associated with secondary fungaemia (OR_{adj} 2.59, 95% CI 1.07–6.25; p=0.033 and OR_{adj} 3.52, 95% CI 1.33–9.32; p=0.011, respectively).

Susceptibility testing

The antifungal susceptibility profile of the strains isolated from blood cultures is detailed in Table 6 (the endpoints

Table 5 Predisposing factors associated to an unfavourable 30-day outcome after the first episode of fungaemia. Univariable and multivariable analysis (logistic regression) was considered

Variables	Outcome 30 d no (%)	Univariable analysis			Multivariable analysis		
	Died	OR*	CI 95%**	*p*	$OR^*_{adjusted}$	CI 95%**	*p*
Albumin support	25 (60)	3.75	1.651–8.55	0.001	–	–	ns
Parenteral nutrition	20 (63)	3.54	1.49–8.40	0.003	–	–	ns
ICU>24 h	24 (56)	2.78	1.24–6.19	0.011	–	–	ns
Gastric protection	30 (51)	2.70	1.19–6.13	0.016	–	–	ns
Low platelet count[a]	35 (50)	3.25	1.29–8.16	0.010	29.30	6.10–140.60	<0.001
CVC	41 (46)	.3.90	1.05–14.53	0.032	–	–	ns
Surgical drainage	21 (60)	3.19	1.38–7.40	0.006	–	–	ns
Age>70 years	20 (63)	3.59	1.48–8.68	0.004	–	–	ns
Urinary catheter	35 (54)	4.27	1.76–10.35	0.001	8.55	2.27–32.13	0.001
Gastrointestinal pathology	29 (60)	4.47	1.96–10.20	<0.001	5.50	1.59–19.05	0.007
With previous hospitalisation	21 (33)	0.45	0.21–1.00	0.05	0.23	0.07–0.74	0.014
Nosocomial fungaemia	42 (48)	4.65	1.46–14.78	0.006	5.91	1.11–31.61	0.038
Inotropic/vasoactive support	15 (58)	2.44	0.99–6.02	0.048	–	–	ns
Low total proteins level[a]	36 (50)	3.42	1.31–8.95	0.010	–	–	ns
Low albumin level[a]	39 (49)	9.75	2.13–44.53	0.001	–	–	ns
Without antifungal prophylaxis	36 (48)	2.76	1.10–6.94	0.027	–	–	ns

[a] When compared to reference values
ns=not significant
*OR=odds ratio)
**CI 95%=confidence interval at 95%

Table 6 In vitro susceptibility of fungal isolates to fluconazole, voriconazole, itraconazole, caspofungin and amphotericin B, determined according the Clinical Laboratory Standard Institute (CLSI) M27-A2 protocol

Strains (no. of isolates)	Antifungals	MIC range (μg/ml)	No. of S-DD and R strains (%)
C. albicans (41)	Fluconazole	0.125–>64	8 (20)
	Voriconazole	0.015–>8	4 (10)
	Itraconazole	0.015–>8	11 (27)
	Caspofungin	0.03–4	2 (5)
	Amphotericin B	0.03–0.5	0
C. glabrata (9)	Fluconazole	2–16	1 (11)
	Voriconazole	0.125–0.5	0
	Itraconazole	0.25–2	3 (33)
	Caspofungin	0.03–>8	2 (22)
	Amphotericin B	0.125–0.5	0
C. parapsilosis (30)	Fluconazole	0.25–4	0
	Voriconazole	0.015–0.25	0
	Itraconazole	0.015–1	1 (3)
	Caspofungin	2–>32	30(100)
	Amphotericin B	0.06–1	0
C. tropicalis (15)	Fluconazole	1–>64	4 (27)
	Voriconazole	0.06–>8	3 (20)
	Itraconazole	0.06–>8	5 (33)
	Caspofungin	0.5–32	3 (20)
	Amphotericin B	0.25–1	0
C. neoformans (12)	Fluconazole	0.125–16	0
	Voriconazole	0.015–0.125	0
	Itraconazole	0.125–0.5	0
	Caspofungin	4–>32	12 (100)
	Amphotericin B	0.015–0.5	0
All organisms (117)	Fluconazole	0.125–≥64	17 (15)
	Voriconazole	0.015–>8	9 (8)
	Itraconazole	0.015–>8	23 (20)
	Caspofungin	0.03–>32	56 (48)
	Amphotericin B	0.03–2	1 (1)
Quality control strains			
C. albicans ATCC 90028	Fluconazole	1	
	Voriconazole	0.25	
	Itraconazole	0.5	
	Caspofungin	0.5	
	Amphotericin B	0.5	
C. parapsilosis ATCC 22019	Fluconazole	4	
	Voriconazole	0.06	
	Itraconazole	0.5	
	Caspofungin	2	
	Amphotericin B	0.5	
C. krusei ATCC 6258	Fluconazole	>64	
	Voriconazole	0.25	
	Itraconazole	0.5	
	Caspofungin	0.5	
	Amphotericin B	0.5	

S-DD=susceptible dose dependent; R=resistant

shown were obtained after 48 h; they differed no more than one dilution from those obtained after 24 h). With the exception of *C. lusitaniae*, all of the strains were susceptible to amphotericin B. The largest resistance to azoles was detected among *C. tropicalis* isolates (four strains out of 15 strains being R or S-DD to fluconazole). With regards to caspofungin MICs, a good correlation was obtained between microdilution and the Etest; all *C. parapsilosis*,

C. neoformans, *Rhodotorulla* spp. and *C. guilliermondii* showed high MIC values, although most of the other species showed a susceptible pattern (Table 6). Forty percent of strains with high MIC values to caspofungin were isolated from patients receiving caspofungin within the first fungaemia episode. The MICs for the control strains were within the expected range (Table 6).

Cases of dissociation between clinical and laboratory results were found; the patients died despite the in vitro susceptibility of the strain to the administered drug (11.4% for azoles and 43% for amphotericin B), while in some other cases, despite in vitro resistance, the outcome was favourable. Eighty-one percent of patients with fungaemia due to resistant strains had been submitted to antifungal treatment (mostly with fluconazole) within the first episode of fungaemia (p=0.017). Although *Candida* spp. isolated from patients who had received azole prophylaxis were less susceptible to azoles, no significant correlations were observed.

Discussion

The incidence of invasive fungal infection is increasing in most hospitals around the world. Patients with fungaemia show a promoted risk of death during hospitalisation compared to haematogenous infections by other agents [16]. Apart from the fact that no national epidemiological data is yet available, it is important to compare our data with other countries.

In this study, fungi represented the fourth cause of septicaemia, corresponding to 3.5% of all of the microorganisms isolated from blood cultures, similar to some North American studies [16, 17]. A remarkable finding of our study was the high incidence of fungaemia. The rate of 2.7 cases per 1,000 hospital admissions were higher than those reported from the North and South America and Europe studies [18, 19, 20]. Fungaemia occurred more frequently among males, which is in agreement with previously published studies [18, 21–23], and might be related to a larger male population admitted to the ICU. In patients aged between 20 and 60 years old, fungaemia was more frequent in ICU patients, similarly to what has been found in England and Spain [22, 23].

C. albicans was the most frequent isolate, but over half of the cases were due to *Candida* non-*albicans* (59%), which confirms the increasing importance of such species as emerging agents of fungaemia [2, 3, 19, 21, 24]. Although *C. albicans* is still the most frequent cause of fungaemia in the US, the incidence of candidaemia due to *Candida* non-*albicans*, mainly *C. glabrata*, is increasing [5, 19]. This species represents the second cause of fungaemia in the US and in some European countries, with prophylaxis with fluconazole being considered as responsible for the observation [21, 25]. In our study, *C. parapsilosis* was the second most common agent, being isolated particularly in the youngest patients and in haematological patients. This happened also in Spain, Latin American and Japan, but not in other European and American studies [3, 18, 23, 26–28], where *C. glabrata* was the second most frequent isolate. In a study carried out at a Thai hospital between 1981 and 2000, *C. tropicalis* was the second most common agent after *C. albicans*, a fact considered to be related to the absence of antifungal prophylaxis [29]. Regarding this topic, our results showed a higher proportion of patients (27%) under prophylaxis, compared to the published results [18, 23].

C. neoformans was isolated from HIV patients aged between 20 to 40 years old. Our results showed an increase of these species compared to other published studies [4, 30]. This fact could be explained by a large number of HIV patients admitted at our hospital. In neoplasic patients, like in other studies, non-*albicans* strains were the predominant species (70%), with *C. parapsilosis* being the most common [18]. Around 30% of such patients did not receive antifungal treatment, probably due to the fact that candidaemia was detected in the terminal stage of the underlying disease.

Our study showed a considerable incidence of nosocomial fungaemia (75%), with *C. albicans* being the main agent of infection, which could be explained by its endogenous nature. In some cases, the colonisation/infection might have started prior to hospital admission, but evolved to clinical infection closely following the initial 48 h after admittance, although it has been defined as nosocomial according to Garner et al. [10]. It is important to emphasise that most of our patients were admitted to an ICU or surgical wards, in which CVCs or other medical implanted devices were widely used.

Earlier studies consider neutropaenia, antimicrobial therapy and the presence of bacteraemia as important risk factors for fungaemia [28, 31]. In our study, half of the patients had positive bacterial blood cultures prior to the first fungaemia episode.

The route of the acquisition of infection is interesting to determine the risk of nosocomial transmission and to introduce appropriate preventive measures. Fungaemia by *Candida* spp. in critical patients is considered to have an endogenous origin in the gastrointestinal tract [32]. In our study group, 34 patients had been submitted to gastrointestinal surgery, while, later, 33 had developed nosocomial fungaemia. Interestingly, patients treated with omeprazol showed a three-fold greater risk of developing nosocomial fungaemia. The change of the gastrointestinal protective barrier could be an explanation favouring this form of

adherence and colonisation. Another method for the acquisition of *Candida* infection results from an exogenous source, like the colonisation of indwelling catheters. Parenteral nutrition, the presence of a CVC and surgical devices were significantly associated in our study with nosocomial fungaemia. Vasoactive/inotropic amines support therapy was highly associated to secondary fungaemia, possibly due to promotion of the growth rate of *Candida* spp. (authors' unpublished data). Our data showed that, as with other recent studies [33, 34], there was a significant correlation between thrombocytopaenia and mortality due to fungaemia, a topic deserving further investigation.

The crude mortality rate of fungaemia was 39.3%, which is similar to the European Confederation of Medical Mycology (ECMM) survey and other recent studies [5, 18, 21]. Mortality by *C. glabrata* was 78%. Such a high value is most probably related to its nosocomial nature in most cases, and with the underlying neoplasic disease, patient age (over 40 years old) and the lack of antifungal treatment when comparing to *C. albicans* cases. In addition, more than 50% of *C. glabrata* fungaemia patients had been submitted to surgery. The crude mortality of nosocomial fungaemia was similar to previous descriptions [1, 35].

Regarding susceptibility profiles, it is important to notice the high level of resistance to fluconazole (15%) compared to other studies, mainly in cases of *C. albicans* [15, 18, 23]. This might be related to the wide use of this drug in treatments as well as in prophylaxis [36]. Strong resistance was also found regarding *C. tropicalis* (27%), similar to that reported by Péman et al. [23] (23%) and St-Germain et al. (27%) [37]. In relation to *C. albicans*, some of the isolates showed cross-resistance between azoles, a fact that can be explained by the presence of efflux pumps [38]. In this study, susceptibility to caspofungin was determined by microdilution and the Etest, as it has not been yet standardised. High MIC values for caspofungin were detected among *C. parapsilosis* and *C. neoformans* (considered as intrinsically resistant) in accordance with other authors [15, 39]. Several aspects in need of further investigation have been raised with this study. The dissociation between susceptibility testing and the outcome of the patient deserves further attention. Presently, we are focussing on the impairment produced by concomitantly administered drugs upon the susceptibility pattern.

The high mortality rate as well as the incidence of nosocomial fungaemia found in this study represents important issues in terms of public health and economics. This research also stresses the need for surveillance studies for monitoring changes in species distribution and antifungal susceptibility profiles.

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