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Original Article

Microbiologic and clinical characteristics of biofilm-forming *Candida parapsilosis* isolates associated with fungaemia and their impact on mortality☆

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ABSTRACT

Objectives: Biofilm formation (BF) by fungal isolates may dramatically complicate infection. We determined the ability of *Candida parapsilosis* isolates from single fungaemia episodes to form biofilms and we analysed biofilm subgroups for antifungal susceptibility and pathogenic potential. We then correlated BF with clinical characteristics and outcomes of the episodes.

Methods: BF was measured using the crystal violet biomass assay. Antifungal susceptibility of preformed biofilms was assessed, and virulence was studied using the *Galleria mellonella* model. A retrospective analysis of patients' clinical records was performed.

Results: Of 190 patient-unique isolates, 84, 38 and 68 were identified as having high BF (HBF), moderate BF (MBF) or low BF (LBF), respectively. Among 30 randomly selected isolates, nine (eight HBF and one MBF), six (all HBF) and one (HBF) isolates had elevated sessile minimum inhibitory concentrations to fluconazole, anidulafungin or amphotericin B; all HBF and MBF isolates had elevated voriconazole sessile minimum inhibitory concentrations. *G. mellonella* killing rates of HBF isolates were significantly greater than MBF (or LBF) isolates (50% vs. 20%, 2 days from infection). By comparing HBF/MBF (106 patients) and LBF (84 patients) groups, we found that HBF/MBF patients had more central venous catheter-related fungaemias (62/106 (58.5%) vs. 29/84 (34.5%), p 0.001) and were more likely to die at 30 days from fungaemia onset (61/106 (57.5%) vs. 28/84 (33.3%), p 0.01). In the HBF/MBF group, azole antifungal therapy and central venous catheter removal were significantly associated with a higher and lower 30-day mortality rate, respectively.

Conclusions: *C. parapsilosis* BF influences the clinical outcome in patients with fungaemia. **S. Soldini, Clin Microbiol Infect 2017;▪:1**

Introduction

In southern European surveys of *Candida* bloodstream infections (BSIs), *C. parapsilosis* was the second most commonly isolated species after *C. albicans* [1–4], and many isolates have been shown to exhibit decreased susceptibility to antifungal agents *in vitro* [5–8]. *Candida* BSIs are complicated by the propensity of isolates to adhere to and grow as biofilms on indwelling medical devices, such as intravascular catheters [9]. From these reservoirs, persister (antifungal tolerant) *Candida* cells [10,11] tend to migrate into the

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bloodstream [12]. Biofilm-forming *Candida* BSIs have been associated with the highest hospital mortality [13,14]. Thus, removal of the central venous catheter (CVC) is the only viable treatment option [15].

According to their different biofilm formation (BF) capabilities [16], clinical *C. parapsilosis* isolates feature distinct biofilm structures [17]. BF by *C. parapsilosis* should be considered within well-defined categories [18], especially when looking for clinical correlations with this phenotypic property [19]. Previously we found that hospital mortality was significantly greater in patients with biofilm-forming *Candida* BSI than in patients with non-biofilm-forming *Candida* BSI, but we reported only very restricted categories, i.e. biofilm formers and non-biofilm formers, of *Candida* isolates in our analysis of association with clinical outcomes [20].

The aim of the present study was to characterize BF by a large number of *C. parapsilosis* bloodstream isolates and subsequently analyse biofilm subgroups with respect to *in vitro* antifungal susceptibility and *in vivo Galleria mellonella* pathogenicity. Furthermore, a retrospective analysis of the relative cohort of patients was performed to determine if isolates' biofilm levels were related to clinical characteristics of fungaemias associated with these isolates.

Materials and methods

Study design and setting

We carried out a retrospective study of *C. parapsilosis* isolates consecutively recovered from BSIs at a 1200-bed tertiary care institution in Rome, Italy, between January 2005 and December 2015. All the first episodes of fungaemia were included in the study. Patients with polymicrobial fungaemia ($n = 10$) or with incomplete clinical data ($n = 2$) were excluded. Twenty-five patients and isolates have been previously studied [20]. For each patient, demographic and clinical information included hospital ward, underlying medical conditions, date of fungaemia onset (i.e. the day of the first positive blood culture), risk factors for fungaemia, time at risk (i.e. the number of hospital days from admission to the onset of fungaemia), details of antimicrobial therapy, length of hospital stay after the onset of fungaemia and 30-day outcome (i.e. assessed from the first positive blood culture until 30 days or death). The study protocol was approved by the institutional ethics committee (no. 1401/16), but informed consent from all patients was waived because of the observational nature of this study.

Isolate collection and identification

C. parapsilosis isolates from patients' blood cultures were identified as described elsewhere [5]. Isolates were kept frozen in glycerol and were subcultured at the time of this study. Before testing, isolates were analysed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [21] to include only isolates ($n = 190$) identified as *C. parapsilosis sensu stricto* (hereafter referred to as *C. parapsilosis*). Isolates ($n = 9$) identified as *C. orthopsilosis*, which together with *C. metapsilosis* belongs to the *C. parapsilosis* species complex [22], were excluded.

BF and antifungal susceptibility testing

All *C. parapsilosis* isolates were tested for BF according to Marcos-Zambrano et al. [18]. Briefly, 100 μL of each isolate's cell suspension standardized to 1×10^6 cells/mL in RPMI 1640 broth medium (Sigma-Aldrich, St. Louis, MO, USA) was allowed to grow as biofilms in 96-well microtitre plates at 37°C for 24 hours. After BF, the wells were washed with 100 μL of a phosphate-buffered saline (PBS) solution to discard nonadhered cells. The biomass level of each isolate was quantified using the crystal violet assay [23]. Isolates were then categorized using optical density at 540 nm ($OD_{540\ nm}$) cutoff values (<0.44, 0.44–1.17 and >1.17), recently proposed to classify *Candida* species isolates [18], as having low BF (LBF), moderate BF (MBF) and high BF (HBF), respectively.

Isolates with LBF ($n = 10$), MBF ($n = 10$) and HBF ($n = 10$), which were chosen randomly among others, were tested for antifungal susceptibility using 24-hour-old biofilms individually treated with each antifungal agent (fluconazole, voriconazole, anidulafungin or amphotericin B) for 24 hours in 96-well microtitre plates. All antifungal agents were prepared according to the Clinical and Laboratory Standards Institute (CLSI) [24] and were used at drug concentration ranging 0.03 to 64 mg/L. Sessile minimum inhibitory concentrations (SMICs) were assessed on the basis of the metabolic reduction of 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide [25]. The SMICs were expressed as the lowest drug concentrations at which a 50% decrease in absorbance was detected compared to that of the biofilms formed in the absence of drug [26]. The minimum inhibitory concentrations (MICs) for planktonic cells were determined by the CLSI reference method and were defined as the lowest drug concentrations that caused either an approximately 50% (for fluconazole, voriconazole and anidulafungin) or 100% (for amphotericin B) growth inhibition compared to that of the drug-free growth control [24].

In vivo killing assay using the G. mellonella model

Killing assays in *G. mellonella* were performed to assess the virulence of randomly selected LBF ($n = 3$), MBF ($n = 3$) and HBF ($n = 3$) *C. parapsilosis* isolates [19,27,28]. Briefly, sixth-instar larvae (300–350 mg in weight; RedBug.it, Milan, Italy) were inoculated within 24 hours of receipt. A Hamilton syringe fitted with a 26-gauge blunt needle was used to inject 10 μL of each isolate's suspension in sterile PBS (5×10^5 cells per larva) into the larval haemocoel. At least ten larvae were inoculated per isolate per experiment; experiments used three independent isolates of each group. Control groups of larvae receiving 10 μL of sterile PBS in exactly the same manner or mock-inoculated larvae pierced on the proleg with a sterile needle (noninjected larvae) were also included in each experiment. Larvae were incubated at 37°C. Survival was recorded at 24-hour intervals for 7 days. Larvae were considered dead when they displayed no movement in response to touch together with a dark discoloration of the cuticle. According to previous studies [29,30], histologic analysis of the larvae after infection was performed by killing representative larvae from each group at 24, 48 and 72 hours after infection. Briefly, the larvae were inoculated with buffered formalin and processed to obtain transverse-cut sections for microscopic evaluation. Tissue sections were stained with periodic acid–Schiff and examined by a technician and a pathologist. Image acquisition was performed by the NanoZoomer-XR C12000 series (Hamamatsu Photonics, Hamamatsu City, Japan).

Definitions of clinical data and therapeutic measures

An episode of fungaemia was defined as the isolation of *C. parapsilosis* in one or more blood cultures obtained from a peripheral vein of a patient with consistent clinical manifestations. The episode was defined as catheter related if the same *Candida* species was also isolated from a catheter tip [31]. In most episodes, the differential time of positivity was also used as an indicator of catheter-related fungaemia [32]. Fungaemia was defined as nosocomial if it occurred more than 48 hours after admission to the hospital and if no signs or symptoms of infection were noted at admission (https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/0512-TED-PPS-HAI-antimicrobial-use-protocol.pdf).

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Septic shock was defined as refractory hypotension despite adequate fluid resuscitation [33]. CVC and total parenteral nutrition were considered to be risk factors if they were present at the onset of fungaemia. Previous antimicrobial therapy was defined as any antimicrobial agent administration within 30 days of the onset of fungaemia. Surgery and immunosuppressive therapy were considered to be risk factors if the patient had undergone any major surgical procedure or had received prednisone (or other immunosuppressant agents) or any chemotherapy within 3 months of the onset of fungaemia. With regard to the therapeutic measures to be used as variables, adequate antifungal therapy was defined as the initiation of antifungal therapy provided at a recommended dosage within 48 hours after the first blood culture was obtained [15], with isolation of an organism that was found to be susceptible *in vitro* to the antifungal agent used. Source control was defined adequate if, within 48 hours from the first blood culture, any CVCs were removed or when a surgical or radiologic procedure to drain abscess or fluid collection was performed.

Statistical analysis

Data are presented as median (interquartile range) or as counts (%) as appropriate. To assess for differences between patient groups, categorical variables were compared by the chi-square test or Fisher's exact test, as appropriate, and continuous variables were compared by Student's *t* test. Odds ratios and 95% confidence intervals were calculated. To identify predictive factors for 30-day mortality, variables with $p < 0.2$ in univariate analysis were included in multivariate analyses, which were conducted using stepwise logistic regression. The Kaplan-Meier method with the log-rank test was used to assess the effect of variables on patient survival. Survival curves adjusted for the variables we identified that were independently associated with mortality were estimated and compared by a Cox proportional hazards regression model. One-way analysis of variance or Student's *t* test were used, as appropriate, to measure statistical differences between two or more groups assessed in microbiologic studies, whereas *G. mellonella* survival curves were analysed by the log-rank test. In all analyses, $p < 0.05$ was considered statistically significant. Calculations were performed with either Stata 11.1 (StataCorp, College Station, TX, USA) or GraphPad Prism 7.03 (GraphPad Software, La Jolla, CA, USA).

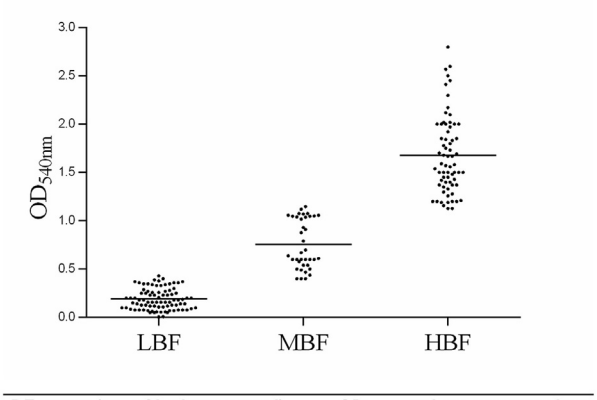
Results

Microbiologic characteristics of C. parapsilosis isolates

We first characterized 190 *C. parapsilosis* BSI isolates for the ability to form biofilms *in vitro*. According to cutoff values established previously [18], isolates were grouped into LBF (84/190; 44.2%), MBF (38/190; 20.0%) and HBF (68/190; 35.8%) categories (Fig. 1). Two further groups, LBF ($OD_{540\ nm} < 0.44$, 84 isolates) and HBF/MBF ($OD_{540\ nm} \geq 0.44$, 106 isolates), were defined.

The distributions of antifungal MICs and SMICs for *C. parapsilosis* isolates among LBF ($n = 10$), MBF ($n = 10$) and HBF ($n = 10$) groups are shown in Supplementary Table S1. According to CLSI breakpoint and epidemiologic cutoff MIC values [34], all 30 isolates grown planktonically were antifungal susceptible. Although MICs could not be compared to SMIC values, we found that SMICs to anidulafungin and fluconazole were >4 mg/L for six (all HBF) and nine (eight HBF and one MBF) isolates, respectively, and one HBF isolate had a SMIC of >2 mg/L to amphotericin B; in contrast, voriconazole SMICs for isolates from the HBF and MBF groups were all >0.5 mg/L.

A shown in Fig. 2(a), inoculation of *G. mellonella* larvae with HBF isolates was shown to kill 50% after 2 days and 80% after 6 days, whereas larvae inoculated with MBF or LBF isolates exhibited only 20% mortality after 2 days. Differences in survival of HBF larvae compared to MBF (or LBF) larvae were found to be statistically significant. Furthermore, microscopy studies of larvae at 24, 48 and 72 hours after infection with HBF, MBF and LBF representative isolates showed substantial differences in pathogenicity (Fig. 2(b)), particularly at 48 hours, where a strong recruitment of haemocytes in the infected area was detected in both HBF and MBF larvae.



BF group (no. of isolates tested)	$OD_{540\ nm}$ values expressed as geometric mean ± SD/range
Low BF (84)	0.153 ± 0.108/0.006–0.400
Moderate BF (38)	0.701 ± 0.252/0.442–1.150
High BF (68)	1.618 ± 0.406/1.201–2.820

Fig. 1. Distribution of 190 *Candida parapsilosis* isolates into biofilm formation (BF) groups. Isolates were grouped depending on levels of biomass production, as quantified spectrophotometrically by $OD_{540\ nm}$ reading (ELx808 Absorbance Microplate Reader instrument; BioTek Instruments, Winooski, VT, USA). The following groups were established: $OD_{540\ nm}$ cutoff value <0.44, low BF; $OD_{540\ nm}$ cutoff value from 0.44 to 1.17, moderate BF; and $OD_{540\ nm}$ cutoff value >1.17, high BF. $OD_{540\ nm}$, optical density at 540 nm.

Clinical characteristics of patients infected by C. parapsilosis isolates

The characteristics of patients with HBF/MBF ($n = 106$) or LBF ($n = 84$) *C. parapsilosis* infections are shown in Table 1. The majority of patients had a CVC in place, had received parenteral nutrition and had previously been exposed to antibacterial drugs at the BSI diagnosis, and 91 (47.9%) of 190 fungaemias were due to a catheter-related infection. Patients infected with LBF *C. parapsilosis* isolates had more preexposure to cephalosporins, whereas patients infected with HBF/MBF *C. parapsilosis* isolates had more CVC-related infections and were likely to die within 30 days of onset of BSI.

Relationship between BF and mortality in C. parapsilosis fungaemia

Supplementary Table S2 compares patients with different 30-day outcome (101 alive and 89 dead). Multivariate logistic regression analysis showed that infection by HBF/MBF *C. parapsilosis*, presentation with septic shock and adequate therapeutic measures were significantly and independently associated with 30-day mortality (Table 2).

To better address the association between BF and mortality in *C. parapsilosis* fungaemia, we monitored the survival of patients from the HBF/MBF and LBF groups over a period of 30 days. Kaplan-Meier curves showed that patients infected with HBF/MBF isolates had a significantly lesser likelihood of survival than patients infected with LBF isolates (Fig. 3(a)). After adjustments for those factors identified as independent predictors of death (Table 2),

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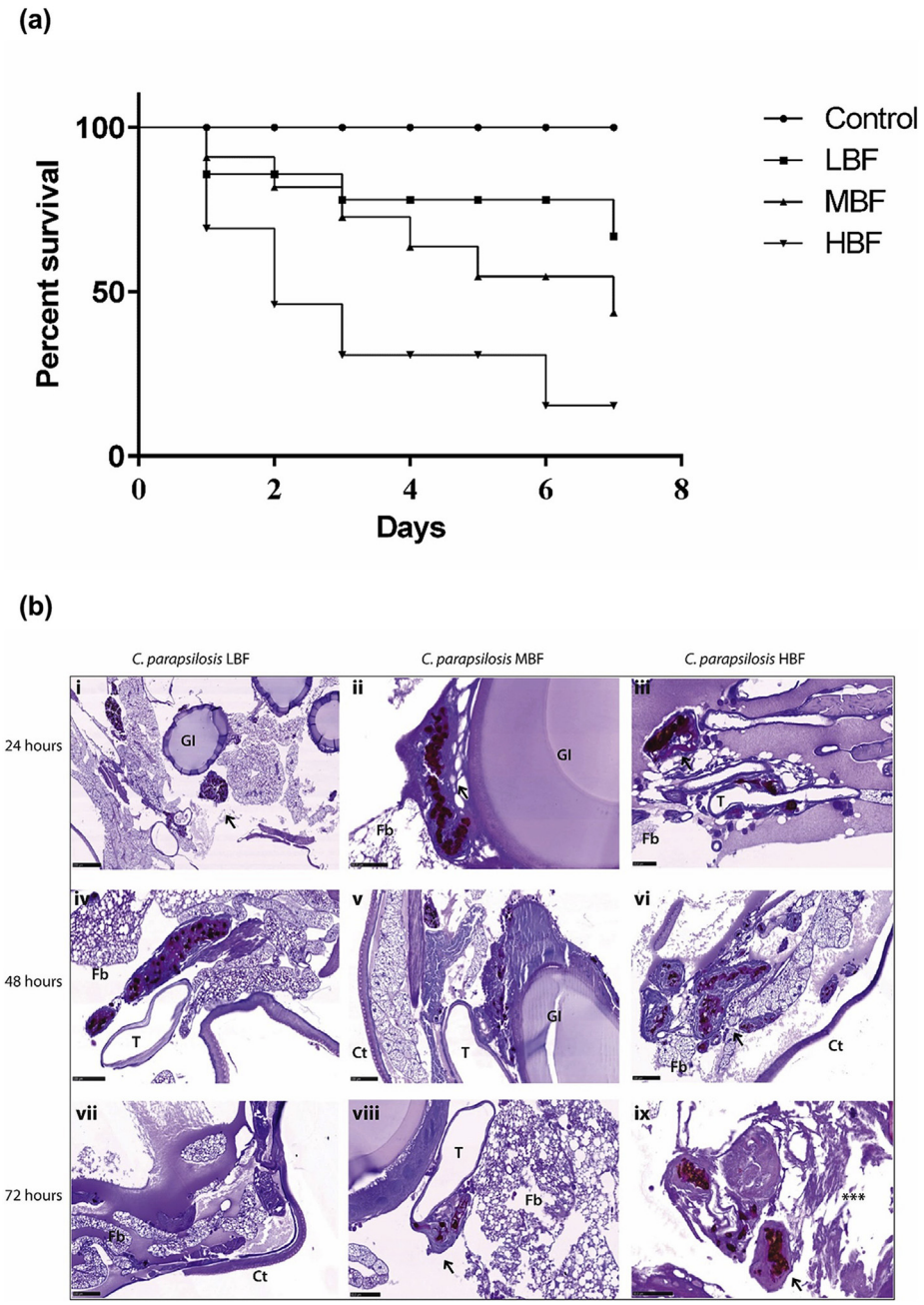

Fig. 2. Comparison of virulence from HBF, MBF and LBF isolates of *Candida parapsilosis* using *G. mellonella* as infection model. (a) Kaplan-Meier plots of *Galleria mellonella* survival after injection with 1×10^5 CFU of larvae of indicated group of isolates. Three isolates were tested per group, with ten larvae per isolate. Experiments were performed in duplicate; plots represent combined (additive) data from all isolates and all experiments. No larval killing was observed in phosphate-buffered saline–injected (or uninjected) larvae, which were included as negative control. Differences were statistically significant ($p < 0.0001$). (b) Histologic images of formalin-fixed infected larvae. At 24 hours, LBF (i) and MBF (ii) infected larvae had several melanization spots, with nodules mostly present under cuticle (Ct) and in peripheral fat body (Fb), whereas HBF (iii) infected larvae had larger nodules with greater melanin deposition also involving tracheal system (T). At 48 hours, LBF (iv) and MBF (v) infected larvae had small nodules containing both yeast and some pseudohyphae in deeper larval tissues (GI tract), whereas HBF (vi) had elongated hyphae targeting GI walls. At 72 hours, LBF (vii) and MBF (viii) infected larvae showed signs of persistent infection and increased haemocyte circulation. HBF (ix) infected larvae were also characterized by intense tissue damage with wide necrosis of fat body. Arrows indicate nodules; asterisks indicate necrosis. CFU, colony-forming unit; GI, gastrointestinal; HBF, high biofilm formation; LBF, low biofilm formation; MBF, moderate biofilm formation.

differences in survival between the two groups of patients were again statistically significant (Fig. 3(b)). Furthermore, we analysed the 106 HBF/MBF *C. parapsilosis* infections according to the patients' outcome (61 dead and 45 alive). We found that the receipt of initial antifungal treatment with azoles and the initiation of antifungal therapy beyond first 48 hours were significantly associated with death (Table 3).

Discussion

C. parapsilosis isolates were able to produce biofilms characterized by high or moderate biomass, and HBF/MBF affected *in vitro* (i.e. antifungal susceptibility) phenotypic and *in vivo* (i.e. *G. mellonella*) pathogenic features. We identified HBF/MBF as a risk factor for mortality after the *C. parapsilosis* fungaemia diagnosis,

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Table 1
Demographic and clinical characteristics of patients with *Candida parapsilosis* fungaemia according to level of biofilm formation by blood culture isolates

Characteristic	All patients (n = 190)	Patients infected with isolates categorized as:		p
		HBF/MBF (n = 106)	LBF (n = 84)	
Age, years, median (interquartile range)	67 (58–77)	67 (59–77)	67.5 (55–76.5)	0.73
Male sex	115 (60.5)	70 (66.0)	45 (53.6)	0.08
Department				
Medical	117 (61.6)	66 (62.3)	51 (60.7)	0.82
Surgical	45 (23.7)	28 (26.4)	17 (20.2)	0.31
Intensive care unit	28 (14.7)	12 (11.3)	16 (19.0)	0.13
Underlying disease				
Diabetes	46 (24.2)	25 (23.6)	21 (25.0)	0.82
Chronic obstructive pulmonary disease	39 (20.5)	23 (21.7)	16 (19.0)	0.65
Cardiovascular disease	21 (11.0)	13 (12.3)	8 (9.5)	0.54
Chronic liver failure	22 (11.6)	14 (13.2)	8 (9.5)	0.43
Chronic renal failure	43 (22.6)	23 (21.7)	20 (23.8)	0.72
Inflammatory bowel disease	19 (10.0)	11 (10.4)	8 (9.5)	0.84
HIV infection	6 (3.1)	4 (3.7)	2 (2.4)	0.58
Risk factor				
CVC	152 (80.0)	88 (83.0)	64 (76.2)	0.24
Total parenteral nutrition	132 (69.5)	70 (66.0)	62 (73.8)	0.24
Surgery	80 (42.1)	48 (45.3)	32 (38.1)	0.31
Mechanical ventilation	22 (11.6)	10 (9.4)	12 (14.3)	0.29
Previous other-than cephalosporin antibacterials	177 (93.1)	101 (95.3)	76 (90.5)	0.19
Previous cephalosporins	33 (17.4)	13 (12.3)	20 (23.8)	0.03*
Previous antifungals	16 (8.4)	12 (11.3)	4 (4.8)	0.10
Corticosteroids	60 (31.6)	34 (32.1)	26 (30.9)	0.86
Chemotherapy	35 (18.4)	18 (17.0)	17 (20.2)	0.56
Multifocal *Candida* colonization	26 (13.7)	16 (15.1)	10 (11.9)	0.52
Neutropenia (<500 cells/mm^3)	14 (7.4)	6 (5.7)	8 (9.5)	0.31
Clostridium difficile-associated diarrhoea	9 (4.7)	6 (5.7)	3 (3.6)	0.50
Time between hospitalization and onset of fungaemia, days, median (interquartile range)	19 (10–36)	18.5 (10–37)	19 (10–35)	0.98
Type/severity of infection				
In-hospital infection	186 (97.9)	104 (98.1)	82 (87.6)	0.81
Septic shock	45 (23.7)	26 (24.5)	19 (22.6)	0.76
Bacteria in incident blood culture	22 (11.6)	15 (14.1)	7 (8.3)	0.21
Source of infection				
CVC	91 (47.9)	62 (58.5)	29 (34.5)	0.001*
Primary	81 (42.6)	35 (33.0)	46 (54.8)	0.002*
Abdomen	8 (4.2)	5 (4.7)	3 (3.6)	0.69
Urine	6 (3.1)	2 (1.9)	4 (4.8)	0.26
Other	4 (2.1)	2 (1.9)	2 (2.4)	0.002*
Therapeutic measures (≤48 hours)				
Adequate antifungal therapy	74 (38.9)	44 (41.5)	30 (35.7)	0.42
CVC removal[b]	133/152 (87.5)	77/88 (87.5)	56/64 (87.5)	1
Length of hospital stay (days), median (interquartile range)	40 (26–62)	41 (26–63)	39 (26–60)	0.79
Mortality				
At 7 days	40 (21.0)	31 (29.2)	9 (10.7)	0.001*
At 30 days	89 (46.8)	61 (57.5)	28 (33.3)	0.01*

Data are presented as n (%) unless otherwise indicated.
CVC, central venous catheter; HBF, high biofilm formation; LBF, low biofilm formation; MBF, moderate biofilm formation.
*Statistically significant ($p < 0.05$).
[a]Including carbapenems, aminoglycosides, glycopeptides, fluoroquinolones, β-lactams except cephalosporins and other antimicrobial agents.
[b] In subset of patients with CVCs.

and we correlated adequate therapeutic measures with the mortality in fungaemias caused by HBF/MBF isolates.

Compared to *C. albicans* and other so-called CUG species (i.e. related species that use a nonstandard translation for that codon), *C. parapsilosis* should be not only less virulent in animal models of disseminated infection but also less fit in *in vitro* assays of BF [35]. Nonetheless, we found the approximately 56% of our isolates (68 HBF and 38 MBF) robustly adhered to microplate wells in RPMI 1640, as measured by the retention of crystal violet. Similar to our findings, Marcos-Zambrano et al. [18] found that many of their *C. parapsilosis* bloodstream isolates were MBF (66/162, 41%), followed by HBF (27/162, 17%), thereby supporting the high strain-to-strain variability in BF. However, strain differences can have important clinical repercussions with respect to treatment and pathogenicity [19,36].

Whereas voriconazole was equally ineffective against HBF and MBF isolates, higher SMICs to anidulafungin and fluconazole were primarily found in the HBF group. Only one HBF isolate had a higher SMIC to amphotericin B. As in *C. albicans* [19,37], HBF isolates displayed significantly greater killing rates of *G. mellonella* larvae than

Table 2
Regression analysis of variables associated with 30-day mortality.

Characteristic	OR	95% CI
HBF/MBF *Candida parapsilosis* infection	3.85	1.88–7.87
Presentation with septic shock	3.78	1.63–8.75
Receipt of adequate antifungal therapy (≤48 hours)	0.26	0.13–0.54
CVC removal (≤48 hours)	0.21	0.10–0.45

All variables with univariate p values of <0.20 were included for multivariate stepwise logistic regression analysis.
CI, confidence interval; CVC, central venous catheter; HBF, high biofilm formation; MBF, moderate biofilm formation; OR, odds ratio.

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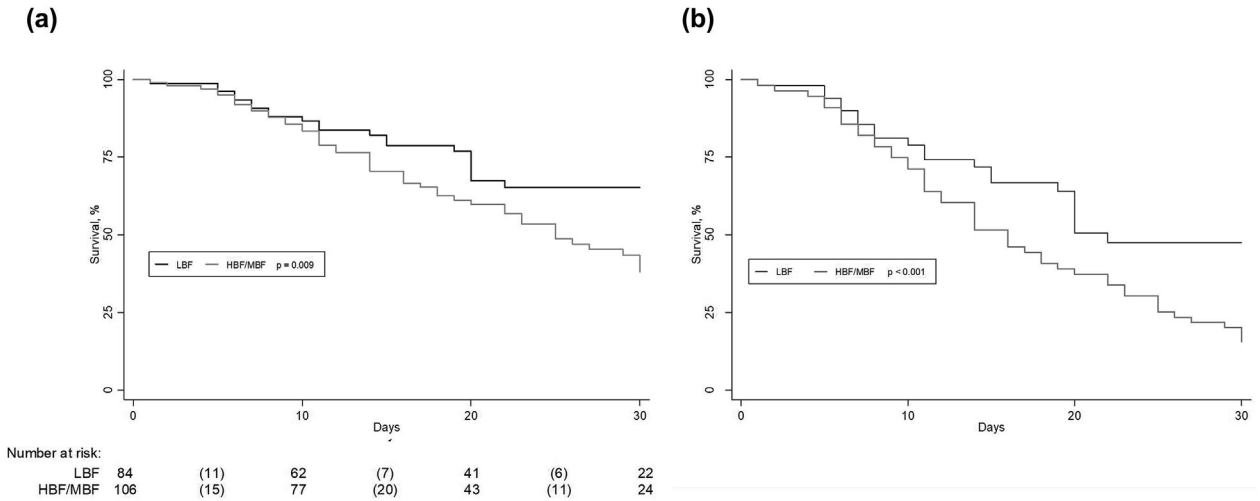

Fig. 3. Survival of patients with either LBF or HBF/MBF *Candida parapsilosis* fungaemia who were monitored over 30 days from first positive blood culture. (a) Kaplan-Meier survival curve. (b) Kaplan-Meier survival curve adjusted for presentation with septic shock, receipt of adequate antifungal therapy (≤48 hours) and removal of central venous catheter (≤48 hours). Comparison between these curves showed statistically significant difference in mortality rate. Shown are number of patients in both LBF and HBF/MBF groups who were available for follow-up at beginning of each interval (10, 20, and 30 days) of survival curves depicted in (a). HBF, high biofilm formation; LBF, low biofilm formation; MBF, moderate biofilm formation.

Table 3
Therapeutic measures affecting 30-day mortality rate in 106 patients with HBF/MBF *Candida parapsilosis* fungaemia

Characteristic	Died ($n = 61$)	Alive ($n = 45$)	OR (95% CI) for mortality
Initial antifungal therapy			
Azole based	36 (59.0%)	11 (24.4%)	4.63 (1.83–12.03)
Non-azole based[a]	24 (39.3%)	34 (75.5%)	0.21 (0.08–0.54)
Switch to azole therapy[b]	4/24 (16.7%)	14/34 (41.2%)	0.29 (0.05–1.14)
Causes of inadequate antifungal therapy			
Initiation beyond first 48 hours	43 (70.5%)	18 (40.0%)	3.58 (1.47–8.75)
No antifungal therapy	1 (1.6%)	0 (0.0%)	—
CVC removal (≤48 hours)[c]	36/47 (76.6%)	39/41 (95.1%)	0.17 (0.02–0.86)

CI, confidence interval; CVC, central venous catheter; HBF, high biofilm formation; MBF, moderate biofilm formation; OR, odds ratio.
[a] Includes treatment with echinocandin (anidulafungin or caspofungin) or polyene (amphotericin B).
[b] In subset of patients who were initially treated with azoles.
[c] In subset of patients with CVCs ($n = 88$).

LBF isolates; there were also significant differences in larval survival between HBF and MBF isolates, with higher survival in the MBF group. Histologic analysis of infected larvae revealed a similar cell morphology of yeast and pseudohyphae among LBF, MBF and HBF isolates, which is consistent with the grouping of *C. parapsilosis* with the less virulent *Candida* species [35].

We showed that intense BF was an independent factor of poor prognosis in patients with *C. parapsilosis* fungaemia (30-day mortality, 46.8%). A CVC-related *C. parapsilosis* fungaemia was diagnosed more frequently in HBF/MBF patients than in LBF patients. Interestingly, in the HBF/MBF patient subgroup, initial antifungal treatment with echinocandins or polyenes tended to be associated with lower 30-day mortality, whereas treatment with azoles was associated with higher 30-day mortality. Similarly, CVC removal and inadequate antifungal therapy had the opposite effects on the 30-day mortality rate.

Our study is limited by its retrospective design, its single centre nature and its limited size. We were unable to test other methods of biofilm development owing to the large number of isolates studied. However, standardized protocols for the *in vitro* testing of BF on clinical *Candida* isolates are still lacking.

This study is of interest for clinicians who treat patients with non-*albicans Candida* species BSIs according to the current guidelines that support the empirical use of an echinocandin, pending final identification and susceptibility testing [15,38]. Although the initial use of echinocandins does not necessarily translate into clinical failure even if the isolated *Candida* is later identified as *C. parapsilosis* [39], the knowledge of the BF status of the *Candida* isolate [40] could support patient treatment with polyenes or echinocandins, which have long been known to have better activity against biofilms than the azoles [41].

In conclusion, HBF or MBF from *C. parapsilosis* isolates is associated with a high mortality rate in patients with fungaemia. Further clinical studies and microbiologic analyses with larger cohort of patients and isolates are justified to better interpret the relevance of our findings.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2017.11.005.

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Transparency declaration

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