BMC Infectious Diseases

RESEARCH

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# Biofilm formation and increased mortality among cancer patients with candidemia in a Peruvian reference center

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## Abstract

**Background** Candidemia is an invasive mycosis with an increasing global incidence and high mortality rates in cancer patients. The production of biofilms by some strains of *Candida* constitutes a mechanism that limits the action of antifungal agents; however, there is limited and conflicting evidence about its role in the risk of death. This study aimed to determine whether biofilm formation is associated with mortality in cancer patients with candidemia.

**Methods** This retrospective cohort study included patients treated at Peru's oncologic reference center between June 2015 and October 2017. Data were collected by monitoring patients for 30 days from the diagnosis of candidemia until the date of death or hospital discharge. Statistical analyses evaluated the association between biofilm production determined by XTT reduction and mortality, adjusting for demographic, clinical, and microbiological factors assessed by the hospital routinary activities. Survival analysis and bivariate and multivariate Cox regression were used, estimating the hazard ratio (HR) as a measure of association with a significance level of $p < 0.05$.

**Results** A total of 140 patients with candidemia were included in the study. The high mortality observed on the first day of post-diagnosis follow-up (81.0%) among 21 patients who were not treated with either antifungal or antimicrobial drugs led to stratification of the analyses according to whether they received treatment. In untreated patients, there was a mortality gradient in patients infected with non-biofilm-forming strains vs. low/medium and high-level biofilm-forming strains (25.0%, 66.7% and 82.3%, respectively, $p = 0.049$). In treated patients, a high level of biofilm formation was associated with increased mortality (HR, 3.92; 95% $p = 0.022$), and this association persisted after adjusting for age, comorbidities, and hospital emergency admission (HR, 6.59; CI: 1.87–23.24, $p = 0.003$).

**Conclusions** The association between candidemia with in vitro biofilm formation and an increased risk of death consistently observed both in patients with and without treatment, provides another level of evidence for a possible causal association. The presence of comorbidities and the origin of the hospital emergency, which reflect the fragile clinical condition of the patients, and increasing age above 15 years were associated with a higher risk of death.

**Keywords** Biofilms, Candidemia, Mortality, Cancer, Risk factors

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## Background

Candidemia is a nosocomial fungal infection worldwide [1–5] with rising numbers of cases due to the increase in immunocompromised patients in recent decades [6–10], and mortality rates between 30 and 50% among cancer patients [11–16]. There are known risk factors for death among patients with candidemia, such as advanced age, high Acute Physiology and Chronic Health Evaluation (APACHE II) score, presence of a central venous catheter (CVC), and lack of or inadequate antifungal treatment [17–26]. Additionally, the intrinsic characteristics of each *Candida* strain may also influence the clinical course of the disease [27–29].

Biofilms, which are microbial communities contained in an extracellular matrix, allow *Candida* yeasts to tolerate high concentrations of antifungal agents and evade the host immune response [30–35]. Evidence suggests that candidemia caused by biofilm-producing strains leads to an increased risk of death. Removal of CVCs, the main source of biofilm formation in clinical settings [36, 37], and treatment with echinocandins, an effective antifungal against *Candida* biofilms [38, 39], are associated with lower mortality in patients with candidemia [40–43]. However, these studies have not accounted for the role of biofilm formation by *Candida* spp. strains on disease outcomes.

In addition, there is conflicting evidence regarding biofilm formation and mortality in patients with candidemia [44–51], as some studies were unable to find an association [49–51], possibly due in part to sample size limitations and failure to adjust for confounding variables.

The high mortality of candidemia among immunocompromised patients, the potential additional mortality risk of biofilm-producing *Candida* strains, and the limited and contradictory evidence from previous studies, warrant a better understanding of the role of biofilms in the survival of patients with candidemia. Therefore, we evaluated whether the formation of biofilms is associated with increased mortality among cancer patients with candidemia.

## Methods

### Study design

In this retrospective cohort study, we compared the mortality of patients with candidemia caused by biofilm- and non-biofilm-forming strains. We analyzed samples and data collected from cancer patients at Peru's National Oncology Reference Center (Instituto Nacional de Enfermedades Neoplasicas, INEN as per its acronym in Spanish) at candidemia diagnosis and during the following 30 days. The analyzed data was routinely collected in medical records by the treating physician, and in the INEN Microbiology Laboratory records. In addition, biofilm production assays were conducted on the strains collected during the study period. This test is not part of the laboratory routine evaluations.

### Population and sample

The study population included hospitalized patients who were evaluated for blood stream infection and had at least one of the following: (1) fever or chills or leukopenia or leukocytosis, (2) Diagnosis of candidemia by positive blood cultures, and (3) Candida strain isolated at the INEN Microbiology Laboratory. Cases of repeated candidemia within a period of less than one month from a previous episode were excluded.

### Microbiological methods

Peripheral blood samples were obtained from patients with suspected nosocomial infections by venipuncture or venipuncture and central venous catheter. Samples were taken into at least one vial of liquid culture medium and incubated in the BD BACTEC™ FX automated system (BD Diagnostics, Sparks, MD, USA) for up to 5 days [52–54]. Once a positive blood culture was detected by the system and yeasts were observed by Gram staining, they were isolated in the Sabouraud Glucose Agar culture medium. The identification of the isolate, at the genus and species levels, was carried out by morphological tests according to the Dalmaut Technique [55] on Rice Starch Agar and chromogenic tests using CHROMagar *Candida* (CHROMagar Microbiology, France) [56]. Confirmatory identification was performed by biochemical analysis of carbohydrates using the commercial API 20 C yeast identification method (bioMérieux) [57, 58]. In addition, fluconazole susceptibility was evaluated using agar-based methods: diffusion disk as screening method and E-test (AB Biodisk, Solna, Sweden) as a confirmed method, obtaining the categories of sensitive, sensitive dose-dependent, and resistant [59, 60]. Biofilm formation assays were performed using the standardized microplate method and quantified by reducing 2, 3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2 H-tetrazolium-5-carboxanilide (XTT) [61, 62]. *Candida* strains with optical densities (OD) $<0.1$ were categorized as non-biofilm-formers and strains with OD $\geq 0.1$ as biofilm-formers [45, 63, 64]. In addition, we categorized the levels of biofilm formation according to OD: no biofilms ($<0.1$), low level (0.1 to 0.25), medium level (0.26 to 0.5), and high level ($>0.5$). Low and medium level biofilm production categories were merged because the OD values of those two categories were similar and considerably lower than the OD values of the high category. Therefore, biofilm formation was compared between low/medium versus high levels.

### Data collection and study variables

Laboratory information (*Candida* species and fluconazole sensitivity profile) was obtained from the INEN

Laboratory Information System. The demographic information of the patients (sex and age), underlying medical conditions such as type of cancer, oncological diagnosis, comorbidities, hospital derivation, intensive care unit (ICU) stay, severe neutropenia status (absolute neutrophil count $\leq 0.5 \times 10^9$/L), invasive procedures (mechanical ventilation, parenteral nutrition), presence of central venous catheter (CVC), immunosuppressive therapy (corticosteroids), antimicrobial and antifungal therapy data were collected through review of medical records. Adequate antifungal therapy was defined as therapy received within 48 h of diagnosis and for at least five consecutive days [46, 65]. Biofilm production capacity of *Candida* isolates was evaluated at the Mycology Laboratory of the "Daniel Alcides Carrion" Instituto de Medicina Tropical, Universidad Nacional Mayor de San Marcos, as described in the text.

The outcome of interest, in-hospital mortality or survival, was measured from the time of candidemia diagnosis to the date of death (from any cause). Patients who survived after 30 days of follow-up or were discharged were censored and considered to be survivors in our analyses.

#### Statistical analysis

The relationship between in-hospital mortality and the differences across covariates was evaluated using the Chi2 test so mortality can be presented as a proportion using a direct, clear estimate. Survival curves were estimated with the Kaplan-Meier method and they were compared between covariate categories using the Log-rank test. Survival analysis was performed to identify possible differentiated patterns of mortality. Cox's bivariate regression analysis was performed, estimating the crude mortality hazard ratio (HR), as a measure of association between in-hospital mortality, and the formation of biofilms as well as the HR for each of the covariates considered in the analysis. A manual forward stepwise process was used to build a parsimonious nested model, sequentially adding variables to the model based on likelihood ratio tests ($p<0.05$). The goodness of fit of the model was determined through the analysis of Schoenfeld and Cox-Nell residuals and ties were handled using the partial likelihood method proposed by Efron. The assumption of proportionality of the hazards in the global model and for each covariate in the bivariate analyses were evaluated using graphical (Schoenfeld residuals and observed versus expected survival) and analytical (proportionality test) methods. The final multiple Cox regression model evaluated the possible association between the time to in-hospital mortality and biofilm formation, adjusted for all other covariables significantly associated with death. All data analysis was performed using Stata version 14.0 software (StataCorp, College Station, Texas 77845 USA).

## Results

From June 2015 to October 2017, 21,820 blood cultures were evaluated at the INEN Microbiology Laboratory, resulting in 412 (1.89%) contaminated blood cultures. Among the valid cultures, 148 were positive for *Candida* spp. (0.69%), and were obtained from 137 patients. Three patients had two episodes of candidemia separated by more than one month and eight had positive blood cultures within less than one month of the previous episode. Therefore, the final available sample size was 140 patients with positive blood culture results for *Candida* spp. (Fig. 1).

#### General characteristics of the sample

Most of the participants (58.6%) were adults between 16 and 59 years of age, and more than half were women (54.3%). Patients frequently presented with comorbidities (32.1%) and severe neutropenia (40.7%). The most common type of neoplasia was hematological malignancy (57.9%), and the most frequent oncological diagnoses were acute lymphocytic leukemia (25.7%) and gastrointestinal tumors (22.2%). A high percentage of the patients received antimicrobial therapy (79.3%) and immunosuppressive therapy (42.9%). In addition, 64.3% of the patients had a CVC and 22.1% had an ICU stay. Candidemia cases were treated with antifungals (76.4%), almost half of which were treated with antifungals with activity against biofilms, such as echinocandins and lipid formulation of amphotericin B (47.1%), and 62.1% of the patients received adequate antifungal therapy (therapy received within 48 h of diagnosis and for at least five consecutive days).

Subsequent identification of the isolates revealed that the predominant species were *C. tropicalis* (47.9%) and *C. albicans* complex (34.3%). More than a quarter of the isolates (27.2%) were resistant to fluconazole and the majority (75.7%) formed biofilms in vitro. Almost half of the patients (65, 46.4%) died within 30 days of the candidemia diagnosis. Most deaths occurred early, with almost a third of the fatalities taking place in the first day (32.3%). An additional 20% occurred between the second and fifth days, reaching 81.5% on day 15 of follow-up (Table 1).

#### Factors associated with in-hospital mortality

According to the medical records, mortality was at least 28% higher in patients>15 years old ($p=0.034$). Higher mortality was also observed in patients with comorbidities (64.4% vs. 37.9%, $p=0.003$), in those admitted for emergencies (87.5% vs. 43.9%, $p=0.016$), and in patients who did not receive therapy and/or received inappropriate therapy (60.4% vs. 37.9%, $p=0.010$). In addition, patients with candidemia that formed biofilms had a marginally higher mortality (50.9% vs. 32.3%, $p=0.059$), with

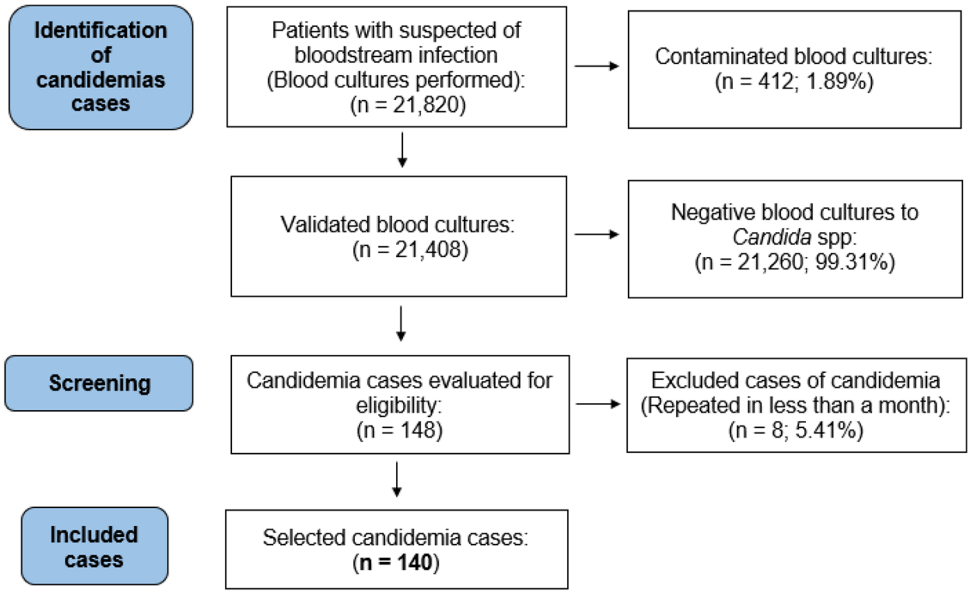


**Fig. 1** Selection of the study sample

a significant positive correlation between higher biofilm formation and higher mortality ($p=0.033$). On the other hand, we observed lower mortality in both patients that received antifungal therapy (41.1% vs. 63.6%, $p=0.023$) and those who received antimicrobial therapy (41.4% vs. 65.5%, $p=0.021$). Interestingly, mortality rates were very similar in treated and non-treated patients for both types of therapy, since according to the clinical records, both antifungal and antimicrobial therapy prescription in the same patient coincided much more often that they differed (90% vs. 10%, $p<0.001$). Patients with CVC also had lower mortality (40.0% vs. 58.0%, $p=0.041$), as well as those receiving adequate antifungal therapy (37.9% vs. 60.4%, $p=0.010$). In addition, there was no proportionality of hazards for antimicrobial ($p=0.001$) or antifungal ($p=0.033$) treatment, while the hazards were proportional for all other covariates. (Table 2). In an additional analysis, we found that biofilm production differed by *Candida* species ($p=0.015$), with more frequent biofilm-producing strains in *C. tropicalis* (57/67=85.1%) and fewer among *C. parapsilosis* complex (4/9=44.4%). Among the nine high-level biofilm-producing strains, seven were identified as *C. tropicalis* and two as *C. glabrata complex.*

### Survival analysis

Decreased 30-day survival was observed with higher biofilm production of *Candida* isolates. (Fig. 2). A substantial decrease in survival was observed on the first day of follow-up, while deaths were more sporadic after day two, and survival decreased more slowly and progressively since (Fig. 3). This differentiated pattern of mortality early during follow-up and the lack of proportional hazards for antifungal and antimicrobial treatment, warranted for stratification by treatment status and estimation of separate survival curves for patients with different antifungal and antimicrobial treatment. For both types of therapy, the survival curves of the patients showed substantially higher mortality on the first day of follow-up among untreated patients but a lower, more homogeneous mortality rate across the 30-day period in treated patients (Fig. 4). This survival pattern was compatible with the disproportionate hazards associated with antifungal and antimicrobial treatment. When exploring in detail the characteristics of patients who died on the first day, the vast majority of deaths occurred in patients who did not receive either antifungal or antimicrobial therapy (17/21=81.0%, Table 3). In contrast, deaths after the second day occurred almost exclusively in patients who received both therapies, and became more progressive.

Given the large difference in first-day mortality between untreated and treated patients, we hypothesized that treatment could be an effect modifier for the association between biofilm formation and survival. Therefore, we stratified by treatment, following Kleinbaum recommendations [66], to be able to apply Cox regression in treatment-defined groups with proportional hazards. Therefore, the results were analyzed separately in two strata:1) patients that did not receive antifungal or antimicrobial treatment, and 2) patients who received antifungal and/or antimicrobial treatment. The association with mortality was analyzed dichotomously in the first stratum because deaths were mostly concentrated on the first day, while in the second stratum the assumptions for Cox regression as a time-to-event approach were valid since mortality occurred more progressively.

**Table 1** Demographic, clinical, and microbiological characteristics and outcome of 140 cases of candidemia

| Characteristics | *N* (%) |
|---|---|
| **Gender** | |
| Male | 64 (45.7) |
| Female | 76 (54.3) |
| **Age groups (years)** | |
| 0–15 | 28 (20.0) |
| 16–59 | 82 (58.6) |
| ≥ 60 | 30 (21.4) |
| **Type of cancer** | |
| Solid tumors | 59 (42.1) |
| Hematological malignancies | 81 (57.9) |
| **Oncological diagnosis** | |
| Acute myeloid leukemia | 19 (13.6) |
| Acute lymphoid leukemia | 36 (25.7) |
| Non-Hodgkin lymphoma | 15 (10.7) |
| Gastrointestinal tumor | 31 (22.2) |
| Genitourinary tumor | 15 (10.7) |
| Others* | 24 (17.1) |
| **Comorbidities** | |
| No | 95 (67.9) |
| Infectious disease | 17 (12.2) |
| Respiratory disease | 10 (7.1) |
| Others** | 9 (6.4) |
| More than one comorbidity | 9 (6.4) |
| **Hospital derivation** | |
| Hospitalization | 132 (94.3) |
| Emergency | 8 (5.7) |
| **Severe neutropenia** | |
| No | 83 (59.3) |
| Yes | 57 (40.7) |
| **Antimicrobial therapy** | |
| No | 29 (20.7) |
| Yes | 111 (79.3) |
| **Antifungal therapy** | |
| No | 33 (23.6) |
| Yes | 107 (76.4) |
| **Adequate antifungal therapy** | |
| No | 53 (37.9) |
| Yes | 87 (62.1) |
| **Anti-biofilm antifungal therapy** | |
| No | 74 (52.9) |
| Yes | 66 (47.1) |
| **ICU stay** | |
| No | 109 (77.9) |
| Yes | 31 (22.1) |
| **Presence of CVC** | |
| No | 50 (35.7) |
| Yes | 90 (64.3) |
| **Etiology** | |
| *C. albicans complex* | 48 (34.3) |
| *C. tropicalis* | 67 (47.9) |
| *C. parapsilosis complex* | 9 (6.4) |
| *C. glabrata complex* | 10 (7.1) |
| Others*** | 6 (4.3) |
| **Fluconazol resistance** | |
| No | 91 (72.8) |
| Yes | 34 (27.2) |
| **Biofilm formation level** | |
| Non-formation | 34 (24.3) |
| Low-medium | 97 (69.3) |
| High | 9 (6.4) |
| **Mortality rate** | |
| No | 75 (53.6) |
| Yes | 65 (46.4) |

*(7) Head and neck cancer (6) chronic myeloid leukemia (3) Hodgkin lymphoma, osteosarcoma (2) Histiocytosis (1) liver cancer, mama cancer, skin cancer

**(3) Genitourinary disease, gastrointestinal disease (2) diabetes (1) Down syndrome

***(4) *C. lusitaniae* (2) *Meyerozyma guilliermondii* (*C. guilliermondii*) complex

## Mortality in patients with candidemia according to treatment stratum

### *Stratum 1: patients without antimicrobial or antifungal treatment (n = 24)*

Out of the 24 patients who did not receive these two treatments, 17 (70.8%) died. Higher mortality was observed in patients with hematological malignancies (100.0% vs. 46.1%, $p=0.006$) and among patients infected with low-medium and high-level biofilm-forming strains of *Candida* spp. versus infections with strains that did not form biofilms (82.3% vs. 25.0% and 66.7% vs. 25.0%, $p=0.049$, Table 4). No other statistically significant differences in mortality were observed, perhaps because of the small sample size and the resulting low power that also prevented exploration of possible multivariate associations.

### *Stratum 2: patients who received antimicrobial and/or antifungal treatment (n = 116)*

A total of 48 patients died within this stratum (41.4%). Bivariate analysis showed that there was a higher instantaneous risk of death in patients in 16–59 and 60 + years old compared to 0–15 years old (HR 3.61, $p=0.008$; HR 3.87, $p=0.012$, respectively), with some comorbidity (HR 1.82; $p=0.038$), hospital emergency (HR 4.84; $p=0.001$), receiving inadequate antifungal therapy (HR 2.18; $p=0.013$), mechanical ventilation (HR 1.83; $p=0.037$), and infection with high-level biofilm-forming strains of *Candida* (HR 3.92; $p=0.022$).

Exploratory multiple regression analyses in this second stratum found independent associations between a greater instantaneous risk of death and older age (16–59 years with HR 4.27; $p=0.003$ and 60 + years with HR 5.88; $p=0.001$ compared to 0–15 years), presence of comorbidities (HR 2.72; $p=0.001$), hospital emergency derivation

(HR 7.58; $p<0.001$), and a high level of biofilm formation (HR 6.59; $p=0.003$). Hazards were proportional for each covariate evaluated and for all covariates together ($p>0.2$ and $p=0.848$, respectively, Table 5).

## Discussion

We observed a significant association between high levels of in vitro biofilm formation and increased mortality among candidemia cases. This association was consistently present both in patients who did not receive antimicrobial nor antifungal treatment in whom mortality was high and deaths occurred very early, and also in patients who received at least one of these treatments, among whom mortality was lower and occurred progressively during follow-up. In the latter group, the association remained significant after multiple regression adjustment.

Our findings confirm hypotheses raised by previous studies that observed higher mortality in candidemia with biofilm formation [44–48], but contradict other studies that found no significant association [49–51]. In both cases, the results may be inconclusive because the studies had small sample sizes [44, 47, 49, 50] and did not include potential confounding variables in their analysis, such as patients' baseline clinical status [44, 48–50], presence of comorbidities [46, 47], administration of antifungal therapy, and in vitro resistance of isolates to fluconazole [49, 50]. Finally, no previous studies [44–51] had assessed the timing and temporal pattern of survival in these frail patients, which was critical to detect basal differences in the survival probability.

Our study design and analysis attempted to overcome the challenges faced by previous studies, and our findings suggest a possible role of biofilm formation on increased mortality among patients with candidemia. There are some possible mechanisms that could explain how candidemia with high biofilm formation could lead to higher mortality. For example, antifungal drugs penetrate poorly into biofilm structures, allowing *Candida* spp. yeasts to resist their action [30]. Additionally, biofilms can act as a physical barrier that prevents phagocytosis, allow yeasts to evade the host immune response, and alters the profile of cytokines secreted by immune cells [35]. Therefore, infections with candida strains that form biofilms could hinder effective treatment and represent a risk factor for a complicated clinical course and potentially fatal outcomes. Although candidemia mortality rate is lower outside cancer patients, biofilm formation remains a concern as it can play a role in fatal outcomes in non-tumor populations. Three studies documented lower but non-negligible mortality rates among non-tumor hospitalized patients with candidemia compared to patients with malignancies. Among 126 Korean ICU patients, mortality was lower among non-tumor patients (43/81; 53.1% versus 32/45; 71.1%, $p=0.048$) and remained lower after adjusting for hemodialysis, mycological failure, and septic shock (OR, 0.12, 95% CI: 0.03–0.45, $p=0.002$) [67]. Similarly, in 60 hospitalized patients from a 750-bed Korean tertiary medical center, mortality was lower in non-cancer patients (6/23; 26.1% versus 19/37; 51.4%, $p=0.05$), and remained lower after adjusting for APACHE II score and receipt of antifungal treatment (OR, 0.07; 95% CI, 0.01–0.40; $p=0.003$) [68]. Finally, among 341 patients with candidemia at 14 major hospitals in Spain, mortality was lower in patients without hematologic malignancy (27; 13% versus 9; 24%, RR 0.5, $p=0.1$), and remained lower after excluding *C. parapsilosis* cases and deaths occurring at days 1 to 2, and adjusting for severity of illness category (OR, 0.29; 95% CI, 0.01 to 0.91, $p=0.03$) [69]. In summary, there is considerable mortality in hospitalized patients with candidemia, even in the absence of malignancies, an important risk factor. Prospective studies of potential mechanisms of increased virulence, such as drug inactivation and immune evasion, could help to elucidate the role of biofilms in the clinical course of candidemia.

Our survival analysis identified a subgroup of patients with very high, early mortality, suggesting they had a highly vulnerable baseline clinical condition at cohort entry and a worse prognosis [70, 71]. The identification of two groups with dramatic differences in mortality rates and time of death suggests a differential risk of death associated with antifungal or antimicrobial treatment. Therefore, the study methodology was adjusted by introducing stratification by treatment, but higher mortality associated with biofilm formation was consistently observed, regardless of the treatment received and the level of associated mortality. The high mortality observed in patients infected with biofilm-producing strains but were not treated is important evidence that suggests how severe this condition can be among frail, vulnerable patients. This is an important finding that should be confirmed in future studies with larger sample sizes.

The lower mortality rate observed among patients receiving antimicrobial therapy has not been described previously and may be an artificial effect of prophylactic treatment in the hospital. Patients with candidemia receiving antifungal drugs often receive antimicrobial therapy as well because they are at risk of developing severe bacterial infections [72]. In our study, almost all patients who received an antifungal drug also received antimicrobial therapy (102/107=95.3%), and most patients who did not receive an antifungal drug also did not receive antimicrobial therapy (24/33=72.7%). However, it is unlikely that the antimicrobial treatment received could directly reduce mortality from candidemia.

**Table 2** Demographic, clinical, and microbiological characteristics of 140 cases of candidemia according to mortality, and proportional hazards assessment

| Variables | Survivors *N* (%) | Deceased *N* (%) | *p* value | Proportionality (*p* value) |
|---|---|---|---|---|
| **Gender** | | | 0.560 | 0.163 |
| Male | 36 (56.3) | 28 (43.7) | | |
| Female | 39 (51.3) | 37 (48.7) | | |
| **Age groups (years)** | | | 0.034 | 0.624 |
| 0–15 | 23 (82.1) | 5 (17.9) | | |
| 16–59 | 36 (43.9) | 46 (56.1) | | |
| ≥ 60 | 36 (43.9) | 46 (56.1) | | |
| **Type of cancer** | | | 0.132 | 0.333 |
| Solid tumors | 36 (61.0) | 23 (39.0) | | |
| Hematological malignancies | 39 (48.1) | 42 (51.9) | | |
| **Oncological diagnosis** | | | 0.674 | 0.393 |
| Acute myeloid leukemia | 11 (57.9) | 8 (42.1) | | |
| Acute lymphoid leukemia | 19 (52.8) | 17 (47.2) | | |
| Non-Hodgkin lymphoma | 6 (40.0) | 9 (60.0) | | |
| Gastrointestinal tumor | 20 (64.5) | 11 (35.5) | | |
| Genitourinary tumor | 7 (46.7) | 8 (53.3) | | |
| Others* | 12 (50.0) | 12 (50.0) | | |
| **Comorbidity** | | | 0.003 | 0.745 |
| No | 59 (62.1) | 36 (37.9) | | |
| Yes | 16 (35.6) | 29 (64.4) | | |
| **Type of comorbidity** | | | 0.011 | 0.426 |
| No | 59 (62.1) | 36 (37.9) | | |
| Infectious disease | 6 (35.3) | 11 (64.7) | | |
| Respiratory disease | 1 (10.0) | 9 (90.0) | | |
| Others** | 5 (55.6) | 4 (44.4) | | |
| More than one comorbidity | 4 (44.4) | 5 (55.6) | | |
| **Hospital derivation** | | | 0.016 | 0.993 |
| Hospitalization | 74 (56.1) | 58 (43.9) | | |
| Emergency | 1 (12.5) | 7 (87.5) | | |
| **Severe neutropenia** | | | 0.223 | 0.256 |
| No | 48 (57.8) | 35 (42.2) | | |
| Yes | 27 (47.4) | 30 (52.6) | | |
| **Antimicrobial therapy** | | | 0.021 | 0.001 |
| No | 10 (34.5) | 19 (65.5) | | |
| Yes | 65 (58.6) | 46 (41.4) | | |
| **Antifungal therapy** | | | 0.023 | 0.033 |
| No | 12 (36.4) | 21 (63.6) | | |
| Yes | 63 (58.9) | 44 (41.1) | | |
| **Adequate antifungal therapy** | | | 0.010 | 0.034 |
| No | 21 (39.6) | 32 (60.4) | | |
| Yes | 54 (62.1) | 33 (37.9) | | |
| **Anti-biofilm antifungal therapy** | | | 0.370 | 0.041 |
| No | 37 (50.0) | 37 (50.0) | | |
| Yes | 38 (57.6) | 28 (42.4) | | |
| **ICU stay** | | | 0.141 | 0.503 |
| No | 62 (56.9) | 47 (43.1) | | |
| Yes | 13 (41.9) | 18 (58.1) | | |
| **Presence of CVC** | | | 0.041 | 0.087 |
| No | 21 (42.0) | 29 (58.0) | | |
| Yes | 54 (60.0) | 36 (40.0) | | |
| **Etiology** | | | 0.541 | 0.549 |
| *C. albicans complex* | 24 (50.0) | 24 (50.0) | | |

**Table 2** (continued)

| Variables | Survivors *N* (%) | Deceased *N* (%) | *p* value | Proportionality (*p* value) |
|---|---|---|---|---|
| *Candida* no *albicans complex* | 51 (55.4) | 41 (44.6) | | |
| ***Candida* species** | | | 0.332 | 0.264 |
| *C. albicans complex* | 24 (50.0) | 24 (50.0) | | |
| *C. tropicalis* | 33 (49.3) | 34 (50.7) | | |
| *C. parapsilosis complex* | 6 (66.7) | 3 (33.3) | | |
| *C. glabrata complex* | 7 (70.0) | 3 (30.0) | | |
| Others | 5 (83.3) | 1 (16.7) | | |
| **Fluconazol resistance** | | | 0.834 | 0.731 |
| No | 50 (55.0) | 41 (45.0) | | |
| Yes | 18 (52.9) | 16 (47.1) | | |
| **Biofilm formation** | | | 0.059 | 0.790 |
| No | 23 (67.7) | 11 (32.3) | | |
| Yes | 52 (49.1) | 54 (50.9) | | |
| **Biofilm formation level** | | | 0.033 | 0.652 |
| Non-formation | 23 (67.7) | 11 (32.3) | | |
| Low-medium | 49 (50.5) | 48 (49.5) | | |
| High | 3 (33.3) | 6 (66.7) | | |

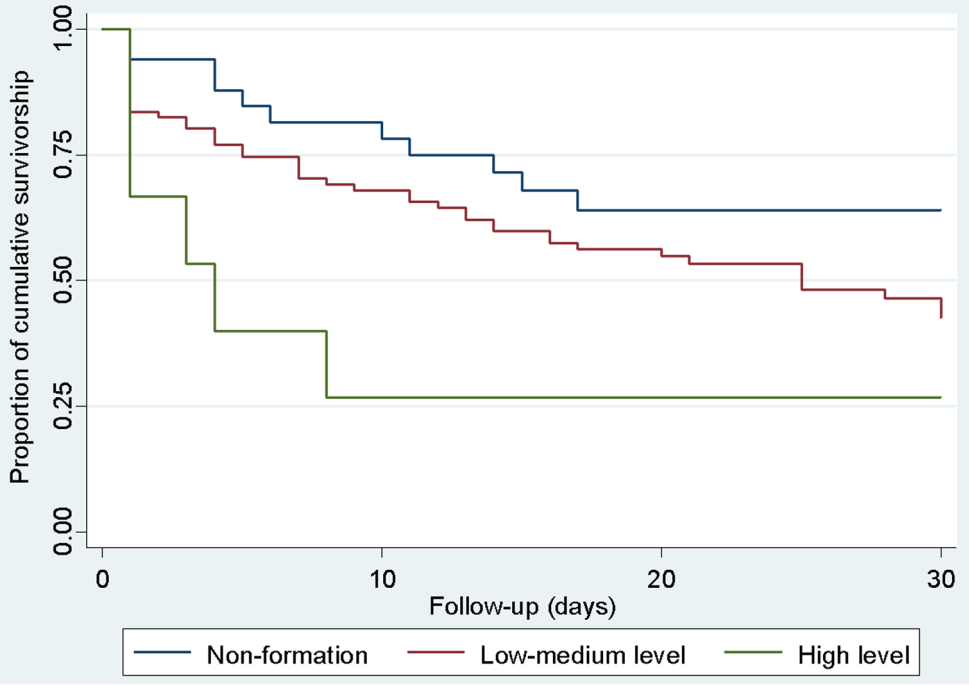


**Fig. 2** Survival of patients with candidemia according to the level of biofilm formation of the isolates estimated by the Kaplan Meier method

We observed that late antifungal treatment or inadequate dosing was associated with higher mortality in candidemia, in consistency with studies showing the efficacy of antifungal therapy in candidemia depends on whether or not it is properly administered [26, 40, 43]. Antifungal therapy can reduce mortality, particularly if administered within 48 h of diagnosis and for at least 5 consecutive days. Similarly, the lipid formulation of amphotericin B and echinocandins has documented activity against *Candida* spp. biofilms [38, 39]. However, we found no association between the use of these antifungal agents and mortality from candidemia. Limited statistical power may have prevented detecting small reductions in mortality associated with antifungal therapies against biofilms. Evaluation of these antifungal agents in prospective studies with a larger sample size could better document the effect of these antifungal agents on candidemia caused by biofilm-producing strains. Antifungal prophylaxis was not part of oncologic therapeutic management at the Institute at that time except for fluconazole and

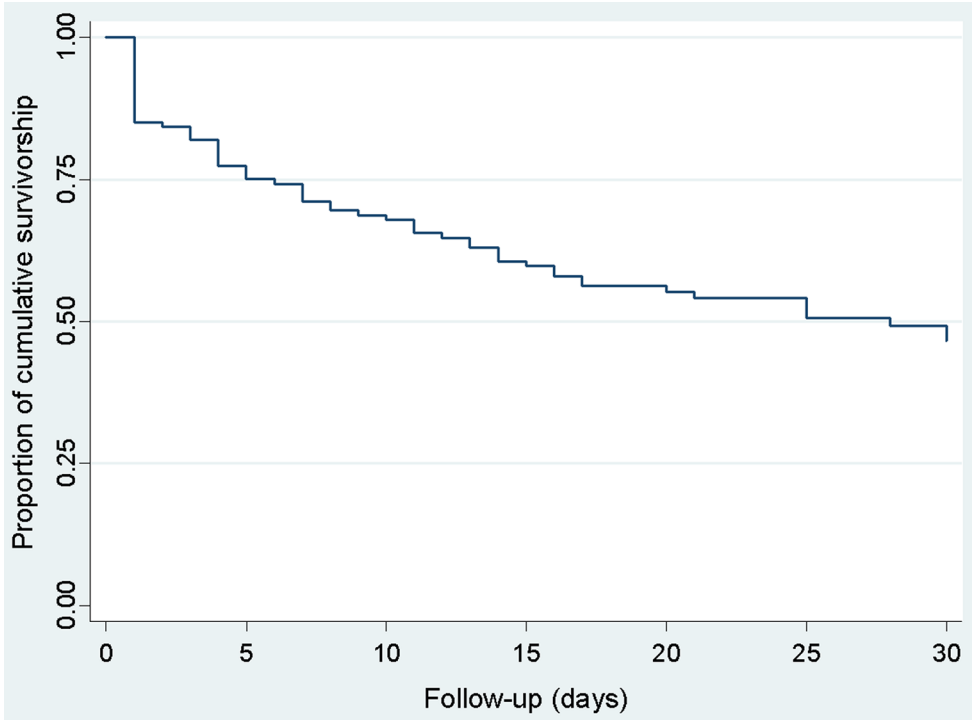


**Fig. 3** Overall survival of patients with candidemia estimated by the Kaplan Meier method

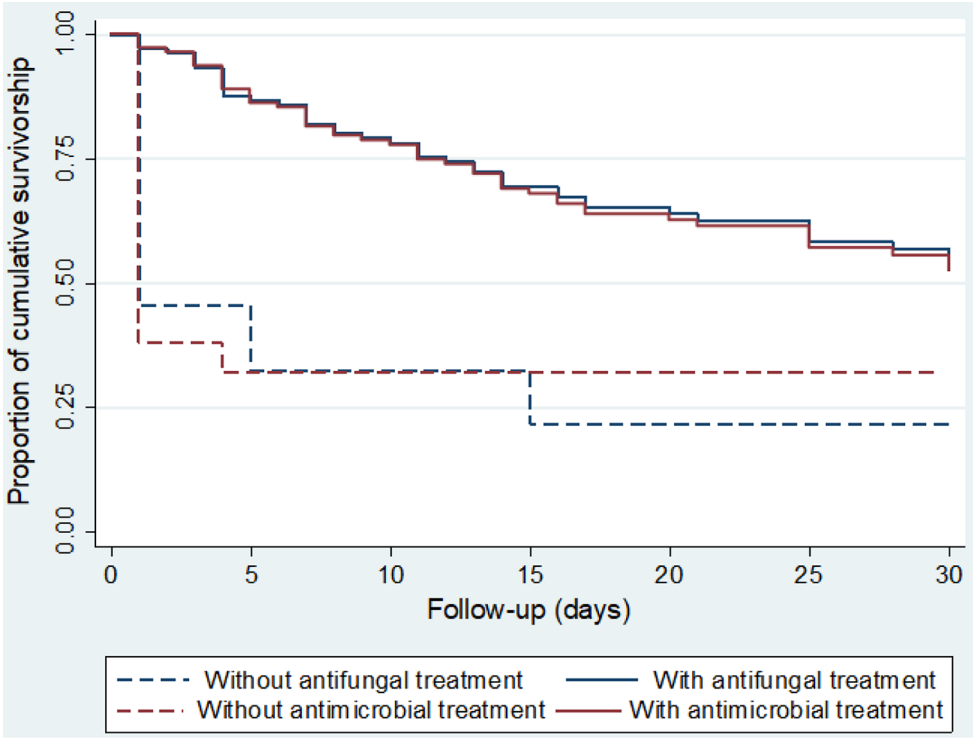


**Fig. 4** Survival of patients with candidemia according to having received antifungal and antimicrobial treatment estimated by the Kaplan Meier method

**Table 3** Day of death and total number of deaths during follow-up, stratified according to having received antifungal and antimicrobial treatment

| Groups | No treatment (*n* = 24) | Only antimicrobial treatment (*n* = 9) | Only antifungal treatment (*n* = 5) | Both treatments (*n* = 102) | Total (*n* = 140) |
|---|---|---|---|---|---|
| Total deaths | 17 (71%) | 4 (44%) | 2 (40%) | 42 (41%) | 65 (46%) |
| Death day | | | | | |
| 1 | 17 (100%) | 1 (25%) | 1 (50%) | 2 (5%) | 21 (32%) |
| 2–5 | 0 (0%) | 2 (50%) | 1 (50%) | 10 (24%) | 13 (20%) |
| 6–15 | 0 (0%) | 1 (25%) | 0 (0%) | 18 (43%) | 19 (29%) |
| 16–30 | 0 (0%) | 0 (0%) | 0 (0%) | 12 (28%) | 12 (19%) |
| Total no deaths | 7 | 5 | 3 | 60 | 75 |
| % Deaths at the day 1 | 17/24 (71%) | 1/9 (11%) | 1/5 (20%) | 2/102 (2%) | 21/140 (15%) |

**Table 4** Factors associated with mortality in patients without antimicrobial or antifungal treatment (*n* = 24)

| Variables | Survivors *N* (%) | Deceased *N* (%) | *p* value |
|---|---|---|---|
| **Gender** | | | 1.000 |
| Male | 4 (33.3) | 8 (66.7) | |
| Female | 3 (25.0) | 9 (75.0) | |
| **Age groups (years)** | | | 0.077 |
| 0–15 | 0 (0) | 0 (0) | |
| 16–59 | 3 (17.7) | 14 (82.4) | |
| ≥ 60 | 4 (57.1) | 3 (42.9) | |
| **Type of cancer** | | | 0.006 |
| Solid tumors | 7 (53.9) | 6 (46.1) | |
| Hematological malignancies | 0 (0) | 11 (100.0) | |
| **Comorbidity** | | | 0.065 |
| No | 7 (41.2) | 10 (58.8) | |
| Yes | 0 (0) | 7 (100.0) | |
| **Hospital derivation** | | | 1.000 |
| Hospitalization | 7 (31.8) | 15 (68.2) | |
| Emergency | 0 (0) | 2 (100.0) | |
| **Severe neutropenia** | | | 0.130 |
| No | 7 (38.9) | 11 (61.1) | |
| Yes | 0 (0) | 6 (100.0) | |
| **ICU stay** | | | 1.000 |
| No | 7 (30.4) | 16 (69.6) | |
| Yes | 0 (0) | 1 (100.0) | |
| **Presence of CVC** | | | 0.507 |
| No | 6 (27.3) | 16 (72.7) | |
| Yes | 1 (50.0) | 1 (50.0) | |
| **Etiology** | | | 1.000 |
| *C. albicans complex* | 3 (33.3) | 6 (66.7) | |
| *Candida* no *albicans complex* | 4 (26.7) | 11 (73.3) | |
| **Fluconazol resistance** | | | 0.826 |
| No | 3 (23.1) | 10 (76.9) | |
| Yes | 3 (42.9) | 4 (57.1) | |
| **Biofilm formation** | | | 0.059 |
| No | 3 (75.0) | 1 (25.0) | |
| Yes | 4 (20.0) | 16 (80.0) | |
| **Biofilm formation level** | | | 0.049 |
| Non-formation | 3 (75.0) | 1 (25.0) | |
| Low-medium | 3 (17.7) | 14 (82.3) | |
| High | 1 (33.3) | 2 (66.7) | |

**Table 5** Factors associated with mortality in patients with antimicrobial and antifungal treatment ($n = 116$)

| Variables | Raw model | | | | Adjusted model† | | |
|---|---|---|---|---|---|---|---|
| | HR | CI 95% | *p* value | Propor. | HR | CI 95% | *p* value |
| **Gender** | | | | 0.148 | | | |
| Male | Ref. | | | | Ref. | | |
| Female | 1.17 | 0.66–2.10 | 0.576 | | 0.94 | 0.50–1.76 | 0.846 |
| **Age groups (years)** | | | | | | | |
| 0–15 | Ref. | | | 0.977 | Ref. | | |
| 16–59 | **3.61** | **1.40–9.27** | **0.008** | | **4.27** | **1.64–11.11** | **0.003** |
| ≥ 60 | **3.87** | **1.34–11.16** | **0.012** | | **5.88** | **1.98–17.44** | **0.001** |
| **Type of cancer** | | | | 0.161 | | | |
| Solid tumors | Ref. | | | | Ref. | | |
| Hematological malignancies | 1.02 | 0.56–1.83 | 0.958 | | 1.31 | 0.66–2.57 | 0.437 |
| **Comorbidity** | | | | 0.540 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | **1.82** | **1.03–3.22** | **0.035** | | **2.72** | **1.47–5.01** | **0.001** |
| **Hospital derivation** | | | | 0.741 | | | |
| Hospitalization | Ref. | | | | Ref. | | |
| Emergency | **4.84** | **1.88–12.45** | **0.001** | | **7.58** | **2.67–21.49** | **< 0.001** |
| **Severe neutropenia** | | | | 0.374 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 1.19 | 0.67–2.09 | 0.553 | | 1.29 | 0.72–2.34 | 0.394 |
| **Antimicrobial therapy** | | | | 0.189 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 0.76 | 0.18–3.13 | 0.702 | | 0.41 | 0.95–1.79 | 0.236 |
| **Antifungal therapy** | | | | 0.958 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 0.43 | 0.15–1.20 | 0.107 | | 0.38 | 0.12–1.15 | 0.086 |
| **Adequate antifungal therapy** | | | | 0.295 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | **0.46** | **0.25–0.85** | **0.013** | | 0.61 | 0.30–1.20 | 0.151 |
| **Anti-biofilm antifungal therapy** | | | | 0.405 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 0.88 | 0.50–1.57 | 0.670 | | 0.93 | 0.52–1.68 | 0.816 |
| **ICU stay** | | | | 0.614 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 1.60 | 0.88–2.89 | 0.121 | | 1.51 | 0.80–2.85 | 0.208 |
| **Presence of CVC** | | | | 0.754 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 0.67 | 0.35–1.26 | 0.214 | | 0.90 | 0.45–1.80 | 0.763 |
| **Etiology** | | | | 0.568 | | | |
| *C. albicans complex* | Ref. | | | | Ref. | | |
| *Candida* no *albicans complex* | 0.77 | 0.42–1.38 | 0.372 | | 0.75 | 0.41–1.39 | 0.361 |
| **Fluconazol resistance** | | | | 0.477 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 1.05 | 0.62–1.76 | 0.861 | | 1.59 | 0.89–2.84 | 0.116 |
| **Biofilm formation** | | | | 0.340 | | | |
| No | Ref. | | | | Ref. | | |
| Yes | 1.39 | 0.69–2.78 | 0.351 | | No analizable | | |
| **Biofilm formation level** | | | | 0.208 | | | |
| Non-formation | Ref. | | | | Ref. | | |
| Low-medium | **1.30** | **0.64–2.62** | **0.472** | | **1.30** | **0.62–2.75** | **0.492** |
| High | **3.92** | **1.22–12.57** | **0.022** | | **6.59** | **1.87–23.24** | **0.003** |

posaconazole prophylaxis for patients with acute myeloid leukemia, which accounted for only 13.6% of the study sample.

On the other hand, confirmatory laboratory diagnosis of candidemia is mainly limited by performing microbiological culture of multiple blood samples. The *Candida* spp isolates in our study are very likely to be the true cause of the candidemia since more than 90% of the blood cultures were performed with multiple specimens from at least two sites sampling from both arms and had concordant results, reducing the possibility of skin-contaminated samples. Also, a second infection was detected in only 12.2% of the patients. On the other hand, non-culture-based tests would have been useful in diagnosing candidemia, such as the (1–3)-β-D-glucan test, but limited resources prevented their use. However, having multiple, concordant results from different cultures, there are lower chances of contamination and less need for further supplementary testing.

Candidemia is the most common invasive mycosis and has challenging diagnosis. Its clinical course of this fungal infection is severe and life-threatening, with a high mortality rate among immunocompromised populations. Therefore, early suspicion and consideration among the differential diagnoses of hospital-acquired infections are critical in at-risk patients, such as our oncologic population. The role of empirical treatment and antifungal prophylaxis in resource-limited settings requires further research. Adulthood, age older than 15 years [18, 24], and the presence of some comorbidity [73–75] were identified, similar to previous studies, along with emergency hospital admission as factors strongly associated with higher mortality from candidemia. These factors likely characterize frail patients with candidemia with a worse prognosis. Such empirical results reflect the important role of patients' baseline clinical condition in their potential survival and should be further investigated in future prospective studies.

The study had some particularities that must be considered when interpreting the results, but do not invalidate our conclusions. First, the stage and status of the cancer were not evaluated, variables that may provide an indication of the severity of the patient's clinical condition. However, other variables capture the potential frailty of the baseline clinical condition, such as treatment in the hospital emergency department, presence of chronic disease, and advanced age. Second, patient health status severity was not assessed because this is not routinely done for all patients treated at INEN. In addition, there is no uniform scale for assessing severity in patients admitted to different hospital units. For example, the scale APACHE II, cannot be used in patients outside the ICU (77.9% of the sample) or in the pediatric population (20.0% of the sample). In addition, the effective sample size was partially reduced by separately analyzing patients who received antifungal or antimicrobial treatment (82.9% of the sample), then no conclusions could be drawn about the analysis of patient mortality by antifungal and antimicrobial treatment received due to the small sample size. Although several significant differences were found, including those related to the study hypothesis, there might be other associations that statistical power was insufficient to detect. Finally, we have no information on CVC removal, which some studies have found to be associated with survival among patients with candidemia, although it is difficult to understand retrospectively the reasons for CVC removal among hospitalized patients. CVC removal in patients with good prognosis may aim to prevent hospital-acquired infections but in patients under palliative therapy may be the beginning of less invasive interventions.

## Conclusions

- The association between candidemia with in vitro biofilm formation and an increased risk of death consistently observed in both treated and untreated patients, provides an additional level of evidence for a possible causal association.
- Survival analysis in a time-to-event study is essential to detect differences in the probabilities of developing the outcome. In our study, this analysis allowed us to ensure an adequate assessment of the true risk of death that patients had since their entry into the cohort.
- The presence of comorbidities and the origin of the hospital emergency, which reflect the fragile clinical condition of the patients, and increasing age above 15 years were associated with a higher risk of death.

### Abbreviations

| | |
|---|---|
| APACHE | Acute Physiology and Chronic Health Evaluation |
| CVC | Central venous catheter |
| INEN | Instituto Nacional de Enfermedades Neoplasicas |
| OD | Optical densities |
| ICU | Intensive care unit |
| HR | Hazard ratio |

### Acknowledgements

The authors thank professors of the master's program for their contributions in the study design, data analysis, and manuscript preparation. AGL and the Master's program are supported by program D43 TW007393 "Emerge, Emerging Diseases Epidemiology Research Training," supported by the Fogarty International Center of the United States National Institutes of Health. We thank the Belgian Development Agency - CTB grant program of Belgium Government for funding the master's degree program, Dr. Gustavo Giusiano, from the Instituto de Medicina Regional at Universidad Nacional del Nordeste, and Dr. Alexis Holguín, from the Infectious Disease Department at INEN, for advice on laboratory and hospital data analysis, respectively.

### Author contributions

This research was the thesis of FV-C for his Masters of Science in Epidemiological Research at Universidad Peruana Cayetano Heredia. FV and

AGL conception and design of the study; FV, JG, VB, IC, CM and AM biofilm assay; FV, IC, CM and AM data collection; FV and AGL data analysis; FV, AGL, JG and VB writing, review and editing of the manuscript. All authors read and approved the final version of the manuscript.

**Funding**
All activities involving this study were self-financing without any organizational support.

**Data availability**
Data is provided within the manuscript or supplementary information files.

## Declarations

**Ethical approval**
This is a secondary analysis of clinical and microbiological data available in the hospital records and primary analysis derived from the biofilm assay in Candida isolates, respectively; performed without any use of human specimens nor contact with human subjects. The data was compiled and analyzed anonymously using unique study codes at all times, and the investigators did not have access to any personal identifiers. Prior to its execution, the study protocol was approved by the INEN Research Department Protocol Review Committee (INEN 16–45) and by the Universidad Peruana Cayetano Heredia Ethics Committee (SIDISI 101763). The research was conducted respecting all ethical principles outlined in the Helsinki Declaration.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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Received: 30 January 2024 / Accepted: 2 October 2024
Published online: 12 October 2024

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