Microbes and Infectious Diseases 2024; 5(3): 1020-1031

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Invasive candidiasis in intensive care units in Tunisia: A prospective matched case-control study

Maher Ben Brahim [1]*, Meriam BenHamida-Rebai [1], Nihel Haddad [1,2], Lamia Tilouche [1], Sameh Boughattas[1], Farah Azouzi [1], Abdelhalim Trabelsi [1], Soumaya Ketata [1]

1- *Laboratory of microbiology, University Hospital Sahloul, Sousse, Tunisia*
2- *Department of preventive and community medicine. Faculty of Medicine of Sousse, University of Sousse*

ARTICLE INFO

Article history:
Received 5 February 2024
Received in revised form 23 February 2024
Accepted 26 February 2024

Keywords:
Invasive candidiasis
ICU
Risk factors
Early diagnosis
Species distribution

ABSTRACT

Background: Invasive candidiasis (IC) is widely recognized as a major cause of morbidity and mortality especially among patients hospitalized in intensive care units (ICU). Hence, we conducted a prospective matched case-control study in three ICUs of the university hospital Sahloul in Sousse region (Tunisia), with the aim of describing the epidemiology, microbiology and risk factors of IC, in order to provide a risk predictive model for early diagnosis of these infections in ICU. **Methods:** A prospective matched case-control study was conducted jointly between the microbiology laboratory and three ICUs for a period of seven months (February-August 2020). A case report form was used to collect data prospectively for each included patient. Patient's characteristics and risk factors were analyzed using univariate and multivariate conditional logistic regression models. **Results:** Out of 112 included patients, 30 patients had IC. The incidence rate of IC in ICU was 11.7 episodes per 1000 patient-days (86.4 per 1000 ICU admissions). Prolonged ICU stay (≥14 days) had a 3-fold increase in the risk of IC (p=0.003). Multivariate analysis showed that diabetes (p=0.035), cardiovascular disease (p=0.028) and recent surgery (p=0.004) were independent risk factors for IC. Non-*albicans Candida* species were responsible for 60% of IC cases and 70% of candidemia cases. Resistance to fluconazole was mainly seen among *C. glabrata* and uncommon *Candida* species. **Conclusion:** This study provides a risk predictive model for early diagnosis of IC in ICU, which can improve the diagnosis of these infections and contribute to guide targeted preventive and therapeutic antifungal strategies.

Introduction

Invasive candidiasis (IC) is the most common invasive fungal infection among hospitalized patients worldwide. Traditionally, IC has been widely reported in immunocompromised patients and critically ill patients in the intensive care unit (ICU) [1,2]. IC become a major global health threat due to its high associated morbidity, mortality and hospital costs [2]. In recent years, the incidence of IC has increased to become the third most common cause of infection in ICU worldwide, accounts for 17% of infections [3]. This evolution is probably related to the constant progress of medical and surgical care including invasive procedures and massive use of broad-spectrum antibiotics [2,4]. IC is caused by *Candida* species and encompasses both candidemia and deep-seated candidiasis, which may

DOI: 10.21608/MID.2024.267990.1789
* *Corresponding author:* Maher Ben Brahim
E-mail address: *maherov9@gmail.com*

occur concurrently or independently [5]. Historically, *Candida albicans* is the primary cause of IC. However, the proportion of non-*albicans* *Candida* species has increased rapidly since the 21st century [6]. These species represent a heterogeneous group with varying epidemiology, virulence and antifungal susceptibilities [6]. However, species distribution varies considerably with geographical location, institutions and even within hospital wards [7]. Therefore, the analysis of local epidemiological trends including species distribution and antifungal susceptibility profile of *Candida* species is essential to guide the adequate preventive and therapeutic strategies. In the past years, only few studies used a prospective matched case-control design to assess risk factors for IC in ICU [8,9]. Unmatched studies identified risk factors such as a central venous catheter (CVC), prior surgery, broad-spectrum antibiotic therapy, or total parenteral nutrition (TPN) which are present in a large number of ICU patients [10]. Furthermore, in Tunisia, the majority of local studies was retrospective, some were limited only to candidemia and others focused on population of neonates [11-13].

In this work, we conducted a prospective study in three ICUs of the university hospital Sahloul in Sousse region (Tunisia), with the aim of describing the epidemiology, microbiology and risk factors of IC in ICU. These data should be considered in clinical practices and health policies in order to improve the management and outcome of IC cases.

Material and Methods

Study design and patients' inclusion criteria

We carried out a prospective matched case-control study jointly between the microbiology laboratory and three ICUs (Medical ICU, Surgical ICU and Cardiovascular and Thoracic Surgery ICU) of the Sahloul university hospital (Sousse, Tunisia), for a period of seven months (February-August 2020). The study population included all patients hospitalized for at least 7 days in the ICU, or as soon as they were admitted if they were transferred from another ward or another care institution.

Data collection

A case report form was used prospectively for each included patient to collect data from the medical records and the internal database of the microbiology laboratory. The data included demographic characteristics, underlying medical conditions, hospital management, clinical risk factors, recent surgery, previous invasive procedures, previous treatments and laboratory data. We also calculated weekly for all included patients the *Candida* colonization index (CCI) and *Candida* score (CS) by using the method described by Pittet et al. [14] and Léon et al. [10]. The CCI was defined as the ratio of the number of distinct non-blood body sites colonized by *Candida* to the total number of body sites cultured. Patients with CI $\geq$ 0.5 were considered heavily colonized [14]. The CS was calculated by adding points provided by each component: two points for sepsis or septic shock and one point for each remaining variable including total parenteral nutrition, initial surgery, and multifocal *Candida* colonization. A CS $\geq$ 3 was considered significant [10].

Definitions and inclusion criteria for invasive candidiasis cases

IC encompasses both candidemia and deep-seated candidiasis, which may occur concurrently or independently. We identify three entities: candidemia in the absence of deep-seated candidiasis, candidemia associated with deep-seated candidiasis and deep-seated candidiasis in the absence of candidemia [5].

Proven IC was defined with reference to the latest version of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the Mycoses Study Group (EORTC/MSG) criteria for the diagnosis of invasive fungal infections. It requires the presence of at least one of the following diagnostic criteria: (i) Histopathological, cytopathological or direct microscopic confirmation of yeast cells in a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes). (ii) At least one peripheral blood culture positive for *Candida* and (iii) positive *Candida* culture from a sample obtained by sterile technique from a normally sterile site (e.g. cerebrospinal, pleural, peritoneal or peritoneal abscess fluid) [15].

Probable IC refers to deep-seated candidiasis in the absence of candidemia. It includes patients having a CS $\geq$ 3, with sepsis or septic shock in the absence of any bacterial infection evidence, and a CCI $\geq$ 0.5, with the isolation of the same *Candida* species from urinary, rectal and respiratory samples.

Patients were excluded if they presented a positive blood culture from a catheter but negative peripheral blood cultures, IC diagnosed before ICU

admission or non-IC (e.g., respiratory tract secretions with *Candida*, candiduria).

Laboratory tests

Candida isolates from blood and other sterile body fluid (including pleural, peritoneal and ascitic fluids), according to the manufacturer's instructions, were collected into both aerobic and anaerobic vials and incubated for at least five days in the BacT/AlerT 3D automated system (bioMérieux, Marcy l'Etoile, France) [16]. Biopsy specimens from deep organs (liver, kidney, spleen, pancreas and bones) and central venous catheters tips were inoculated onto blood agar and Sabouraud dextrose agar media. All positive cultures were manually sampled and inoculated onto CAN2® chromogenic agar plates (bioMérieux, France) to ensure the purity and the viability of the cultures. The following samples were collected for the included patients to assess CCI: respiratory secretions, rectal swab, armpit skin swab, mouth swab, nasal swab and urine [16]. Specimens were inoculated into Sabouraud dextrose agar media for 48h. The identification of all species was confirmed by the VITEK®2 automated system (bioMérieux, Marcy-l'Etoile, France) [5].

Antifungal susceptibility testing was performed using the VITEK® 2 AST-YSO7 cards (bioMérieux) to determine in-vitro susceptibility to five antifungals (Amphotericin B, Fluconazole, Voriconazole, Micafungin and Caspofungin). For some resistant fungal strains, an E-test® (Biodisk AB, Solna, Sweden) was also performed to confirm the results, using the RPMI medium [17]. The analysis and interpretation of data were performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [18].

In case of multiple episodes of IC in the same patient, only the first episode was included in our analysis. We also eliminated redundant specimens and considered only one strain of *Candida* for each patient when isolated from multiple sites presented the same susceptibility profile.

Statistical analysis

Statistical analyses were performed using IBM SPSS software version 23.0. The chi-squared test or Fisher's exact test was used in the comparison of categorical variables. Factors associated with IC were analyzed by using univariate and multivariate conditional logistic regression models. Factors with a $p < 0.05$ in univariate tests were analyzed with a binary logistic regression model to identify the independent risk factors. The difference was statistically significant when $p < 0.05$.

Approval of the ethics committee

The study protocol was approved by the ethics committee of the hospital.

Results

Incidence and patient characteristics

Between January 1, 2020 and August 31, 2020, 347 patients were hospitalized in the three ICUs, of whom 112 respected the inclusion criteria. Of these, 30 patients were diagnosed with IC. IC's cases distribution according to diagnosis criteria is summarized in **Table 1**. The incidence rate of IC in ICU was 11.7 episodes per 1000 patient-days (86.4 per 1000 ICU admissions), and that of candidemia was 3.9 episodes per 1000 patient-days (28.8 per 1000 ICU admissions). The baseline characteristics of the patients with IC and matched controls are presented in **Table 2.** Median age was 55 years (26–84). Female and male patients had approximately the same rates (53.3% vs 46.7%). IC occurred within a median duration of 30 days after ICU admission. Patients who stayed for at least 14 days in ICU were three times more likely to develop IC (p=0.003). Patients admitted for surgical (p<0.001) or traumatic (p=0.003) reasons were at greater risk of developing IC than those admitted for medical reasons.

Risk factors for invasive candidiasis

Univariate and multivariate analyses of risk factors for IC in ICU are summarized in **Table 2** and in **Table 3**, respectively.

The most common underlying diseases were diabetes (p<0.001) and cardiovascular disease (p<0.001). Other risk factors for IC included: concomitant bacterial infections (p=0.001), particularly *Staphylococcus aureus* infections (p=0.016), previous surgery (p<0,01), especially digestive surgery (p=0.006) and cardiovascular surgery (0.027), exposure to corticosteroids (p=0.014), a long exposure (≥ 7 days) to invasive mechanical ventilation (p=0.04) or central venous catheterization (CVC) (p=0.012) and broad-spectrum antibiotic therapy ≥14 days (p=0.001). Exposure to carbapenems (p=0.002) and the association of cephalosporins and aminoglycosides (p=0.033) were significantly associated with IC.

Independent risk factors for IC in ICU included recent surgery (Odds ratio (OR) = 4.74, 95% confidence interval (CI) = 1.64-13.68,

p=0.004), cardiovascular disease (OR = 3.41, CI =1.13-10.22 *p*=0.028) and diabetes (OR = 3.13, CI= 1.08-9.06, *p*=0.035).

Microbiology

A total of 114 *Candida* isolates from 112 patients were identified and analyzed in the microbiology laboratory. Of these, 34 samples (urine and respiratory) were considered as colonization and thus were eliminated. Among 30 IC cases, 10 patients had candidemia (seven patients with only positive peripheral blood cultures and other mucous samples yielding *Candida* and three patients with both positive blood cultures and pleural effusion samples yielding *Candida*). Twenty probable IC cases were diagnosed according to the modified definitions **(Table 1):** Invasive respiratory samples (BAL, FBAS) yielding *Candida* associated to clinical signs and multisite *Candida* colonization. IC was mostly caused by C. *albicans* (40%), followed by *C. tropicalis*, *C. glabrata* (16.7% each) and *C. parapsilosis* (10%). Other species were *C. guilliermondii* (3.3%), *C. dubliniensis* (3.3%), *C. ciferrii* (3.3%) and unidentified *Candida spp.* (6.7%). Regarding samples distribution, *C. albicans* was the most common isolated species in all different samples. Non-*albicans Candida* species were responsible for 60% of IC cases and 70% of candidemia cases. The results of in vitro antifungal susceptibilities of *Candida* strains are summarized in **Table 4**. All *C. albicans* strains were susceptible to the five tested antifungals. For *C. glabrata*, two strains were resistant to fluconazole. One *C. parapsilosis* strain was resistant to fluconazole and another strain was resistant to echinocandins. Among other species, *C. ciferrii* was resistant to both amphotericin B and voriconazole and two strains of *Candida spp.* (unidentified species) were resistant to fluconazole.

Evolution and treatment outcomes

The mortality rate among patients with IC was 66.7% (n=20/30). The median time from IC diagnosis to death was 12 days. The most commonly used antifungal agent was fluconazole (90%), followed by the echinocandins (10%). The duration of antifungal treatment ranged from seven days to 20 days depending on the location of the infection, the clinical course and the negativity of the mycological findings.

Table 1. Distribution of patients according to invasive candidiasis diagnosis criteria.

	Invasive candidiasis entities	**Parameters**	**Number of patients (%)**
Proven IC	Candidemia in the absence of deep-seated candidiasis	Blood culture that yields *Candida spp.*	7 (23.3)
	Candidemia associated with deep-seated candidiasis	Blood culture that yields *Candida spp.* and recovery of *Candida spp.* by culture from a sample obtained by a sterile procedure from a normally sterile site (other than mucous membranes)	3 (10)
Probable IC (modified definitions)	Deep-seated candidiasis in the absence of candidemia	CS ≥ 3 including the presence of sepsis or septic shock AND CCI ≥ 0.5 including urinary, rectal and invasive respiratory samples[1] yielding the same *Candida* species	20 (66.7)
Overall			**30 (100)**

Abbreviations: IC: Invasive Candidiasis; CS: Candida Score; CCI: *Candida* Colonization Index
[1]Invasive respiratory samples include: Bronchoalveolar lavage (BAL), fiberoptic-bronchoscopy aspirate (FBAS)
Source: Modified from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the Mycoses Study Group (EORTC/MSG) criteria [17].

Table 2. Baseline characteristics of patients with invasive candidiasis and matched controls.

Characteristics	**Invasive candidiasis (n=30)** M (min, max) or n (%)	**Matched controls (n=82)** M (min, max) or n (%)	**Total (n=112)** M (min, max) or n (%)	**OR (95%CI)**	***p***
Demographic data					
Age (years)	55.2 (26, 84)	49.3 (5, 92)	50.88 (5, 92)	0.9 (0,96-1)	0.145
Age ≥ 65 years	8 (26,7)	16 (19,5)	24 (21,4)	1.3 (0.6-2.6)	0.418
Male	14 (46,7)	52 (63,4)	66 (58,9)	0.6 (0.3-1.1)	0.113
Female	16 (53,3)	30 (36,6)	46 (41,1)	1.4 (0.9-2.2)	
Diagnosis on admission					
Surgical	14 (46,7)	9 (11)	23 (20,5)	3.3 (2-5.8)	**< 0.001**
Medical	9 (30)	28 (34,1)	37 (33)	0.8 (0.4-1.7)	0.683
Traumatic	7 (23,3)	45 (54,9)	52 (46,4)	1.7 (1.1-2.5)	**0.003**
ICU length of stay (days)	29.97 (7, 180)	20.13 (7, 97)	22.77 (7, 180)	2.1 (1.2-3)	**0.043**
ICU length of stay ≥14 days	24 (80)	40 (48,8)	64 (57,1)	3 (1.3-6.7)	**0.003**
Concomitant disease					
Diabetes	20 (66,7)	24 (29,3)	44 (39,3)	3 (1.6-5.9)	**< 0.001**
Cardiovascular disease	17 (56,7)	17 (20,7)	34 (30,4)	3 (1.6-5.4)	**< 0.001**
Respiratory disease	15 (50)	36 (43,9)	51 (45,5)	1.2 (0.6-2.2)	0.566
Hypertension	13 (43,3)	28 (34,1)	41 (36,6)	1.3 (0.7-2.4)	0.371
Chronic/acute renal failure	10 (33,3)	17 (20,7)	27 (24,1)	1.5 (0.8-3)	0.167
Gastrointestinal pathology	6 (20)	8 (9,8)	14 (12,5)	1.7 (0.8-3.5)	0.196
Solid cancer	1 (3,3)	4 (4,9)	5 (4,5)	0.7 (0.1-4.3)	0.999
Coma	18 (60)	27 (32,9)	45 (40,2)	2.2 (1.1-4.1)	**0.01**
Laboratory data					
Temperature > 38 °C	30 (100)	30 (36,6)	60 (53,6)	2.7 (2-3.6)	**< 0.001**
Anemia (Hemoglobin < 12 g/l)	23 (76,7)	51 (62,2)	74 (66,1)	1.2 (0.9-1.5)	0.152
C-reactive protein > 8 mg/L	29 (96,7)	48 (58,5)	77 (68,8)	1.6 (1.3-2)	**< 0.001**
Leucocyte count (> 10 x10^3/mm3)	28 (93,3)	36 (43,9)	64 (57,1)	2.1 (1.6-2.7)	**< 0.001**
Concomitant bacterial infections	27 (90)	45 (54,9)	72 (64,3)	5 (1.6-9.4)	**0.001**
S. aureus concomitant infection	7 (23,3)	7 (8,5)	14 (12,5)	2.1 (1.1-4)	**0.016**
Previous surgery (within 30 days)	20 (66,7)	23 (28)	43 (38,4)	3.2 (1.7-6.2)	**< 0.001**
Digestive tract surgery	8 (26,7)	5 (6,1)	13 (11,6)	2.7 (1.5-4.8)	**0.006**
Neurosurgery surgery	5 (16,7)	13 (15,9)	18 (16,1)	1 (0.4-2.3)	0.917
Cardiovascular surgery	3 (10)	1 (1,2)	4 (3,6)	3 (1.5-5.7)	**0.027**
Urinary tract surgery	2 (6,7)	2 (2,4)	4 (3,6)	1.9 (0.6-5.4)	0.291
Previous treatment (within 30 days)					
Corticosteroid therapy	15 (50)	21 (25,6)	36 (32,1)	2.1 (1.1-3.8)	**0.014**
Broad-spectrum antibiotic therapy	30 (100)	74 (90,2)	104 (92,9)	1.4 (1.2-1.5)	0.106
Broad-spectrum antibiotic therapy ≥14 days	21 (70)	28 (34,1)	49 (43,7)	3 (1.5-6)	**0.001**
Cephalosporins G3/G4	9 (30)	15 (18,3)	24 (21,4)	1.5 (0.8-3)	0.181
Aminoglycosides	18 (60)	37 (45,1)	55 (49,1)	1.5 (0.8-2.9)	0.163
Cephalosporins + Aminoglycosides	8 (26,7)	8 (9,8)	16 (14,3)	2.2 (1.2-4)	**0.033**
Fluoroquinolones	7 (23,3)	8 (9,8)	15 (13,4)	2 (1-3.7)	0.113
Carbapenems	22 (73,3)	33 (40,2)	55 (49,1)	2.8 (1.4-5.8)	**0.002**
Glycopeptides	9 (30)	14 (17,1)	23 (20,5)	1.7 (0.8-3.1)	0.134
Polymyxines (Colimycine)	4 (13,3)	10 (12,2)	14 (12,5)	1 (0.4-2.6)	0.872
Previous invasive procedures					
Total parenteral nutrition	16 (53,3)	24 (29,3)	40 (35,7)	2 (1.1-3.7)	**0.019**
Peripheral venous catheter	2 (6,7)	18 (22)	20 (17,9)	0.3 (0.1-1.2)	0.061

Characteristics	**Invasive candidiasis (n=30)** M (min, max) or n (%)	**Matched controls (n=82)** M (min, max) or n (%)	**Total (n=112)** M (min, max) or n (%)	**OR (95%CI)**	***p***
Arterial catheter	25 (83,3)	48 (58,5)	73 (65,2)	2.7 (1.1-6.4)	**0.015**
Mechanical ventilation	30 (100)	74 (90,2)	104 (92,9)	1.4 (1.2-1.6)	0.106
Central venous catheter duration	4 (13,3)	31 (37,8)	35 (31,3)	0.3 (0.1-0.9)	**0.012**
≤ 7 days	26 (86,7)	50 (61)	76 (67,9)	3 (1.1-8.1)	
> 7 days					
Mechanical ventilation duration	4 (13,3)	27 (32,9)	31 (27,7)	0.3 (0.1-0.8)	**0.04**
≤ 7 days	26 (86,7)	47 (57,3)	73 (65,2)	3.4 (1.3-9.2)	
> 7 days					

Abbreviations: OR: Odds Ratio; CI: Confidence Interval; G3/G4: Third/Fourth generation

Table 3. Independent risk factors associated with invasive candidiasis.

Risk factors	**Whole population**[1] (n=112)		
	OR	**IC95%**	***p***
Diabetes	3.13	1.08-9.06	**0.035**
Cardiovascular disease	3.41	1.13-10.22	**0.028**
Previous surgery (within 30 days)	4.74	1.64-13.68	**0.004**
Central venous catheter duration > 7 days	1.67	0.25-11.13	0.593
Total parenteral nutrition	1.97	0.64-6.02	0.232
Corticosteroid therapy	2.03	0.42-9.72	0.376

Variables in multivariate models were selected by stepwise regression, using a cutoff *p* value of 0.05
Abbreviations: OR: Odds Ratio; CI: Confidence Interval

Table 4. In vitro antifungal susceptibility results of 30 *Candida* strains.

Species and antifungal agents	MIC (µg/mL)			Number (%) of isolates in each category		
	Range	**50%**	**90%**	**S**	**I/SDD**	**R**
Candida albicans ***(n=12)***						
Amphotericin B	≤0,25-2	0,5	1	12		
Fluconazole	≤0,5-1	0,5	0,5	12		
Voriconazole	≤0,12	≤0,12	≤0,12	12		
Caspofungin	≤0,12-025	≤0,12	≤0,12	12		
Micafungin	≤0,06-0,19	≤0,06	0,19	12		
Candida parapsilosis ***(n=3)***						
Amphotericin B	≤0,25-0,5	0,25	0,5	3		
Fluconazole	1-8	1	8	2		**1**
Voriconazole	≤0,12	≤0,12	≤0,12	3		
Caspofungin	0,5-8	0,5	8	2		**1**
Micafungin	0,5-8	0,5	8	2		**1**
Candida tropicalis ***(n=5)***						
Amphotericin B	≤0,25-0,5	0,25	0,5	5		
Fluconazole	≤0,5-1	1	1	5		
Voriconazole	≤0,12	≤0,12	≤0,12	5		
Caspofungin	≤0,12	≤0,12	≤0,12	5		
Micafungin	≤0,06-0,19	0,06	0,19	5		
Candida glabrata ***(n=5)***						
Amphotericin B	≤0,25-1	0,5	1	5		

Fluconazole	1-8	8	8		**3**	**2**
Voriconazole	≤0,12	≤0,12	≤0,12	5		
Caspofungin	≤0,12	≤0,12	≤0,12	5		
Micafungin	≤0,06	≤0,06	≤0,06	5		
Others[a] ***(n=5)***						
Amphotericin B	≤0,25-16	0,5	16	4		**1**
Fluconazole	1- 8	8	8	3		**2**
Voriconazole	≤0,12-8	≤0,12	8	4		**1**
Caspofungin	≤0,12-0,5	0,25	0,5	5		
Micafungin	≤0,06	≤0,06	≤0,06	5		

Notes: [a] Others includes: *Candida* spp. (2), *C. ciferrii* (1), *C. dubliniensis* (1) *C. guilliermondii* (1)
Abbreviations: S: susceptible; I: intermediate; SDD: susceptible dose-dependant; R: resistant

Discussion

This prospective, matched case-control study was designed to analyze epidemiology, risk factors and microbiology data of IC in ICU patients in Sahloul University hospital in Sousse (Tunisia). Data on IC in Tunisia ICUs are scarce and this is the first prospective study of IC in Tunisia.

In this study, the incidence rate and the cumulative incidence of IC in ICU were 11.7 episodes per 1000 patient-days and 86.4 per 1000 ICU admissions, respectively. Unfortunately, no data to compare were found in Tunisia. Local studies were retrospective and focused only on candidemia or some specific populations, like neonates [11-13]. Worldwide, the cumulative incidence of IC in ICU ranges from two to 138 cases per 1000 admissions [19-21]. The incidence found in our study is much higher than most of these studies which focused mainly on candidemia cases. In many studies, we noticed the word 'underestimated' referring to the incidence of IC [22,23]. The term "invasive candidiasis" is applied to different defined clinical conditions as we explained in "definitions" section. Some of these, require a complex diagnostic approach including risk factors, clinical and microbiological expertise, while others, such as candidemia, represent a clear-cut entity [5]. In fact, the majority of IC is diagnosed using blood culture, but two studies reported respectively that only 17% or 45% of cases of deep-seated candidiasis were detected by blood culture, suggesting that many cases could be undetected [24,25]. For this reason, in our study, we chose to join both probable and proven IC cases in order to enhance the sensitivity of detecting IC and thus reflect the real burden of these infections in routine practices.

Our choice to monitor patients by calculating the CS and the CCI was based on the results of several studies, and two previous studies from our work, which have proven the effectiveness of these tools to help identifying patients at high risk of IC [10,14,26,27]. Léon et al. reported that the relative risk of developing IC was significantly elevated in patients having stool, urine and respiratory swabs compared to other sites, leading us to include it in the criteria [10].

Recently, the detection of 1,3-β-D-glucan, a cell wall constituent of *Candida*, was approved as mycological evidence for the diagnosis of probable IC [15]. Unfortunately, this test is unavailable in our laboratory; otherwise, we would have added it to the criteria of probable IC.

A novelty of our work is the application of a matched case-control design in ICU patients. The advantage of this observational method is to separate risk factors that are specific for IC from those that are common among other ICU patients. In our study, we showed that IC can occur in all patients, regardless age (p=0.145) and gender (p=0.113). Overall, our study confirms the well-established risk factors for IC, such as prolonged stay in ICU, recent surgery, total parenteral nutrition, central venous catheter, mechanical ventilation, as well previous exposure to broad-spectrum antibiotics (without class specification) [1,4,10].

In addition, our study showed more specific correlations between these risk factors and IC. For example, the simple exposure to mechanical ventilation does not lead to IC (p=0.106), but the long exposure (≥7 days) to this risk factor was significantly associated to IC (p=0.04). Moreover, we highlighted the specific types of surgery linked to IC. Digestive surgery was significantly associated with IC (p=0.006). As known, the systemic diffusion of *Candida* yeasts generally results from the translocation starting from the digestive lumen [28]. The disruption of the gut microbiota is

consequently a major determinant for the transition from colonization to infection [28]. Moreover, cardiovascular surgery was an important risk factor for IC (p=0.027). In patients who have undergone this type of surgery, the risk of IC increases with the use of broad-spectrum antibiotics, the presence of heart failure, and prolonged stay in the ICU after surgery [29]. Regarding exposure to broad-spectrum antibiotics, we highlighted the importance of the duration of broad-spectrum antibiotic therapy which was significantly associated with IC (p=0.001). In particular, patients who have taken broad-spectrum antibiotic therapy for at least 14 days were three times more likely to develop IC. In fact, the presence and behavior of the bacterial microbiota seem to condition the relative virulence of *Candida*. In the absence of endogenous bacterial flora, *Candida* spp. is able to multiply, infect and invade epithelial surfaces [28]. In general, the risk of developing IC is higher the wider the antibiotic spectrum, the longer the duration of exposure and the higher the number of different antibiotics used [9].

One of the most striking findings of our study was the implication of some classes of antibiotics compared to others. The use of cephalosporins or aminoglycosides alone was not associated with IC (p=0.181, p= 0.163 respectively) but their use in association significantly increased the risk of infection (p=0.033). In addition, the use of carbapenems was significantly associated with IC (p=0.002). This finding is consistent with the recent study of Poissy et al. that also reported a significant association between the occurrence of candidemia in ICU patients and previous exposure to carbapenems, aminoglycosides and fluoroquinolones [9]. In our study, IC was significantly associated with concomitant bacterial infections, especially *Staphylococcus aureus* infections. The association between IC and the presence of *S. aureus* is probably explained by the ability of *S. aureus* to bind to the hyphae form of *Candida*, which will allow it to reach deeper tissues thus increasing the risk of infection [30]. The independent risk factors significantly associated with IC found in our study were diabetes, cardiovascular disease and recent surgery. In the literature, multivariate analyses of IC risk factors considered only candidemia and not all IC entities. Yet, Blumberg et al. and Poissy et al. both reported recent surgery and cardiovascular disease as independent risk factor for candidemia [9,31]. In the other hand, it is well known that diabetic patients are at greater risk of infections, whether bacterial or fungal, than the general population [32]. Diabetics are particularly vulnerable to fungal infections and the risk of mycoses is increased by a factor of 1.38 [33]. Indeed, prolonged hyperglycemia is a favorable environment for the growth of *Candida*, and carbohydrates increases its virulence, by stimulating the transition from blastospore to hyphae form [33]. Recently, Lao M et al. in a study conducted on diabetic patients, proved the increased risk of contracting IC in this population requiring thus more rigorous monitoring [33].

In our study, *C. albicans* was the most frequently isolated *Candida* species (40% of the isolates). Non-*albicans Candida* species were responsible for 60% of IC cases and 70% of candidemia cases. Similar rates were reported by Sellami et al. in Sfax (Tunisia) (77.7%) [11], Arrache et al. in Algeria (68.4%) [34] and other global studies (54.3% to 67.9%) [35,36]. In recent years, we are witnessing a change in the ecology of *Candida* species with a trend towards non-*albicans* species [6]. This fact was also confirmed by our local retrospective study conducted in the same Sahloul University hospital in 2020. In this 5-year surveillance study of IC, non-*albicans Candida* species represented over than 63% of IC cases in ICU [37]. This high non-*albicans Candida* species in ICU is linked to the selective pressure associated with the excessive and inappropriate use of antifungals, to individual patient risk factors and clonal outbreaks of some *Candida* species [38]. As we reported, fluconazole is the most antifungal drug used in our institution due to its availability and low cost compared to other drugs [39]. In fact, excessive use of azoles leads to select resistant *Candida* strains [38]. Indeed, in the present study, five among all strains (16.7%) were resistant to fluconazole. As seen previously in our center, fluconazole resistance rate was found to be high (6.7% of all isolates) [37]. These results should alarm us in order to change the routine treatment management of IC. Bassetti et al.'s finding that limiting fluconazole use can reduce non-*albicans* candidemia, which are often more resistant to antifungals, should spur reconsideration of the use of fluconazole [40]. Recent guidelines highlighted the importance of earlier antifungal therapy and improved clinical outcomes in IC patients [15]. Thus, an improved prediction of the risk of IC, based on our findings, may contribute to guide targeted preventive and therapeutic antifungal

strategies.

Conclusion

The results from this prospective matched case-control study could be used to establish a risk predictive model for early diagnosis of IC. Based on our findings, we recommend the following: greater focus on early, multisite diagnostic sampling, by monitoring both CCI and CS. Patients with significant scores who are diabetic, have cardiovascular disease or undergone recent surgery, especially digestive, should be closely monitored and aggressively treated. In addition, patients on prolonged broad-spectrum antibiotic therapy including carbapenems or association of cephalosporins and aminoglycosides, presenting concomitant bacterial infections, especially invasive *S. aureus* infections, or prolonged invasive mechanical ventilation are at high risk for IC. Furthermore, we recommend timely removal of drainage catheter after the first positive sample and prescribing antifungal therapy in accordance with local epidemiological patterns. As we highlighted, non-*albicans Candida* species are frequently isolated in ICU. Given the high fluconazole resistance rate among these species in our institution, we recommend the early use of echinocandins as first-line treatment, as recommended worldwide [15]. Continuous surveillance of local epidemiology as well as improving diagnosis means is essential to reduce morbidity and mortality of IC in ICU.

Acknowledgments

None

Funding

No financial support was used for the completion of this study.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

1- Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Rev Dis Primers 2018;4:1-20.

2- Barantsevich N, and Barantsevich E. Diagnosis and Treatment of Invasive Candidiasis. Antibiotics (Basel) 2022 May 26;11(6):718.

3- Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD et al. EPIC II Group of Investigators. International study of the prevalence and outcomes of infection in intensive care units. JAMA. 2009 Dec 2;302(21):2323-9.

4- Paiva JA, Pereira JM, Tabah A, Mikstacki A, De Carvalho FB, Koulenti D, et al. Characteristics and risk factors for 28-day mortality of hospital acquired fungemias in ICUs: data from the EUROBACT study. Crit Care 2016;20:1-13.

5- Clancy CJ, and Nguyen MH. Diagnosing Invasive Candidiasis. J Clin Microbiol 2018 Apr 25;56(5):e01909-17.

6- Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty Years of the SENTRY Antifungal Surveillance Program: Results for Candida Species From 1997-2016. Open Forum Infect Dis 2019 Mar 15;6(Suppl 1):S79-S94.

7- Ricotta EE, Lai YL, Babiker A, Strich JR, Kadri SS, Lionakis MS et al. Invasive Candidiasis Species Distribution and Trends, United States, 2009-2017. J Infect Dis. 2021 Apr 8;223(7):1295-1302.

8- Playford EG, Lipman J, Jones M, Lau AF, Kabir M, Chen SC et al. Problematic Dichotomization of Risk for Intensive Care Unit (ICU)-Acquired Invasive Candidiasis: Results Using a Risk-Predictive Model to Categorize 3 Levels of Risk From a Multicenter Prospective Cohort of Australian ICU Patients. Clin Infect Dis. 2016 Dec 1;63(11):1463-1469.

9- Poissy J, Damonti L, Bignon A, Khanna N, Von Kietzell M, Boggian K et al. Risk factors for candidemia: a prospective matched case-control study. Crit Care. 2020 Mar 18;24(1):109.

10- León C, Ruiz-Santana S, Saavedra P, Galván B, Blanco A, Castro C et al. Usefulness of the "Candida score" for discriminating between Candida colonization and invasive candidiasis in non-neutropenic critically ill patients: a prospective multicenter study. Crit Care Med. 2009 May;37(5):1624-33.

11- Sellami A, Sellami H, Néji S, Makni F, Abbes S, Cheikhrouhou F et al. Antifungal susceptibility of bloodstream Candida isolates in Sfax hospital: Tunisia. Mycopathologia. 2011 Jun;171(6):417-22.

12- Saghrouni Fatma, Bougmiza Iheb, ben abdeljelil Jihene,Yacoub A., Khammari, Imen et al. (2011). Epidemiological trends in invasive candidiasis: Results from a 15-year study in Sousse region, Tunisia. Journal De Mycologie Medicale - J MYCOLOGIE MEDICALE. 21. 123-129.

13- Ben Abdeljelil J, Saghrouni F, Nouri S, Geith S, Khammari I, Fathallah et al. Neonatal invasive candidiasis in Tunisian hospital: incidence, risk factors, distribution of species and antifungal susceptibility. Mycoses. 2012 Nov;55(6):493-500.

14- Pittet D, Monod M, Suter PM, Frenk E, Auckenthaler R. Candida colonization and subsequent infections in critically ill surgical patients. Ann Surg 1994; 220(6):751–758.

15- Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis. 2020 Sep 12;71(6):1367-1376.

16- Horvath LL, George BJ, Hospenthal DR. Detection of fifteen species of Candida in an automated blood culture system. J Clin Microbiol 2007 Sep;45(9):3062-3064.

17- Berkow EL, Lockhart SR, OstroskyZeichner L. Antifungal susceptibility testing: current approaches. Clin Microbiol Rev 2020; 33:e00069-19.

18- The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs for antifungal agents. EUCAST, version 10.0, 2020. http://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals.

19- Bassetti M, Giacobbe DR, Vena A, Trucchi C, Ansaldi F, Antonelli M, et al. Incidence and outcome of invasive candidiasis in intensive care units (ICUs) in Europe: results of the EUCANDICU project. Crit Care 2019;23(1): 17.

20- Montagna MT, Caggiano G, Lovero G, De Giglio O, Coretti C, Cuna T, et al. Epidemiology of invasive fungal infections in the intensive care unit: Results of a multicenter Italian survey (AURORA Project). Infection 2013;3:645–53.

21- Massou S, Ahid S, Azendour H, Bensghir M, Mounir K, Iken M, et al. Les candidoses systémiques en réanimation médicale : Analyse des facteurs de risque et intérêt de l'index de colonisation. Pathol Biol 2013;61(3):108-12.

22- Soriano A, Honore PM, Puerta-Alcalde P, Garcia-Vidal C, Pagotto A, Gonçalves-Bradley DC, Verweij PE. Invasive candidiasis: current clinical challenges and unmet needs in adult populations. J Antimicrob Chemother. 2023 Jul 5;78(7):1569-1585.

23- Strollo S, Lionakis MS, Adjemian J, Steiner CA, Prevots DR. Epidemiology of Hospitalizations Associated with Invasive

Candidiasis, United States, 2002-20121. Emerg Infect Dis 2016 Jan;23(1):7-13.

24- Nguyen MH, Wissel MC, Shields RK, Salomoni MA, Hao B, Press EG et al. Performance of Candida real-time polymerase chain reaction, β-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. Clin Infect Dis. 2012 May;54(9):1240-8.

25- Fortún J, Meije Y, Buitrago MJ, Gago S, Bernal-Martinez L, Pemán J et al. Clinical validation of a multiplex real-time PCR assay for detection of invasive candidiasis in intensive care unit patients. J Antimicrob Chemother. 2014 Nov;69(11):3134-41.

26- M. Ben Brahim, N. Haddad, S. Boughattas, A. Farah, L. Tilouche, W. Naija, et al. Abstracts from the 6th international conference on prevention & infection control (ICPIC 2021) . Prospective evaluation of Candida colonization index in intensive care unit patients, in Tunisia. Antimicrob Resist Infect Control 2021; 10(Suppl 1):130.

27- M. Ben Brahim, N. Haddad, S. Boughattas, A. Farah, L. Tilouche, W. Naija, et al. Abstracts from the 6th international conference on prevention & infection control (ICPIC 2021) . Evaluation of « Candida score» in intensive care unit patients: a prospective observational study in Tunisia. Antimicrob Resist Infect Control 2021, 10(Suppl 1):130.

28- Höfs S, Mogavero S, Hube B. Interaction of Candida albicans with host cells: Virulence factors, host defense, escape strategies, and the microbiota. J Microbiol 2016;54(3):149-69.

29- Giacobbe DR, Salsano A, Del Puente F, Miette A, Vena A, Corcione S, et al. Risk Factors for Candidemia after open heart surgery: Results from a multicenter case–control study. Open Forum Infect Dis 2020;7(8):1-7.

30- Bernard C, Girardot M, Imbert C. Candida albicans interaction with Gram-positive bacteria within interkingdom biofilms. J Mycol Med 2020;30(1):1-25.

31- Blumberg HM, Jarvis WR, Soucie JM, Edwards JE, Patterson JE, Pfaller MA, et al. Risk factors for candidal bloodstream infections in surgical intensive care unit patients: The NEMIS prospective multicenter study. Clin Infect Dis 2001;33(2):177- 86.

32- Muller L, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, et al. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005;41(3):281-8.

33- Lao M, Li C, Li J, Chen D, Ding M, Gong Y. Opportunistic invasive fungal disease in patients with type 2 diabetes mellitus from Southern China: Clinical features and associated factors. J Diabetes Investig. 2020;11(3):731-44.

34- Arrache D, Madani K, Zait H, Achir I, Younsi N, Zebdi A, et al. Fungal diagnosed at the parasitology-mycology laboratory of the Mustapha University Hospital in Algiers, Algeria (2004–2014). J Mycol Med 2015;3(25):237-238.

35- Xiao M, Sun ZY, Kang M, Guo DW, Liao K, Chen SC, et al. Five-year national surveillance of invasive candidiasis: Species distribution and azole susceptibility from the China hospital invasive fungal surveillance net (CHIF-NET) Study. J Clin Microbiol 2018;56(7):1-12.

36- Castanheira M, Messer SA, Rhomberg PR, Pfaller MA. Antifungal susceptibility patterns of a global collection of fungal isolates: results of the SENTRY Antifungal Surveillance Program (2013). Diagn Microbiol Infect Dis 2016 Jun;85(2):200-4.

37- BEN BRAHIM M., BenHamida-Rebai M, Haddad N, Tilouche L, Trabelsi A, Ketata S. Surveillance of the epidemiology and antifungal susceptibility of invasive candidiasis: A retrospective study from 2016 to 2020 in a teaching hospital in Sousse, Tunisia. Microbes and Infectious Diseases 2024; 2024; 5(1): 347-358. doi: 10.21608/mid.2023.222594.1562

38- Colombo AL, Júnior JNA, Guinea J. Emerging multidrug-resistant Candida species. Curr Opin Infect Dis 2017;30(6):528538.

39- Grau S, Pozo JC, Romá E, Salavert M, Barrueta JA, Peral C et al. Cost-effectiveness of three echinocandins and fluconazole in the treatment of candidemia and/or invasive candidiasis in nonneutropenic adult patients. Clinicoecon Outcomes Res. 2015 Oct 13;7:527-35.

40- Bassetti M, Ansaldi F, Nicolini L, Malfatto E, Molinari MP, Mussap M et al. Incidence of candidaemia and relationship with fluconazole use in an intensive care unit. J Antimicrob Chemother. 2009 Sep;64(3):625-9.

Ben Brahim M, BenHamida-Rebai M, Haddad N, Tilouche L, Boughattas S, Azouzi F, Trabelsi A, Ketata S. Invasive candidiasis in intensive care units in Tunisia: A prospective matched case-control study. Microbes Infect Dis 2024; 5(3): 1020-1031.