Epidemiology of candidemia in neonatal intensive care units: a persistent public health problem


Key words: Epidemiology, candidemia, neonatal intensive care unit, fluconazole, amphotericin B.

Parole chiave: Epidemiologia, candidemia, terapia intensiva neonatale, fluconazolo, amfotericina B.

Abstract

Background. Candidemia has become an increasingly important problem in infants hospitalized in the Neonatal Intensive Care Units (NICUs). Candida species are the third most common agents of late-onset infections in critically ill neonates and they are associated with high morbidity and mortality rates. In this study we evaluated the epidemiology of Candida bloodstream infections in the NICU of an Italian university hospital during a 15-year period. Our specific aims were to analyze the change in species distribution and the vitro susceptibility of these yeasts to fluconazole (FCZ) and amphotericin B (AmB).

Methods. A retrospective study of candidemia in the NICU of a university hospital in southern Italy, covering the years 2000-2014 was carried out. The isolates were identified using the VITEK2 yeast identification system and antifungal susceptibility was determined using the E-test method.

Results. Among the 57 patients with confirmed candidemia, 60% were males (n = 34 cases) and 82% (n = 47) had a gestational age of 24-32 weeks. Twenty-seven neonates (47%) had a very low birth weight (< 1500 g), 20 (35%) an extremely low birth weight (<1000 g), and 10 (18%) a low birth weight (< 2500 g). The most important potential risk factors were the placement of a central venous catheter, total parenteral nutrition, and endotracheal intubation (100%, each). Candida albicans was the most frequent yeast (47%), followed by Candida parapsilosis (44%). The proportion of Candida non-albicans increased slightly, from 46% in 2000-2004 to 71% in 2010-2014 (χ² test for trend, p = 0.030). All isolates were susceptible to FCZ and AmB.

Conclusions. The detection in this epidemiologic study of an increase in Candida non-albicans highlights the importance of correct species-level identification in the rapid diagnosis for an efficient treatment of candidemia. Knowledge of the local epidemiological trends in Candida species isolated in blood cultures will facilitate therapeutic decision-making.

Introduction

In the neonatal intensive care unit (NICU), blood stream infections (BSI) caused by Candida species are less frequent than those caused by Gram-positive or Gram-negative bacteria; nonetheless, they have higher rates of morbidity and mortality. Among infants with an extremely low birth weight (<1000 g), 4-8% will develop candidemia, which

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has a 30% mortality in this group of patients (1, 2). Even among infants who survive these infections, long-term neurological impairments, including cerebral palsy, blindness, hearing impairment, cognitive deficits, and periventricular leukomalacia, are common (2). The risk factors for neonatal candidemia include prematurity, use of central venous lines (CVCs), intubation, parenteral nutrition, broad-spectrum antibiotics administration (in particular, third-generation cephalosporins), prolonged hospitalization, abdominal surgery, exposure to a H2 blocker and Candida colonization (2, 3). While Candida albicans is the most prevalent species, the incidence of BSI caused by Candida non-albicans, particularly Candida parapsilosis complex and Candida glabrata complex, has increased during the past two decades (3).

Only a few epidemiological studies on candidemia in infants hospitalized in NICUs have been published in Italy (4-7). The aim of this study was to determine: i) the epidemiology of Candida BSI in the NICU of an Italian university hospital during a 15-year period, ii) the changing species distribution, and iii) the in vitro susceptibility to fluconazole (FCZ) and amphotericin B (AmB).

**Materials and methods**

**Study design and definitions**

This retrospective observational study was conducted in the NICU of a university hospital in southern Italy, from January 1, 2000 to December 31, 2014. All neonates who developed candidemia were included in the study. Only the first episode of candidemia was reported for those patients with recurrent or subsequent episodes. Clinical data were collected from the microbiological laboratory database and included sex, age, weight, and predisposing risk factors for Candida BSI (intravascular devices, prematurity, prolonged antibiotics, total parental nutrition, prolonged hospitalization). Candidemia was diagnosed on the basis of the *Candida* positivity of a blood sample drawn from a peripheral vein. Prematurity was defined as a gestational age ≤37 weeks. Prolonged hospitalization was defined as >14 days and prolonged antibiotics use as >14 days (8).

**Laboratory procedures**

Blood cultures were performed using a lysis-centrifugation system (Isolator, DuPont Co., Wilmington, DE, USA). The samples were cultured on two plates of Sabouraud dextrose agar containing 0.05% chloramphenicol (BioRad, Marnes-la-Coquette, France), incubated at 36°±1° and 28°±1°, and examined daily for 10 days. The isolates were identified using standard procedures (morphology on cornmeal agar plates, germ-tube production in serum, and ability to grow at 37° and 42°) and biochemical analyses (VITEK2 System, Biomérieux, Marcy l’Etoile, France). Antifungal minimum inhibitory concentration (MIC) was determined using the E-test method (AB BIODISK, BioMérieux) on RPMI 1640 agar plates (Biolife, Milan, Italy) as recommended by the manufacturer. The drug concentration shown on the E-test strip at the outer border of the elliptical inhibition halo was recorded as the MIC.

**Analysis of the results**

FCZ susceptibility was determined on the species-specific clinical breakpoints suggested by the Clinical and Laboratory Standards Institute subcommittee (9). In *C. albicans* and the *C. parapsilosis* complex, FCZ MIC end points of ≤2, 4, and ≥8 µg/mL were defined as susceptible (S), susceptible dose-dependent (SDD), and resistant (R), respectively. For *C. glabrata*, SDD and R were defined as FCZ MIC end points of ≤32 and ≥64 µg/mL, respectively. For *Candida guilliermondii*, the epidemiological cut-off
value (ECV) indicating resistance was ≥8 µg/mL (10).

An ECV ≥2 µg/mL was used to define the AmB resistance (R) of the isolates (11). MIC data for each species are presented as the MIC$_{50}$ and MIC$_{90}$ (the MIC causing 50% and 90% inhibition of the isolates, respectively). MIC$_{50}$ and MIC$_{90}$ values were calculated for species with 10 or more isolates.

A χ$^2$ test for trend was used to compare changes in species distribution. P values of <0.05 were considered to indicate statistical significance. The data were analyzed with GraphPad Prism version 5.0 for Windows (San Diego, CA, USA).

**Results**

During the 15-year study, there were 57 confirmed cases of candidemia in the NICU. The clinical characteristics of patients are shown in Table 1. Sixty percent were male (34 patients) and 82% (47 patients) had a gestational age of 24–32 weeks. The birth weight of the infected patients was very low (1001–1500 g) in 27 (47%), extremely low (<1000 g) in 20 (35%), and low (1501–2500 g) in 10 (18%). CVC placement, total parenteral nutrition, and endotracheal intubation coincided with the development of candidemia in all 57 patients.

*Candida* species were isolated in the urinary (100%), respiratory (87.5%), and intestinal (50%) tracts. *Candida albicans* (27 patients, 47%) was the most frequent species, followed by *C. parapsilosis* complex (25 patients, 44%), *C. guilliermondii* (3 patients, 5%), and *C. glabrata* complex (2 patients, 4%). The proportion of *Candida* non-*albicans* increased from 46% between 2000 and 2004 to 71% between 2010 and 2014 (p=0.030).

All isolates were highly susceptible to FCZ and AmB. The MIC$_{50}$/MIC$_{90}$ values of FCZ and AmB for *C. albicans* were 0.25/0.75 and 0.19/0.25 µg/mL, respectively; for *C. parapsilosis*, the corresponding values were 0.38/1.5 and 0.125/0.25 µg/mL. For *C. guilliermondii*, the MIC values were below the ECV both for FCZ and for AmB (1 and 0.25 µg/mL, respectively). Two isolates of *C. glabrata* were both SDD to FCZ (8 µg/mL) and S to AmB (0.25 µg/mL).

**Discussion and conclusions**

Candidemia is the third-leading (12.2%) cause of late-onset sepsis in very low birth weight infants. The infections are associated in these patients with high morbidity and mortality rates (3). The most prevalent yeast in neonatal disease is *Candida albicans*, with most series reporting a prevalence of ~50% (12). However, over the years, there has been an epidemiological shift towards the predominance of species other than *C. albicans*, especially *C. parapsilosis* (3, 7, 13, 14). The findings from our 15-year surveillance study confirm this trend. In fact, while *C. albicans* was the most common (~50%) blood isolate, candidemia caused by *Candida non-albicans* increased from 46% in 2000 to 71% in 2014; in the large majority of these infections, *C. parapsilosis* was the most causative agent (~83%). This high rate of *C. parapsilosis* infections may reflect...

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Patients (No)</th>
<th>%</th>
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<tbody>
<tr>
<td>Central venous catheter</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>57</td>
<td>100</td>
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<tr>
<td>Prolonged antibiotic therapy</td>
<td>57</td>
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<tr>
<td>Total parenteral nutrition</td>
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<td>100</td>
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<tr>
<td>Prolonged hospitalization</td>
<td>56</td>
<td>98.26</td>
</tr>
<tr>
<td>Birth weight ≤1500 g</td>
<td>47</td>
<td>82.46</td>
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<tr>
<td>Prematurity</td>
<td>47</td>
<td>82.46</td>
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the ability of this species to form biofilms on catheters and to contaminate glucose-containing solutions, including those used in parenteral nutrition (15). In addition, because the C. parapsilosis complex is a commensal of human skin (horizontal transmission), its transmission from the hands of healthcare workers to neonates has been suggested in cases of CVC-related infections (13, 16).

Regarding the predisposing factors of candidemia in our study, prematurity, CVC, prolonged antibiotic treatment, mechanical ventilation, parenteral nutrition, and prolonged hospitalization resulted the most frequent, in agreement with other studies (8, 12-14). However, the extent to which these factors contributed to the development of candidemia in our patients could not be determined because our survey – based on a historical series - did not include a control, non-Candida-infected population.

Candida colonization is very common among NICU patients not receiving antifungal prophylaxis (17). In 28 out of 57 enrolled neonates with candidemia, Candida species had been isolated at the time of diagnosis, including from endotracheal, rectal, and/or other sites. Manzoni et al. (18) showed that Candida colonization of multiple body sites is an important predictor of progression to candidemia, underlining the need for systematic surveillance cultures in preterm infants.

In agreement with other studies (12-14), none of the strains isolated from our patients exhibited resistance to FCZ and AmB, which are the antifungal drugs of choice used in the prophylaxis and treatment of Candida BSI in neonates (19). No FCZ resistance may be related to the treatment policy in use at our hospital: systemic antifungal prophylaxis and/or empiric therapy with FCZ were not usually employed. In neonates, FCZ prophylaxis as a driver of the emergence of azole resistance has been reported (3, 20). In our opinion, the administration of FCZ as prophylaxis or as therapy should be evaluated according to the epidemiology of the hospital’s NICU: if the incidence of candidemia is low, a better approach may be to use probiotics or lactoferrin as prophylaxis, saving FCZ to treat documented infections (4, 21).

In conclusion, this epidemiological study demonstrated a progressive increase in Candida non-albicans infections and therefore the importance of correct species-level identification to achieve a rapid diagnosis and the efficient treatment of candidemia. Because BSIs with Candida spp. are life threatening (1, 2), affected patients require immediate antifungal treatment with the appropriate agents. Accordingly, knowledge of the local epidemiological trends in Candida species, as determined from hospital blood cultures, is important to guide therapeutic decision-making.

Riassunto

Epidemiologia delle candidemie nelle unità di terapia intensiva neonatale: un costante problema di sanità pubblica

Introduzione. Negli ultimi anni le sepsi da Candida spp. sono diventate un problema sempre più importante nei neonati ricoverati in terapia intensiva (UTIN). Le candidemie sono la terza causa di infezione late-onset nei neonati, associate ad alti tassi di morbilità e mortalità. Scopo di questo studio è valutare l’epidemiologia delle candidemie nella UTIN di un ospedale universitario italiano durante un periodo di 15 anni, analizzare il trend delle diverse specie di Candida e verificare la circolazione di ceppi resistenti nei confronti di fluconazolo (FCZ) e amfotericina B (AmB).


Risultati. Complessivamente, sono stati identificati 57 episodi di candidemia. Il 70% dei pazienti era di sesso maschile (n = 34), 1’82% dei neonati (n = 47) aveva un’età gestazionale di 24-32 settimane. Ventisette neonati (47%) avevano un peso molto basso (< 1500 grammi), 20 (35%) un peso estremamente basso (< 1000 grammi),
e 10 (18%) un peso basso (< 2500 grammi). I principali fattori di rischio sono risultati presenza di un catetere venoso centrale, nutrizione parenterale totale e intubazione endotracheale (100%, ciascuno). Candida albicans è risultata responsabile del 47% degli episodi (n = 27), mentre il 53% (n = 30) è stato sostenuto da Candida non-albicans, di cui Candida parapsilosis è risultata la specie più frequente (n = 25; 83%). La percentuale di C. non-albicans è aumentata dal 46% nel periodo 2000-2004 al 71% nel periodo 2010-2014 (p = 0.030). Tutti gli isolati sono risultati sensibili a FCZ e AmB.

Conclusioni. I nostri dati dimostrano un significativo aumento delle candidemie sostenute da C. non-albicans in UTIN. Un’adeguata conoscenza dell’epidemiologia locale è importante per impostare la terapia appropriata.

References


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