



## CANDIDA PELLICULOSA BLOODSTREAM INFECTION IN A NEONATE PATIENT FROM MÉRIDA, VENEZUELA

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Article Info	ABSTRACT
<p>Received 01/04/2015 Revised 15/04/2015 Accepted 04/05/2015</p> <p><b>Key words:</b> <i>Candida pelliculosa</i>, Fungemia, Nosocomial, Neonate.</p>	<p>The incidence of neonatal systemic candidiasis is associated with significant morbidity and mortality. <i>Candida pelliculosa</i> has been rarely involved as a causative agent of nosocomial fungemia. However, in this study we describe the first case of a <i>C. pelliculosa</i> blood infection in a term neonate hospitalized in a neonatal intensive care unit (NICU) in Mérida, Venezuela. Microbiological identification of the yeast was performed by the VITEK 2 system and confirmed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS). Antifungal susceptibility testing was carried out by the AST-YS07 VITEK 2 card system and Etest methods. The neonate was successfully treated with amphotericin B thanks to prompt antifungal susceptibility tests results. This case highlights the importance of <i>C. pelliculosa</i> as an emerging nosocomial fungal pathogen.</p>

### INTRODUCTION

Invasive fungal infections caused by yeasts are important causes of morbidity and mortality in hospitalized patients globally. *Candida* species have emerged as one of the most common causes of bloodstream infections among neonates and account for 9 - 13% of such infections [1]. Although *Candida albicans* is the primary etiological agent of neonatal candidemia worldwide, during the past decade an increasing trend of systemic and fatal infections with non-*C. albicans* species such as *C. tropicalis*, *C. krusei*, *C. lusitanae*, *C. parapsilosis*, *C. pelliculosa* has been reported [2].

*Candida pelliculosa* (teleomorph *Pichia anomala*) is a yeast frequently found in fruits, tree exudates, soil, vegetables and many other organic compounds [3]. Infections produced by this yeast are infrequent; however it has been implicated as the causative agent of a plethora of

clinical conditions such as nosocomial fungemia, interstitial lung disease, endocarditis, and enteritis, mainly in premature neonates and immunocompromised pediatric patients [4]. In a study on candidemia in the Maracaibo University Hospital, Zulia State, Venezuela, *C. pelliculosa* was the third most common non-*C. albicans* species after *C. tropicalis* and *C. parapsilosis* [5]. Also, a study on *Candida* species isolated from blood samples in the Neonatology Department of the "Concepción Palacios" Maternity and Women's Hospital in Caracas, revealed the predominance of *C. pelliculosa* amongst all *Candida* isolates from neonates with fungemia [6].

The pathogenesis and clinical prognosis of infections caused by *C. pelliculosa* rely on the immunological status of the patient, the virulence and resistance of the strain to antifungals and on the efficacy of antifungal therapy [7].



Usually, the susceptibility patterns of *C. pelliculosa* are variable or unknown and empiric therapy is frequently used to treat such infections [8]. Hence, early identification and rapid antifungal susceptibility testing are pivotal in life threatening cases [9, 10]. Here, we describe the first case of fungemia caused by *C. pelliculosa* in a newborn patient who was admitted to the neonatal intensive care unit (NICU) of The Andes University Hospital, Merida, Venezuela.

### CASE REPORT

A female neonate born at term (39 weeks), weighing 3200 g, to a 16-year-old primigravida mother and delivered by caesarean section, presented breathing difficulty and tachypnea, prompting her admission to a local hospital. The patient was placed in an incubator with oxygen supply, intravenous fluids and administered intravenous ampicillin (150 mg/kg/day) and amikacin (15 mg/kg/day) therapy. Three days later, the patient's clinical condition worsened, requiring her transfer to The Andes University Hospital. On admission to the pediatric emergency service, the baby was in bad general conditions, revealing muco-cutaneous paleness, distal cyanosis and severe respiratory distress which merited immediate intubation with ventilation assistance, multi-organ intensive care monitoring, proper intravenous fluids according to need, as well as parenteral feeding. The baby was admitted in the neonatal intensive care unit (NICU), where specimens were drawn for laboratory studies, including cerebrospinal fluid (CSF) and blood which were collected and sent for culture. The baby was started with first line antibiotics ampicillin/sulbactam (150 mg/kg/day) and gentamicin (5 mg/kg/day) in view of potential risk factors for sepsis. Laboratory results revealed anemia (hemoglobin, 10.7 g/dL) and thrombocytopenia (70.000 mm<sup>3</sup>) prompting transfusion with one unit of globular concentrate. Sepsis screening was positive, with a total leukocyte count of 4500 mm<sup>3</sup> and a C-reactive protein of

44.7 mg/dL. CSF showed pleocytosis (500 cells/mm<sup>3</sup>) with neutrophils predominance (70%), and hypoglycorrhachia (11 mg/dL) with increased protein levels (129 mg/dL). Gram stains and bacterial cultures returned negative. After three days, a blood culture grew *Candida pelliculosa*. The patient was initially treated with fluconazole (6 mg/kg/day) for 4 days and over the following 26 days with amphotericin B (1 mg/kg/day). All previous antibiotics treatments were discontinued. Other complementary studies such as nephrology, ophthalmological and cardiovascular tests, showed no abnormality, but a computerized tomography (CT) of the head with contrast revealed diffuse contrast aspect cytotoxic edema in the right cerebral hemisphere, suggestive of extensive frontoparietal ischemic injury. The baby was discharged well on breast feed and milk formulas after 34 days of hospital stay, and was scheduled for follow up in the pediatric neurology service.

The yeast was recovered in BacT/ALERT 3D 60 (BioMérieux, Marcy l'Etoile, France) culture vials and subcultured on 5% of sheep blood agar (BBL, Becton Dickinson, Cockeysville, MD, USA), and Sabouraud dextrose agar (BBL). Blastospores were observed in microscopic examination. Identification of the yeast isolate was carried out initially by the VITEK 2 system (BioMérieux) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (VITEK MS MALDI-TOF, BioMérieux) was performed to confirm the identification. Antifungal susceptibility testing (AST) was carried out by the AST-YS07 VITEK 2 card system (BioMérieux) and Etest methods (strips, AB Biodisk, Solma, Sweden).

The antifungal compounds tested were the following: amphotericin B, anidulafungin, caspofungin, fluconazole, flucytosine, micafungin, posaconazole and voriconazole. The interpretation of AST was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [11, 12].

**Table 1. Antifungal susceptibility profile of *Candida pelliculosa* isolated from a neonate patient**

Antifungal Agent	Test	MIC (mg/liter) at 24 hours			
	method	Range	Susceptible	SDD	Resistant
Amphotericin B	AST-YS07 VITEK 2	≤0.25-≥16	0.25	-	-
	Etest	0.002-32	0.094	-	-
Anidulafungin	AST-YS07 VITEK 2	ND	-	-	-
	Etest	0.002-32	0.016	-	-
Caspofungin	AST-YS07 VITEK 2	≤0.25-≥4	≤0.25	-	-
	E-test	0.002-32	0.064	-	-
Fluconazole	AST-YS07 VITEK 2	≤1-≥64	2	-	-
	Etest	0.016-256	-	4	-
Flucytosine	AST-YS07 VITEK 2	≤1-≥64	≤1	-	-
	Etest	ND	-	-	-
Micafungin	AST-YS07 VITEK 2	≤0.06-≥4	0.012	-	-
	Etest	0.002-32	0.047	-	-
Posaconazole	AST-YS07 VITEK 2	ND	-	-	-
	Etest	0.002-32	1	-	-



Tabla 1. (Continued)

Antifungal Agent	Test	MIC (mg/liter) at 24 hours			
	method	Range	Susceptible	SDD	Resistant
Voriconazole	AST-YS07 VITEK 2	≤0.12-≥8	0.25	-	-
	Etest	0.002-32	0.19	-	-

MIC: minimum inhibitory concentration; SDD: susceptible-dose dependent; ND: not determined.

## DISCUSSION

*Candida albicans* is the most common fungal isolate in neonatal candidemia. However, despite advances in medical therapeutics and improved methods for detecting and identifying yeasts, it has been shown that many other non-*C. albicans* species have emerged as clinically relevant agents [1]. Although rare, *Candida pelliculosa* has been reported as an emerging pathogen, which has recently gained relevance as an opportunist agent [3, 4, 13].

*C. pelliculosa* has not previously been isolated in our hospital from blood or any other clinical specimens, as well as from environmental sources. This is the first report describing the isolation of *C. pelliculosa* from a blood culture of a newborn in the NICU of The Andes University Hospital, Mérida, Venezuela.

Neonates admitted in neonatal intensive care units are highly prone to develop invasive fungal infections, mostly due to the presence of several unavoidable risk factors. Besides preterm birth, other factors predisposing to the development of disseminated candidiasis include prolonged use of broad-spectrum antibiotics, use of steroids, total parenteral nutrition and intralipid administration, as well as invasive procedures, particularly intravascular arterial or venous catheterization [13, 14]. In our case, the baby had several of these risk factors. An endotracheal tube and assisted ventilation were applied to treat the severe respiratory neonatal distress, indwelling vascular devices used for parenteral nutrition, and administration of broad-spectrum antibiotics for presumed bacterial infection. Regarding the latter, it is widely known that antibiotics may promote fungal overgrowth at the expense of normal bacterial flora and encourage translocation of yeast across the intact mucosa [3, 4], being this a possible scenario that may apply to this case.

Although important progress is being made in the diagnosis of fungal diseases, the signs and symptoms of candidemia in neonates are nonspecific, non-localizing and indistinguishable from those babies with bacterial infection; consequently, the clinical diagnosis of these infections is difficult. Additionally, laboratory results and microbiological trials may be insufficient and contradictory in neonatal fungal infections [14, 15]. In this case, the baby presented thrombocytopenia and elevated values of C-reactive protein (CRP). Both parameters have been considered as predictors of bloodstream infection [7]. In this regard, several studies have reported that high levels of CRP are often associated with blood infection of fungal etiology [15, 16]. Although the identification of yeast isolates has greatly improved over the past several decades

[10], blood cultures are still considered the gold standard for the diagnosis of invasive neonatal candidiasis. However, manual and biochemical conventional methods, commonly used to identify yeast isolates, are time-consuming and may result in low-discrimination identifications that require additional testing [8, 10]. In our case, *C. pelliculosa* was initially identified by VITEK 2 (99% confidence level) and confirmed by VITEK MS MALDI TOF, which based on the spectral fingerprint of the isolate, confirmed the identification with a 99.9% score. Although *C. pelliculosa* is considered an emergent hematogenous yeast pathogen, data about its susceptibility to antifungal drugs are scarce [8, 9] and several studies report a low susceptibility of *C. pelliculosa* strains to azole compounds [3, 4, 13]. In contrast, our isolate showed good susceptibility to all antifungal drugs tested, regardless of AST used. Results of antifungal susceptibilities of the isolate are shown in Table 1. A slight difference was observed in results obtained with the fluconazole, which value in the Etest method was considered susceptible-dose dependent (4 µg/mL) and by the AST-YS07 VITEK 2 card system as susceptible (2 µg/mL). In this respect, it has been reported that some discrepancies in results obtained from different susceptibility methods depend on the *Candida* species studied [9]. In fact, there is still no consensus on breakpoints for amphotericin B and echinocandins therapy in *C. pelliculosa* fungemia. However, the baby was treated with amphotericin B deoxycholate and responded satisfactorily, confirming that amphotericin B remains the drug of choice for the treatment of candidemia in high-risk neonates [2-4, 13].

Systemic infection by *Candida* species in neonates may involve various organs. It has been reported that the central nervous system (CNS) is the second most commonly involved anatomical site, with incidence ranging from less than 10 to 67% [17]. Unfortunately, in this case CT of the head revealed an extensive fronto-parietal ischemic lesion, probably due to inflammatory vasculopathy or microembolic phenomena. Therefore, a follow up of the incidence of CT abnormalities and their relationship with neurological outcomes is recommended.

Although the mechanism of transmission of *C. pelliculosa* was not assessed, we speculate that a nosocomial acquisition through indirect neonatal contact occurred, possibly via transient hand colonization of health-care personnel. Many studies have linked the hands of health-care workers with *Candida* spp. bloodstream infections outbreaks [1-4, 7]. Therefore, the importance of hand washing and compliance with guidelines for preventing nosocomial infections were emphasized to the



personnel at the time this case was studied. We stress the importance of an infection control program, based on active surveillance, and also of strict adherence to hand disinfection and use of gloves in light of infections due to emerging pathogens such as *C. pelliculosa* as seen in this case.

## CONCLUSION

Candidemia maybe a difficult diagnostic task, especially when caused by *C. pelliculosa* in neonates, either as single cases or outbreaks, scenarios which to date, have rarely been reported. This case highlights the importance of *C. pelliculosa* as an emerging nosocomial fungal pathogen and we hope this case will contribute to enrich epidemiological records in our region. The use of

new laboratory techniques, as fluorescent-based technology (VITEK 2) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, significantly helped in reducing delays in species identification. Also, the availability of rapid antifungal susceptibility results allowed early initiation of appropriate antifungal therapy diminishing the risk of mortality. A sustained surveillance of candidemia is essential in developing local guidelines for the prevention and assessment of appropriate treatment according to antifungal susceptibilities profiles.

## Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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