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Neonatal candidiasis in Venezuela: Clinical and epidemiological aspects

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ABSTRACT. A prospective study of the clinical and epidemiological aspects of *Candida* spp. sepsis was performed to assess the frequency, etiology, and risk factors in the neonatology service of the Pediatrics Hospital "Dr. Elías Toro" (2002-2003). Forty four out of 128 neonatal intensive care patients, with clinical sepsis and suspected fungal etiology, were chosen randomly for this study. Infant blood, urine, gastrointestinal tract, oral and skin samples were cultured. Samples were also taken from health care worker hands and the environment. The antifungal susceptibility patterns of the isolates were evaluated. The prevalence of *Candida* spp. from the clinical samples was: *C. albicans* (72.06%), *C. parapsilosis* (13.24%), *C. tropicalis* (10.29 %), *C. guilliermondii* (2.94%), *and C. glabrata* (1.47%). Due to the similarity of the susceptibility pattern of some isolates from infants and health care workers, we speculate a horizontal nosocomial infection. Statistical analysis revealed the following significant risk factors associated with *Candida* spp. isolation: prolonged hospitalization ($p < 0.05$), missing prenatal birth control ($p < 0.05$), and parenteral nutrition ($p < 0.05$). Blood cultures were all negative for bacteria and only 2.90% were positive for *Candida* spp. All 44 neonates receiving empirical therapy with amphotericin B (0.5-1.0 mg/k/day) evolved satisfactorily.

Key words: *Candida* spp, risk factors, nosocomial infection, antifungal susceptibility, sepsis.

RESUMEN. Se realizó un estudio prospectivo sobre los aspectos clínicos y epidemiológicos de sepsis por *Candida* spp. para determinar los factores de riesgo y frecuencia, en el Hospital Pediátrico "Dr. Elías Toro" (2002-2003). De un total de 128 neonatos que cumplían los criterios de inclusión, 44 fueron seleccionados al azar para el estudio y los cultivos de muestras clínicas de sangre, orina, recto, orofaringe y piel; además fueron incluidos en el estudio muestras del personal de salud y del ambiente de la unidad. Se evaluó la sensibilidad antifúngica de las especies de *Candida* spp. aisladas. Se aislaron*: C. albicans* (72.06%), *C. parapsilosis* (13.24%), *C. tropicalis* (10.29%), *C. guilliermondii* (2.94%), *and C. glabrata* (1.47%). El patrón de sensibilidad similar de algunas cepas provenientes de los neonatos y del personal de salud, sugirió una infección nosocomial de transmisión horizontal. Los factores de riesgo estadísticamente significativos fueron: hospitalización prolongada ($p < 0.05$), falta de control prenatal ($p < 0.05$) y nutrición parenteral ($p < 0.05$). El hemocultivo resultó negativo para bacterias en todos los casos y sólo positivo en un 2.90% de los casos para *Candida* spp. Los 44 neonatos estudiados fueron tratados empíricamente con anfotericina B (0.5-1.0 mg/k/día) y respondieron adecuadamente a la terapia.

Palabras clave: *Candida* spp., factores de riesgo, infección nosocomial, susceptibilidad antifúngica, sepsis.

INTRODUCTION

Candida spp. and coagulase negative S*taphylococcus* (CNS) are an increasingly common cause of neonatal nosocomial sepsis, particularly among very low birth weight immunocompromised infants, who may also present signs of gastrointestinal tract disease, compromised skin integrity, long term endotracheal intubations, chronic malnutrition, and other factors that lead to increased risks of acquiring such infections.[61,62]

However, the signs and symptoms of infection with any of these organisms are nonspecific and include temperature instability, respiratory distress, abdominal distension, apnea and bradycardia, lethargy, and decreased perfusion, therefore clinically difficult to differentiate from each other, and not easy to establish a correct diagnosis and treatment.[4,7,22,29,61]

Candida spp. are significant hospital-acquired pathogens in neonatal intensive care units and nurseries, and are responsible for considerable morbidity and mortality (1.6-4.5 %).[4,10,24,29,55,61]

The risk factors associated with this infection include low birth weight, gestational age, extended hospitalization, use of the third generation cephalosporins, central venous catheters, surgical procedures, parenteral nutrition, among others.[6,11,20,25,32,44,62]

Although *Candida albicans* is still the predominant species (80-90%) in most neonatal intensive care units, recent reports indicate a shift toward no-*albicans Candida* sepsis, especially C. *parapsilosis* (12.5%) and *C. tropicalis* (2.5%). Also rapidly growing antimicrobial resistance for

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the most common antimycotics among these microorganisms is worrying.[6,7,14,15,23,56,62]

The most important risk factors for colonization or infection with the various multiresistant microorganisms are common and universal. Nosocomial colonization is the precursor for clinical infection and the risk for nosocomial infection is as high as 38% with colonization by *Candida*.[53] Therefore surveillance programs that focus on anticipatory isolation and monitoring resistant organisms of all patients with risk factors for infection would very likely reduce secondary spread within the hospital and enhance understanding of the epidemiology with the ultimate goal to guide efforts to develop more effective strategies for prevention of fungal opportunist infections.

In Venezuela, no data on the morbidity of this disease are available in the annual report of epidemiology and vital statistics at the ministry (Anuario de epidemiología y estadística vital (MSDS); it only reveals four diseased patients in four years (1996-1999).[1] As consequence, we conducted a prospective study to examine the epidemiology and clinical presentation and outcome of neonatal infection with *Candida* spp. in infants admitted during one year at the Pediatric Hospital "Dr. Elías Toro", in Caracas.

MATERIALS AND METHODS

A prospective, descriptive study of the clinical and epidemiological aspects of *Candida* spp. sepsis was performed to assess the frequency, etiology, and risk factors in the neonatology service of the Pediatric Hospital "Dr. Elías Toro" (2002-2003).

PATIENTS

During the period of study a total of 128 neonates were admitted to the neonatology intensive care unit with clinical signs of systemic infection, coming from different locations of the city along with rural areas. Forty-four (34.38%) infants were randomly selected according to following inclusion criteria: age ranging between 0-29 days, either gender; sepsis criteria on admission or during their clinical evolution in the hospital; without previous antimycotic therapy on admission; with mucocutaneous lesions suggesting mycotic infection; obstetric vaginal yeast infections of the mother; with or without risk factors for mycotic infections, who presented clinical deterioration after 48 h of admission, and with parental consent to carry out the investigation.

All patients were assessed for risk factors associated with fungal colonization and bloodstream infection, as has been previously described.[63] We recorded demographic data, as well as clinical evolution of the patients and laboratory tests, device days for ventilators and central venous catheters; surgical procedures and use of medications, including antibiotics, into a hand-held computer containing a computerized case report sheet.

SAMPLES

In addition to the surveillance cultures, multiple specimens, including blood, throat swabs, urine and rectal samples collected from all the infants included in the study, as well as from hands and throat swabs of mothers who nursed their infants and from the staff working in the unit and ancillary personnel (dietitian) were taken. Environmental samples were also taken to identify the probable mode of *Candida* transmission.

A total of 220 specimens were collected. Infants were screened for gastrointestinal tract colonization by rectal swabs. Standard blood cultures (2 ml) were incubated at least 8 days at 37ºC. *Candida* urinary tract infection was defined as growth of *Candida* in a specimen (1-2 ml) obtained by sterile catheterization. Cutaneous, oropharingeal, and rectal swabs were cultured in bile agar-Feo (Bile Oxgall Bacto, BBL; 20 g oxoid, 1 g agar, 0.25 g chloramphenicol, 1 L distilled water)[19] and incubated at room temperature (24-28ºC) for 24-48 h. This culture medium is selective for *C. albicans*; it forms typical chlamydoconidia.

Additionally, as described previously, cultures were incubated according to standard laboratory methods and fungi were identified by the commercial identification system Rapid Yeast by Dade Behring® with automated lecture with the MicroSCAN4.

MOTHERS

Oropharingeal and vaginal swabs were obtained from eight mothers, who gave their consent to participate in the study.

MEDICAL STAFF AND ANCILLARY PERSONNEL

Sixteen persons (six nurses, six physicians, ancillary personnel: two food handlers (dietitian) and two cleaning personnel) out of a total staff of 48 voluntarily participated in the study. Specimens obtained from staff (16) were throat swabs and samples from hands.

Hand specimens were taken by inserting the hand into a large sterile latex glove containing 15 ml of bile agar medium. The skin of the hand was then vigorously rubbed through the glove for 1 min before decanting the lavage fluid into Sabouraud Petri dish and incubating for 24 h at 37ºC.

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ENVIRONMENT

Samples from the environment (incubator, nursing bottles, oxygen mask, floor and walls of the unit) were taken by immersing a sterile swab in bile agar medium and streaking on Sabouraud plates. Liquid samples (1 ml) from the environment (medicines, milk bottle, and soap) were directly cultured in bilis agar.

In vitro *susceptibility test*

Susceptibility of the strains to ketoconazole, fluconazole, itraconazole and amphotericin B (AMB) was tested by the well-diffusion method described by Magaldi et al.[38-42] Diameters of the inhibition zones were measured in millimeters [42], and interpretation criteria were: 1) azoles: susceptible ≥ 19, susceptible dose dependent 18-13, resistant < 12; 2) AMB: susceptible ≥ 15, susceptible dose dependent 14-10, resistant < 9 mm. Reduction in growth was observed > 80%.

STATISTICAL METHOD

A descriptive analysis of the data was obtained by using SPSS ver.10.0; Pearson's chi-square nonparametric analysis was performed ($\alpha = 5\%$; $p \leq 0.05$). $P \leq 0.05$ was considered significant.

RESULTS

During the period of study a total of 128 neonates coursing with sepsis were admitted, 34.38% (44) of them were selected randomly and fulfilled the inclusion criteria. Demographic and clinical characteristics are described in Table 1. *Candida* spp. accounted for 22.66% (29) of the 128 infants admitted to the hospital during this study.Twenty-two of the 44 neonates had presumptive diagnosis of *Candida* sepsis, which was confirmed in 47.73% (21) neonates.

A total of 220 samples were taken from a total of 44 neonates included in the study, out of these 70.00% (154) were negative and 0.91% (2) harboured bacteria.

In 74.91% (33) infants, different *Candida* spp. strains could be isolated (Table 2). The number of species isolated augmented significantly ($p = 0.039$) with hospitalization > 15 days. No significant differences of *Candida* spp. isolation were observed among infants according to age at admission ($p = 0.62$), birthweight ($p = 0.74$), gender ($p = 0.07$), and gestational age ($p = 0.97$).

The most frequent positive specimens were rectal (52.27%), oropharingeal (36.36%), cutaneous (27.27%) swabs and urine samples (25.00%), blood cultures were all negative for bacteria; isolation of *Candida* spp. was only 2.90%.

The isolated species were: *C. albicans* (72.06%), *C. parapsilosis* (13.24%), *C. tropicalis* (10.29%), *C. guilliermondii* (2.94%), *and C. glabrata* (1.47%).

Candida albicans was isolated from rectal (38.78%), oropharingeal (24.49%), cutaneous (22.45%), and urine (14.29%) samples.

Candida parapsilosis was isolated from rectal (22.22%), oropharingeal (22.22%), urine (22.22%), and cutaneous (11.11%) samples. Additionally, this species was isolated simultaneously from blood and urine specimens of two patients and had an identical susceptibility pattern (AMB dose dependent). This species accounted also for the colonization of left and/or right hand and one oropharingeal swab of four staff members, showing an identical susceptibility pattern.

Table 1. Demographic and clinical characteristics of the neonates.

	n = 44	%
Male (%)	30	68.18
Age (days)	(0-29)	
Gestational age (weeks)	37.4	
Weight (%)		
< 2.500 g	13	29.55
2.500-3.500 g	23	52.27
> 3.500 g	8	18.18
Hospitalization (days)	1-30	
Respiratory distress (%)	23	52.27
Abdominal distension (%)	26	59.09
Enterocolitis (%)	24	54.55
Icterus (%)	11	25.00
Cyanosis (%)	19	43.18
Diarrhea (%)	14	31.82
Dehydration (%)	23	52.27
Hypothermia (%)	14	31.82
Groan (%)	7	15.91
Hypoglycemia (%)	6	13.64
Irritability (%)	14	31.82
Weak suction (%)	9	20.45
Fever (%)	24	54.55
Without prenatal control (%)	16	36.36
Vaginal birth (%)	41	93.18
Maternal urinary infection (%)	20	45.45
Premature (%)	11	25.00
Reanimation (%)	4	9.09
Hypoxia (%)	9	20.45
Broad-spectrum antibiotics (%)	44	100.00
Venoclysis (%)	44	100.00
Central intravascular catheter (%)	7	15.91
Parenteral nutrition (%)	18	40.91
Blood derivates (%)	28	63.64
Parenteral hydration (%)	44	100.00
Type of antibiotics received	Ampicillin-Cefotaxime (Vancomycin-Ceftazidime-Amikacin)	

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Table 2. *Candida* spp. isolated from clinical specimens of the neonates and mothers

Neonates	Skin	Throat	Rectal	Blood	Urine	Mother
1	*C. albicans*	*C. albicans*	*C. albicans*	-	*C. albicans*	
2	*C. albicans*	*C. albicans*	-	-	*C. albicans*	
3	-	*C. parapsilosis, C.albicans*	*C. albicans*	-	-	
4	*C. albicans*	*C. albicans*	*C. albicans*	-	-	
5	*C. albicans*	*C. albicans*	*C. albicans*	-	*C. albicans*	-
6	*C. albicans*	*C. albicans*	-	-	-	
7	*C. albicans*	-	*C. albicans*	-	-	
8	-	*C. albicans*	*C. albicans*	-	-	
9	-	-	*C. albicans*	-	-	
10	-	-	*C. albicans*	-	-	
11	-	-	*C. albicans*	-	-	
12	*C. parapsilosis*	-	-	*C. parapsilosis*	*C. parapsilosis*	-
13	Bacterias	-	-	-	-	
14	*C. tropicales,C. albicans*	*C. tropicalis*	-	-	*C.tropicalis*	
15	-	-	-	*C. parapsilosis*	*C. parapsilosis*	
16	Bacterias	-	-	-	-	
17	*C. albicans*	-	-	-	-	*C. albicans*
18	-	-	-	-	-	*C. albicans*
19	*C. albicans*	-	*C. albicans*	-	-	
20	-	-	-	-	-	
21	-	*C. albicans*	*C. albicans*	-	-	*C. albicans*
22	-	-	-	-	-	
23	*C. albicans*	*C. tropicalis*	*C. guilliermondii* *C. tropicalis*	-	-	
24	-	*C. tropicalis*	*C. tropicalis*	-	-	
25	-	-	-	-	*C. glabrata*	
26	*C. albicans*	-	*C. albicans*	-	*C. albicans*	-
27	-	-	-	-	-	
28	-	*C. albicans*	*C. albicans*	-	-	-
29	-	-	-	-	*C. albicans*	
30	-	*C. albicans*	*C. albicans*	-	*C. albicans*	
31	-	-	*C. albicans*	-	-	
32	-	-	-	-	-	
33	-	-	-	-	-	
34	-	-	*C. albicans*	-	-	
35	-	*C. parapsilosis*	*C. parapsilosis*	-	*C. albicans*	
36	-	-	*C. guilliermondii* C. parapsilosis	-	-	
37	-	-	-	-	-	
38	-	-	*C. albicans*	-	-	
39	-	-	-	-	-	*C. albicans*
40	-	-	-	-	-	
41	-	*C. albicans*	*C. albicans*	-	-	
42	-	*C. albicans*	*C. albicans*	-	-	
43	-	-	-	-	-	
44	-	-	-	-	-	

Candida tropicalis was isolated from oropharingeal (42.86%), rectal (28.57%), cutaneous (14.29%), and urine (14.29%) specimens.

Candida guilliermondii was isolated from two rectal swabs and *C. glabrata* only in one urine sample.

Thirty-four (77.27%) from the 44 neonates presented

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Table 3. *Candida* spp. isolation in relation to risk factors of the neonates.

Risk factors	Positive (n)	Positive %	Negative (n)	Negative %
ATBs use (44)	27	61.36	17	38.64
Venoclysis (44)	27	61.36	17	38.64
Parenteral hydration (44)	27	61.36	17	38.64
Vaginal birth(41)	26	63.41	15	36.59
Blood derivates (28)	18	64.29	10	35.71
Maternal urinary infection (20)	12	60.00	8	40.00
Parenteral nutrition (18)	15	83.33	3	16.67
Missing prenatal maternal control (16)	12	75.00	4	25.00
Premature (11)	8	72.73	3	27.27
Hypoxia (9)	6	66.67	3	33.33
Central intravascular catheter (7)	6	85.71	1	14.29
Reanimation (4)	2	50.00	2	50.00

Table 4. *Candida* spp. isolated from staff.

Staff	Right hand	Left hand	Throat
Attending Physician 10	*C. melobiosica*	*C. guilliermondii*	-
Attending Physician 9	*C. parapsilosis*	*C. parapsilosis*	-
Dietitian	*C. parapsilosis*	*C. parapsilosis*	*C. albicans*
Dietitian 14	*Rhodotorula glutinis*	*R. glutinis*	
Dietitian 13	*C. parapsilosis*	*R. glutinis*	*C. albicans*
Dietitian 13	*R. glutinis*	*C. albicans*	-
Cleaning staff 8	-	*C. parapsilosis*	*C. albicans*
Resident Physician 6	-	-	*C. albicans*
Resident Physician 11	-	-	*C. albicans*
Nurse 2	-	-	*C. albicans*
Nurse 5	-	-	*C. albicans*
Resident Pysician 3	-	-	-
Resident Physician 7	-	-	-
Resident Physician 12	-	-	-
Nurse 1	-	-	-
Nurse 4	-	-	-
Nurse 15	-	-	-
Nurse 16	-	-	-

critical clinical signs. The most common signs were abdominal distension (59.09%), enterocolitis (54.55%), fever (54.55%), dehydration (52.27%), respiratory distress (52.27%), hypothermia (31.82%) and diarrhea (31.82%). Compared with non-isolation, isolation of *Candida* spp. occurred more frequently (rectal swab 36.76%, urine sample 28.00%) in neonates with respiratory distress or abdominal distension, nevertheless it was not statistically significant ($p = 0.599$).

The most common candidiasis related risk factors studied are shown in Table 3. The isolation of *Candida* spp. occurred in patients in the following proportions: central intravascular catheter (85.71%, $p < 0.05$), parenteral nutrition (83.33%, $p < 0.05$), as well as in the absence of prenatal maternal control (75.00%, $p < 0.05$), blood derivates (64.29%, $p < 0.05$), peripheral intravascular catheter (61.60%, $p < 0.05$) parenteral hydration (61.36%, $p < 0.05$), and receiving broad-spectrum antibiotic therapy (61.36%, $p < 0.05$). Besides, a significant statistical difference could only be observed in and in those infants receiving prolonged parenteral nutrition (> 7 days) ($p = 0.005$).

We also observed a notorious clinical deterioration of 18 (40.90%) infants between 24-72 h after admission, although isolation of *Candida* spp. in neither urine ($p = 0.658$) nor rectal swabs ($p = 0.355$) was significant in these children.

All 44 neonates receiving empirical therapy with AMB (0.5-1.0 mg/kg per day) evolved satisfactorily. The mean time of therapy was 9 days. There was a statistically significant difference in negative culture for *Candida,* between patients with or without antifungal treatment ($p = 0.024$).

Environmental samples (incubator, nipple of nursing bottles, oxygen mask, floor and walls of the unit) yielded the following non-*albicans* fungi: *Chrysonilia sitophyla, Geotrichum sp., Penicillium* sp., *Aspergillus versicolor, A. glaucus,* and *Staphylococcus* sp. (< 3-5 CFU/sample/plate). From the remainder liquid samples, only the milk bottle was contaminated with *C. albicans* even before the infants were nursed with it.

Only 16 from the 48 staff personnel caring for the neonates participated voluntarily in the study. In seven (43.75%) of them, oropharingeal *C. albicans* was isolated.

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Eleven (68.75%) had negative fungus cultures on both hands. *Candida parapsilosis, C. melobiosica, C. guilliermondii,* and *Rhodotorula glutinis* were isolated from the remaining five persons (Table 4).

Susceptibility of all strains for the different antifungal tested revealed 10.70% resistance to fluconazole and a 5.30% dose dependence for AMB, mainly for *C. parapsilosis.*

One strain from the right hand of one of the dietitians showed resistance to both fluconazole and itraconazole, the same as the strain coming from urine, oropharingeal, and rectal swabs of one of the patients. The susceptibility pattern of *C. albicans* isolated from the milk bottle coincided with that of *C. albicans* isolated from the hand of one staff (cleaning personnel); however this strain was not found in any neonate.

On the other site, *C. parapsilosis,* isolated from both the left hand of one dietitian and one physician, showed identical susceptibility patterns, undistinguishable from those isolated from the patients blood culture (see above).

DISCUSSION

Nosocomial bloodstream infections due to *Candida* species are a major cause of morbidity and mortality in neonates, especially in the intensive care units of the United States of America and Thailand, where 12% of the nosocomial infections are due to *Candida* spp.[4,48,62]

In Venezuela, no data on the morbidity of this disease are available in the annual report of epidemiology and vital statistics at the ministry of health (Anuario de epidemiología y estadística vital (MSDS), it only reveals four deceased patients from 1996 to 1999.[1] Nevertheless, searching the Venezuelan literature, we found three studies, their data show a morbidity rate of 7.7% in 1989,[11] a mortality rate of 21% in 1998,[50] and a last study showed a mortality rate of 30.6% in 1999.[54] In this sense, we think that the existing official mortality data are underegistered and rather low. On the other hand, the morbidity rate in our study was of 22.6% and the mortality rate was null.

Numerous studies have identified risk factors, among them are: birthweight ≤ 1,000 g and gestational age < 32.[7,9,11,12,28,52] The neonates in this study were older, therefore immunologically more mature. Infants with positive or negative culture showed no difference between birthweight ($p = 0.74$), and gestational age ($p = 0.97$).

Statistical analysis revealed the following significant risk factors associated with *Candida* spp. isolation: central intravascular catheter, parenteral nutrition, prolonged hospitalization, missing prenatal birth control, blood derivates, peripheral intravascular catheter, parenteral hydration and receiving broad-spectrum antibiotic therapy and parenteral nutrition ($p < 0.05$). All of them extensively reported as major factors for sepsis by *Candida* spp.[20,35]

On the other site, the number of species isolated increased significantly ($p = 0.039$) with hospitalization > 15 days, commonly a significant risk factor [62]. About 61.4% of the infants studied was exposed to third-generation cephalosporins even though it was not statistically significant ($p = 0.13$). The exposure to these antibiotics is commonly considered an important risk factor.[49,58,62,63]

Blood cultures were all negative for bacteria. The limited quantity of positive blood cultures (4.55%) for *Candida* spp. may be due to the early onset of empirical antifungal treatment, as well as to the blood sample volume (2.7 ml). Concentration of *Candida* is normally low therefore isolation is difficult. Mermel and Maki,[47] reported that each milliliter of additional blood sample might increase isolation of *Candida* up to 3.00%. *Candida parapsilosis* was the only species isolated. Recent reports suggest an increasing number of infections attributable to this species.[16,26,31]

The most important risk factors for colonization or infection with *Candida* spp. are common and universal. Isolation of *Candida* in any sample, at least, means that the individual is colonized, and colonization is the precursor of clinical infection.[61,63] The risk for nosocomial infection is as high as 38% by *Candida* sp.[61] Central venous catheters are the main port of entry and cause inoculation and hematogenous dissemination.[3,13,14,20,26,27,33,50,64] Bakr in 2003,[3] recovered an identical organism from the cannula hub or tip in 50.00% of the patients before the onset of clinical and laboratory sepsis (peripheral blood) could be established. Another significant *Candida* spp. colonization source is parenteral nutrition that facilitates the growth of a "biofilm" by *Candida* spp. on the latex tubes, which constantly deliver fungal elements to the bloodstream and is highly resistant to most of the antifungal drugs.[17,27] The high content of essential nutrients may favor the proliferation of these organisms on the colonized patients, and consequently penetrate more frequently via invasive treatments,[58,64] explaining the significant statistical difference that we found in the parenteral hydration (61.36%, $p = 0.012$), parenteral nutrition (83.33%, $p = 0.005$), and those patients receiving prolonged parenteral nutrition (> 7 days) ($p = 0.005$).

Makhoul et al.[43] reported that the rate of fungal colonization in neonates < 30 days of life is minimal, regardless of antibacterial therapy at all sites cultured; however, 27.27% of our infants (16.8 days old) were colonized by *Candida* spp., which nonetheless could be part of the normal oral flora of these newborns. Therefore, these microbiological observations merit attention, when empirical therapy is considered in premature infants suspected of having late-onset sepsis.

The rate of *Candida* spp. detected in blood culture specimens among neonates is low (5-10%). *Candida* spp.

growth rate is low under anaerobiosis, and may be inhibited even more by concomitant bacteria.[2,37,59,66] As reported by Schelonka and Moser in 2003,[66] standard routine culture medium sensibility might be increased by aeration, *Candida* was detected in 3% of the infants with sepsis between 37 ± 14 h, the remainder 97% were only detected after at least 72 h, only if they were not previously exposed to antimycotic therapy. Further studies should consider a selective medium for *Candida* spp., like bile agar, to enhance isolation time and suppress bacterial growth.[19]

A sole isolation of *Candida* spp. on one site is not satisfactory to confirm dissemination. Even so, some authors state that isolation of the same *Candida* more than twice on different sites deserves attention and could be indicating colonization, in an asymptomatic infection and in absence of sepsis.[3,9,20,64]

We regarded every infant as coursing with sepsis, when the same *Candida* species was isolated twice in more than two sites. Following this criterion, *Candida* spp. could be confirmed in 21 (47.73%) neonates coursing with sepsis. We infer that *Candida* almost certainly accounted for the sepsis in those patients that had only positive urine samples (*C. glabrata* and *C. albicans*) and/or responded favorable to antifungal therapy.

Gary and Toney in 1998[21] handled this criterion more cautiously, with more than ≥ 3 isolations; on the other hand, presumptive antifungal therapy in high risk patients may be necessary if outcome is to be improved, as many patients with *Candida* sepsis will have died by the time laboratory diagnosis is made.[6,66] None of our infants treated with AMB died or failed to improve clinically. In view of this finding, we urge to consider presumptive therapy.

Vast efforts have been made to get better sensitivity of blood cultures for *Candida*, as well as serologic test that may discriminate colonization and infection, and diagnostic molecular biology techniques to facilitate identification that might offer solutions in the nearby future.[16,67,68]

Furthermore an epidemiological surveillance based exclusively on laboratory diagnosis may distort data and report lower incidence than in fact present.[18,24,67]

The frequencies of species obtained in this study (*C. albicans* 72.06%, *C. parapsilosis* 13.24%, *C. tropicalis* 10.29%, *C. guilliermondii* 2.94%, *and C. glabrata* 1.47%) are similar to those reported by other authors in the literature (*C. albicans* 60.00%, *C. parapsilosis* 14.25%, and *C. glabrata* < 5%).[18,24,32,34,51,52,63]

All *C. parapsilosis* isolated had identical susceptibility pattern, i.e., those isolated from neonates, and those from health care workers, and may imply horizontal transmission from the hands of health care workers to the patients, or vice versa, producing a nosocomial infection. Only further molecular typing may confirm this assumption. Emerging infection disease issues regarding hand hygiene practices among health-care professionals have been widely discussed, research indicates a link between hand hygiene and nosocomial infections.[3,7,16,20,31,34,63,68] Unless patient care involves invasive procedures or extensive contact with blood and body fluids, current guidelines recommend plain soap for hand washing; however, infection rates in adult or neonatal intensive care units or surgery rooms may be further reduced when antiseptic products are used.[34,42] On the other hand, common-source outbreaks have been associated with an increasing number of infections attributable to *C. parapsilosis*. In some intensive care units, *C. parapsilosis* has become the predominant fungal pathogen. However, *C. parapsilosis* seems to be less virulent. Under certain conditions (i.e. intravascular catheters, high intravenous glucose concentrations), *C. parapsilosis* may have both a selective advantage and increased virulence relative to *C. albicans.*[7,36] Infants with *C. parapsilosis* have a longer prior duration of indwelling central venous catheter, and it is relatively difficult to eradicate, therefore increasing the morbidity and mortality of infants.[23] In this study, we were not able to identify statistically significant aspects of the clinical presentation and medication history, in relation with the laboratory findings, which might be helpful in identifying *Candida* sepsis. The only highly significant clinical sign was dehydration ($p = 0.007$). Recent reports have suggested that the use of third generation cephalosporins was strongly associated with candidemia. This may be due to the presumptive antifungal therapy administered occasionally, previous to specimen retrieval, as suggested by the statistically significant difference in negative culture for *Candida*, between patients with or without antifungal treatment ($p = 0.024$). Other clinical reports have also suggested that gastrointestinal involvement due to *Candida* is significant.[5,16,50,64]

When to suspect *Candida* sepsis? A neonate coursing with sepsis, who is deteriorating, despite empiric broad-spectrum antimicrobial therapy, especially third-generation cephalosporins, using central venous catheters or intravenous lipids, and *Candida* colonization, may be at high risk. Presumptive antifungal therapy is mandatory and AMB (0.5-1.0 mg/kg per day) is the treatment of choice.[5,6,8,30,37,45,46,65,69] Some authors suggest standard initial therapy with fluconazole (8-12 mg/kg, 72 h), switching to AMB, as soon as cultures report of *Candida* growth,[46,66] conversely resistance to this drug may be high.[59,56] In our hospital, we found 10.7% resistance to fluconazole, for that reason and given the high mortality rate of candidiasis (up to 50% or more), we would encourage clinicians to consider empiric coverage with AMB. A report by Benjamin et al.[5] avowed that infants with systemic candidiasis, who were started on AMB > 3 days alter the first positive blood culture was drawn had a mortality rate of 50% (3/6) infants. Even though we also found 5.3%

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dose-dependence to AMB, mainly for *C. parapsilosis* isolates, after 40 years of ceaseless use of this drug, human pathogenic *Candida* spp. remains habitually susceptible, and this is a rather unusual event, which was described in highly immunocompromised patients, but hardly ever in infants.[30,46,57,60,65,69] In the near future caspofungin might become an excellent choice in these cases.

Our study had several limitations. Not all maternal cultures were obtained. There was no- standard time for culturing the hands of healthcare workers; some workers could have had transient colonization because staff members were approached throughout their workday, rather than immediately after hand washing. Hand washing habits of the workers were not assessed and not all of them participated in the study. It is also possible that bi-directional spread of *Candida* spp. occurred between staff and infants. Further molecular typing studies may confirm that health care workers shared *Candida* strains with their patients. Other potentially significant risk factors, such as overcrowding, understaffing, underprovided hygienic measures, were not assessed by our study.

Due to the similarity of the susceptibility pattern of some isolates from infants and health care workers, we infer a horizontal nosocomial infection.

Epidemiological data of this study are important for the decision of the appropriate empirical antimicrobial treatment in our hospital and, finally, understanding the importance of previously established risk factors will permit, in the future, for the development of potential strategies to prevent invasive fungal disease. Efforts should be focused in reducing colonization by enhancing the hand hygiene practices among health-care professionals and ancillary personnel in the unit, without underscoring the importance of adequate epidemiological surveillance. Besides, professionals in health-care units should also encourage the antifungal prophylactic strategies in high-risk infants. Despite missing official data, disseminated candidiasis is an important entity in Venezuela; therefore, in this sense, we urge the pediatrician to be alert on the signs of sepsis associated with risk factors and consider initiation of empiric AMB therapy. Futhermore the incidence rate of nosocomial sepsis extends the length of intensive care stay and is a significant economic burden. Special attention should be given to the continuing education of the medical and nursing personnel, and the quality of care for these neonates, by making hospital administrators and policy makers more sensitive to the needs of adopting an continuous monitoring and surveillance of nosocomial sepsis.

REFERENCES

1. Ministerio de Sanidad y Desarrollo Social. Anuario de Epidemiología y Estadística Vital. 1998. Caracas, Venezuela.
2. Bacci, S. 1994. Candidiasis invasiva en pacientes no neutropénicos. Ant E Inf. 2(4):21-37.
3. Bakr, A.F. 2003. Intravenous lines-related sepsis in newborn babies admitted to NICU in a developing country. J Trop Pediatr. 49:295-7.
4. Beck Sague, C.M., W.R. Jarwis & The National Nosocomial Infections Surveillance System. 1993. Secular trends in the epidemiology nosocomial fungal infections in the United States 1980-1990. J Infect Dis. 167:1247-51.
5. Benjamin, D.K., K. Ross, R.K. McKinney, D. Benjamin, .Auten & R.G. Fisher. 2000. When to suspect fungal infection in neonates: a clinical comparison of *Candida albicans* and *Candida parapsilosis* fungemia with coagulase negative staphylococcal bacteremia. Pediatrics. 106(4):712-718.
6. Benjamin, D.K., E.R. DeLong, W.J. Steinbach, C.M. Cotton, T.J. Walsh & R.H. Clark 2003. Empirical therapy for neonatal candidemia in very low birth weight infants. Pediatrics. 112(1):543-7.
7. Botas, C., I. Kurlat, S. Young & A. Sola. 1995. Candidiasis diseminada e hidrocortisona intravenosa en niños pretérmino. Pediatrics (ed. Esp). 39(6): 373-379.
8. Butler, K., M. Rench & C. Baker. 1990. Amphotericin B as a single agent in the treatment of systemic candidiasis in neonates. Pediatr Infect Dis J. 9:51-6.
9. Campbell, J., E. Sacaría & C. Baker. 2000. Candidiasis sistémica en niños de muy bajo peso al nacer tratados por vía tópica con crema de petrolato para el cuidado de la piel: estudio de casos y controles. Pediatrics (ed. Esp.). 49(5): 293-297.
10. Canton, E., A. Viudes & J. 2001. Pernán. Infección Sistémica Nosocomial por Levaduras. Rev Iberoam Micol. 18:51-55.
11. Carrabs M, A. Garcia & A. Romero. 1991. Infecciones Intrahospitalarias Neonatales. MSc thesis in pediatrics, Facultad de Medicina Caracas, Universidad Central de Venezuela.
12. Carrasco Sánchez, P., M.L.Castillo Montero, A. Bejarano Palmna, A. López Sanz, M. Santana Gallinato, C. Sáenz Reguera, L. Durán Vargas & A. González Meneses. 1999. Candidiasis neonatales y tratamiento con anfotericina B liposomal, nuestra experiencia. Anales Españoles de Pediatria. 51(3):273-280.
13. Chacin, G. & H. Rangel. 1990. Infección sistémica por *Candida albicans* en el recien nacido- tratamiento con ketoconazol. Arch Venez Puericul y Pediat. .53:79-84.
14. Chowdhary, A., K. Becker, W. Fegeler, H.C. Gugnani, L. Kapoor, V.S. Randhawa, G. Mehta. 2003. An outbreak of candidemia due to *Candida tropicalis* in a neonatal intensive care unit. Mycoses. 46(8):287-92.
15. Coleman, D., M. Rinaldi, K. Haynes, R. Summerbell, E. Anaissie & L. Sullivan. 1998. Importance of *Candida* species other than *Candida albicans* as opportunistic pathogens. Med Mycol. 36(1):156-65.
16. Da Silva, C.L., R.M. dos Santos & A.L. Colombo. 2001. Cluster of *Candida parapsilosis* primary bloodstream infection in a neonatal intensive care unit. Braz J Infect Dis. 5(1):32-6.
17. Douglas, J.L. 2003. *Candida* biofilms and their role in infection. Rev Trends Microbiol. 11(1):30-36.
18. Fairchild, K.D., S. Tomkoria, E. Sharp & F.V. Mena. 2002. Neonatal *Candida glabrata* sepsis: clinical and laboratory features compared with other *Candida* species. Pediatr Infect Dis J. 21:39-43.
19. Feo, M. 1977. The nascent culture and its use. Mycopathologia. 63:185-186.
20. Fotedar, R., E.T. Banerjee & A.R. Cahudhary. 2000. Outbreak of systemic candidiasis in low birth weight preterm infants at a neonatal intensive care unit. J Mycol Med. 10:100-104.
21. Gary, A.B. & J. Toney. 1998. Infection *versus* colonization in the critical care unit. Pp. 71-90. In: Cunha, B. (Ed). Critical Care Clinics infections in Critical Care I volume 14, (1) Saunders Company, New York.
22. Hall, R.T., S.L. Hall, W.G. Barnes, M.R. Izuegbu, I. Zorbas. 1987. Characteristics of coagulase-negative staphylococci from infants with bacteremia. Pediat Infect Dis J. 6:377-383.

23. Huang, Y.C., T.Y. Lin, R.I. Lien, Y.H. Chou, C.Y. Kou, P.H. Yang & W.S. Hsieh. 2000. Candidaemia in special care nurseries: comparison of *albicans* and parapsilosis infection J Infect. 40(2):171-5.
24. Hugonnet, S., H. Sax, P. Eggimann, J.C. Chevrolet & D. Pittet. 2004. Nosocomial bloodstream infection and clinical sepsis. Emerg Infect Dis [serial online] Jan [*date cited*]. Available from: URL:http://www.cdc.gov/ncidod/EID/voll10no1/03-0407.htm.
25. Hunter, P., G. Harrison & C. Fraser. 1990. Cross-infection and diversity of *Candida albicans* strain carriage in patients and nursing staff on an intensive care unit. J Med Vet Mycol. 28:317-325.
26. Itzhak, L., R. Olry, V. Sanjee, S. Vasishta, V. Tucci & S. Sood. 1998. Emergence of *Candida parapsilosis* as the predominant species causing candidemia in children. Clin Infect Dis. 26:1086-88.
27. Jabra-Rizk, M.A., W.A. Falkler & TF. Meiller. 2004. Fungal Biofilms and Drug Resistance CDC 10 (1) [serial online] [*date cited*]. Available from: http//www.cdc.gov/ncidod/EID/vol10no 1/03-0119.hym.
28. Jaraba Caballero, S., M. P. Jaraba Caballero, F. Fernández, I. Muriel, M.D. Huertas, R. Álvarez, J. Guzmán & M. Zapatero. 1998. Estudio prospectivo de sepsis por *Candida* en el RN. Anales Españoles de Pediatria. 48(6):639-44.
29. Jarvis, W.R. 1995. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. Clin Infect Dis; 20(6):1526-1530.
30. Johansen, H.K. & P.C. Gøtzsche. 2003. Amphotericin B *versus* fluconazole for controlling fungal infections in neutropenic cancer patients (Cochrane Review).pp. 233-257. In: *The Cochrane Library*, Issue 4, Chichester, UK: John Wiley & Sons, Ltd.
31. Kossoff, E., S. Buescher & G. Karlowicz. 1998. Candidemia in a neonatal intensive care unit: trends during fifteen years and clinical features of 111 cases. Pediatr Infect Dis J. 17(5):504-508.
32. Krcméry, V., M. Fric, M. Pisarcikova, M. Huttova, J. Filka, K. Kralinsky, H. Hupkova, J. Hanzen, J. Trupl & M. Liskova. 2000. Fungemia in neonates: report of 80 cases from seven University Hospitals. Pediatrics. 105(4):913-914.
33. Krcmery, V., M. Huttova, F. Mateicka, L. Laho, L. Jurga, A. Ondrusova, Z. Tarekova, K .Kralinsky, J. Hanzen, A. Liskova, M. Mrazova, A. Sabo, M. Pisarcikova, G. Kovacicova, D. Chovancova & Z. Szovenyiova. 2001. Breakthrough fungemia in neonates and infants caudes by *Candida albicans* and *Candida parapsilosis* susceptible to fluconazole *in vitro*. J Antimicrob Chemother. 48(4):521-525.
34. Larson, B.E. 1995. The 1992, 1993, and 1994 APIC Guideline Committees. APIC guideline for hand washing and hand antisepsis in health care settings. Am J Infect Control. 23:251-69.
35. Laskus, A., W. Mendling, K. Runge & L. Schmidt. 1998. *Candida* septicemia in premature babies: a nosocomial infection? Mycoses. 41(2): 37-40.
36. J.B. López Sastre, D. Coto Cotallo, B. Fernandez Colomer & Grupo de Hospitales Castrillo. 2002. Neonatal sepsis of nosocomial origin: an epidemiological study from the "Grupo de Hospitales Castrillo". J Perinat Med. 30(2): 149-157.
37. Macphail, G.L., G.D. Taylor, M. Buchanan-Chell, C. Ross, S. Wilson & A. Kureishi. 2002. Epidemiology, treatment and outcome of candidemia: a five-year review at three Canadian hospitals. Mycoses. 45(5-6):141-5.
38. Magaldi, S., T. Camero, S. Mata, C. Marcano & C. Hartung. 1998. Pruebas de sensibilidad de *Candida albicans* frente a los antimicóticos de uso comercial. Bol Soc Venez Microbiol. 18:16-20.
39. Magaldi, S., T. Camero. 1997. Susceptibilidad de *Candida albicans* "*in vitro*" mediante los pozos de difusión. Bol Venez de Infecto. l7:5-8.
40. Magaldi, S., S. Mata, C. Hartung, G. Verde, L Deibis, Y. Roldan & C. Marcano. 2000. "*In vitro*" susceptibility of 137 *Candida* sp. isolates from HIV positive patients to several antifungal drugs. Mycopathologia. 149:63-68.
41. Magaldi, S., C. Hartung, G. Verde, L. Spencer & S. Mata. 2001. *In vitro* susceptibility to fluconazole of *Candida* spp. isolates comparing three different methods. J Mycol Med. 11:123-126.
42. Magaldi, S., S. Mata Essayag, C. Hartung de Capriles, C. Pérez, M.T. Colella, C. Olaizola & Y. Ontiveros. 2004. Well diffusion for antifungal susceptibility testing. I J I D. 8:39-45.
43. Makhoul, I..R, P. Sujov, L. Ardekian, I. Kassis, T. Smolkin, J. I. Abu-elnaa', A. Tamir & D. Laufer. 2002. Factors influencing oral colonization in premature infants. Isr Med Assoc J. 4(2):98-102.
44. Mata-Essayag, S. 1993. Clamped homogeneous Electric Gel Electrophoresis Typing of *Candida albicans* isolates from HIV patients. PhD Thesis in Microbiology, University of Manchester, Manchester, England.
45. Mata-Essayag, S. 2002. Anfotericina B: algunos aspectos relevantes. Actualización en Infectología. 18(3):2-10.
46. Melville, C., S.T. Kempley, G.L. Darmstadt, J.G. Dinulos & Z. Miller. 2001. Treatment of Invasive *Candida* Infection in Neonates With Congenital Cutaneous Candidiasis. (Letter to the Editor). Pediatrics. 7:45.
47. Mermel, L.A. & D.G. Maki. 1993. Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. Ann Intern Med. 119:270–272.
48. Mongkolrattanothai, K., K. Chokephaibulkit, T. Kolatat, N. Vanprapar, S. Chearskul, N. Srihapol, V. Pumsawan, S. Pkaworawuth & C. Dhiraputra. 2001. Nosocomial bloodstream infection in pediatric patients: Siriraj Hospital, Bangkok; 1996-1999. J Med Assoc Thai. 84(2):160-5.
49. Narang, A., P. Agrawal, A. Chakrabarti & P. Kumar. 1998. Epidemiology of systemic candidiasis in a tertiary care neonatal unit. J Trop Pediatrics. 44:104-108.
50. Omaña Y, Rojas R. 1998. Infecciones por hongos en recién nacidos sépticos. Thesis Pediatrics, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela.
51. Padovani, E.M., F. Michielutte, Dall'Agnole & Dal-Moro K. 1997. Sepsis caused by *Candida* in neonatal period. Pediatr Med Chir. 19:8-83.
52. Pera, A., A. Byun, S. Gribar, R. Schwartz, D. Kumar & P. Pa. 2002. Dexamethasone therapy and *Candida* sepsis in neonates less than 1250 grams. J Perinatol. 22(3): 204-8.
53. Pittet, D., M. Monod, P.M. Suter, E. Frenk & R. Auckenthaler. 1994. *Candida* colonization and subsequent infections in critically ill surgical patients. Ann Surg. 220:751-8.
54. Ramírez, I., H. Mayerling, D. Daniel & M. Manrique. 1999. Candidiasis Neonatal. Estudio Clínico y Epidemiológico realizado en la Ciudad Hospitalaria "Dr. Enrique Tejera" Antib e Inf. 7(1): 21-30.
55. Rangel, F., T. Wiblin, H. Blumberg, L. Saiman, J. Patterson, M. Rinaldi et al. 1999. National Epidemiology of Mycoses Survey (NEMIS): Variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal care units. Clin Infect Dis. 29:253-258.
56. Rex, J.J., M.A. Pfaller, J.N. Galgiani, et al. 1997. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole, and *Candida* infections. Clin Infect Dis. 24:235-247.
57. Richards, M.J., J.R. Edwards, D.H. Culver & R.P. Gaynes. 1999. Nosocomial infections in medical intensive care units in the United States. Crit Care Med. 27:887–92.
58. Rios Fabra, A. 1992. Fungemias en pacientes criticamente enfermos. Ant E Inf. 1(1): 30-37.
59. Robinson, L.G., L. Jain & A.P. Kourtis. 1999. Persistent candidemia in a premature infant treated with fluconazole. Pediatr Infect Dis J. 18:735-737.
60. Rowen, J., J. Tate. 1998. Management of neonatal candidiasis. Pediat Infect Dis J. 17:1007-11.

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61. Safdar, N., D.G. Maki. 2002. The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant *Staphylococcus aureus*, *Enterococcus*, Gram-negative Bacilli, *Clostridium difficile*, and *Candida*. Ann Intern Med. 136:834-844.
62. Saiman, L., E. Ludington, M. Pfaller, et al. 2000. Risk factors for candidemia in NICU patients Pediatr Infect Dis J. 19:319-24.
63. Saiman L, Ludington E, Dawson JD, Paterson JE, Rangel-Frausto S, Wiblin RT, Blumberg HM, Pfalller M, Rinaldi M, Edwards JE, Wenzel RP, Jarvis W & The National Epidemiology of Mycoses Study Group. Risk factors for *Candida* species colonization of neonatal intensive care unit patients. J Pediatr Infect Dis J 2001;20(12):1119-24.
64. Sanchez A, Johnston DA, Myers C, Edwards JE Jr., Mitchell AP, Filler SG. Relationship between *Candida albicans* virulence during experimental hematogenously disseminated infection and endothelial cell damage *in vitro*. Infect Immun 2004;72:598-601.
65. Schwarze R, Penk A, Pittrow L. Treatment of candidal infections with fluconazole in neonates and infants. Eur J Med Res 2000; 5:203-208.
66. Shelonka RL, Moser SA. Time to positive culture results in neonatal *Candida* septicemia. J Pediatr 2003 142 (5): 564-5.
67. Tirodker UH, Nataro JP, Smith S, Las Casas L, Fairchild KD. Detection of fungemia by polymerase chain reaction in critically ill neonates and children. J Perinatol 2003; 23(2): 117-22.
68. Vazquez J, Boikov D, Boikov S, Dajani A. Use of electrophoretic karyotyping in the evaluation of *Candida* infections in a neonatal intensive-care unit. ICHE 1997; 18:32-37.
69. Walsh TJ, Seibel NL, Arndt C, et al. Amphotericin B liquid complex in pediatric patients with invasive fungal infections. Pediatr Infect Dis J. 1999;18:702-708.

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