Cluster of *Candida parapsilosis* Primary Bloodstream Infection in a Neonatal Intensive Care Unit

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***Candida parapsilosis* is an increasingly important bloodstream pathogen in neonatal intensive care units (NICU). We investigated a cluster of bloodstream infections in a NICU to determine whether nosocomial transmission occurred. During a 3-day period, 3 premature infants hospitalized in the same unit presented with sepsis caused by *C. parapsilosis*. Electrophoretic karyotype of the organisms was performed by using pulsed field gel electrophoresis in a countour-clamped homogeneous electric field system. The isolate from 1 newborn could not be typed, and the isolates from the remaining 2 infants had identical patterns. All 3 cases are described. We conclude that nosocomial transmission of *C. parapsilosis* occurred and that neonates under intensive care may represent a risk group for this pathogen.**

Key Words: Candidemia, nosocomial infections, neonate.

The relative number of organisms causing nosocomial bloodstream infections has changed over the last decade, with *Candida* species now being firmly established as one of the most frequent agents worldwide [1, 2]. The number of nosocomial bloodstream infections due to *Candida* species in critically ill neonates is increasing. Taylor, et al., found that 21% of all cases of candidemia from a large institution, evaluated during a 7-year period, were documented in neonatal intensive care units (NICU) [3].

Nosocomial *Candida* infections have been recognized by many investigators as a major cause of morbidity and mortality in neonatal intensive care units [4, 5]. Newborns may acquire *Candida* species secondary to either vertical or horizontal transmission [6]. Risk factors identified by multivariate analysis of NICU populations include prior antibiotic exposure, hyperalimentation, the use of intravenous fat emulsions, and endotracheal intubation [7].

In the NICU setting, there are several reports of *Candida albicans* nosocomial cross infection [7-17]. However, few studies using molecular DNA-based typing methods have demonstrated clusters of hematogenous candidiasis due to non-*albicans* species in such a population [15,18-20]. The present report describes 3 cases of *C. parapsilosis* candidemia documented during a 3-day period in a NICU in Rio de Janeiro, Brazil.

Materials and Methods

Between April 24 and 26, 3 cases of *C. parapsilosis* candidemia were documented in a NICU of a 159 bed private tertiary care hospital in Rio de Janeiro. All clinical and epidemiological data were retrospectively obtained by reviewing patients' clinical charts. In order to determine the background rate of *C. parapsilosis* bloodstream infections, the hospital's laboratory

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records were reviewed to identify all cases of positive blood cultures for *C. parapsilosis* reported from November 1, 1996, to May 31, 1997.

Cultures. Blood cultures of candidemic patients were obtained by venipuncture and incubated in a semi-automated Bactec System. The yeast isolates were identified at the species level by using the API-20C galleries (BioMerieux).

Susceptibility testing. Yeast profiles of susceptibility to amphotericin B, fluconazole and 5-flucytosine were performed by the NCCLS microdilution method [21].

Molecular Typing. In order to investigate the relatedness among *C. parapsilosis* isolates, we performed an electrophoretic karyotype (EK) of the organisms by using pulsed field gel electrophoresis in a countour-clamped homogeneous electric field system (CHEF-DRIII, Richmond-CA), following a previously described method [22, 23].

Briefly, colonies of *C. parapsilosis* were incubated overnight in 1% yeast extract, 2% peptone and 2% dextrose. After centrifugation, the cells were washed with 50 mM EDTA, pH 8.0 and incubated with lyticase. Agarose blocks were prepared by adding 1% low melt agarose to this solution. The blocks were incubated for 12 h in 0.01 M Tris buffer, 0.45M EDTA, 1% laurylsarcosine and 1mg of Proteinase K per mL. The washing procedures were repeated several times the next day and 2 mm of each agarose block were inserted in individual wells of an 8% chromosomic grade agarose gel. A countour-clamped homogeneous electric field was used to separate different molecular sizes of *C. parapsilosis* chromosomal DNA. *Saccharomyces cerevisiae* chromosome-DNA standard (BioRad) was inserted into the gel as a standard. In addition, 1 isolate of *C. parapsilosis* obtained from the blood culture of an adult patient hospitalized at another institution was included. The electrophoretic conditions were: 150 volts; 13°C switch time: 120 sec for 24 h and then 240 sec for 36 h. The gel was stained in ethidium bromide and phothographed. Two isolates were considered to have the same electrophoretic karyotype profile if all of the bands in one isolate matched the bands in another by visual comparison.

Results

According to data obtained by reviewing the laboratory records, only 3 cases of candidemia due to *C. parapsilosis* were documented in the NICU during the study period. No other candidemia was detected during the 6 months prior to the described outbreak The 3 candidemic patients were put side by side in the NICU; 2 of the patients were twin sisters.

Case 1 (Second Twin) April, 24. The neonate was delivered at 28 weeks gestation and weighed 770 g. At the age of 52 days, she developed a sepsis syndrome with blood and urine cultures positive for *C. parapsilosis.*

Prior to fungal infection: Mechanical ventilation and central venous catheter (CVC) were in place since birth. Phototherapy, hyperalimentation, and 38 days of antibiotics were also required.

Outcome: *Candida parapsilosis* was isolated from peripheral blood cultures and from the catheter tip that had been in place since day 34. Amphotericin B was started on day 54 at 1 mg/kg/day, and the CVC was removed on day 55. The last positive blood culture was obtained 3 days after the catheter removal. She received amphotericin B therapy for 18 days and was discharged at 136 days of age, 2 months after the end of treatment, without any sign of infection.

Case 2 (First Twin) April, 26. The neonate was delivered at 28 weeks gestation and weighed 910 g. At the age of 54 days, she developed a sepsis syndrome with blood cultures positive for *C. parapsilosis*.

Prior to fungal infection: Mechanical ventilation and CVC were in place for 42 days. Phototherapy, hyperalimentation, and 38 days of antibiotics were also required. Following case 1, there was a high suspicion for *Candida* infection and amphotericin B at 1 mg/kg/day was started at the very beginning of clinical signs of sepsis. No CVC had been in place 12 days prior to the occurrence of fungal sepsis.

Outcome: The patient received amphotericin B therapy for 18 days, and no additional positive culture was obtained. She was discharged at 127 days of age, almost 2 months after the end of treatment, without any sign of infection.

Case 3 April 26. The patient was delivered at 27 weeks gestation and weighed 700 g. At the age of 65 days, he developed a sepsis syndrome with blood and urine cultures positive for *C. parapsilosis.*

Prior to fungal infection: Mechanical ventilation and CVC were in place since birth. Phototherapy, parenteral nutrition and 46 days of antibiotics were also required. Outcome: Amphotericin B was started at 1 mg/kg/day and the central venous catheter was exchanged. Additional positive cultures for *C. parapsilosis* were obtained from tracheal aspirate on day 67, and blood cultures on days 69 and 77. Blood cultures remained positive up to the eleventh day of antifungal therapy, when the new CVC was removed. After that, all blood cultures were negative. Amphotericin B was maintained for a total of 24 days. The fungal infection was cured, but the patient died at 178 days of age due to bronchodysplasia complications.

Only 2 of the 3 *C. parapsilosis* isolates were available for further susceptibility testing and molecular typing. The amphotericin B, fluconazole and 5-flucytosine MIC values for both yeast isolates were 0.5 μg/mL, 0.25 μg/mL and 0.125 μg/mL, respectively. As illustrated in Figure 1, the electrophoretic karyotype of the 3 tested isolates exhibited 2 different patterns, one of them shared by both clinical isolates from the NICU patients, and the other exhibited by the reference strain.

Discussion

Apart from the increased risk for developing nosocomial infections, neonates admitted to a NICU are at an increased risk for infections due to unusual pathogens [24]. Knowledge of the epidemiology of nosocomial pathogens documented among newborns admitted to intensive care units is useful for clinicians to better treat this population and to anticipate possible threats. The emergence of *Candida* species as etiologic agents of nosocomial infections has been described [6]. In Brazil, recent data obtained from a multicenter study showed that 28 of 145 (19%) episodes of candidemia were reported among children admitted to the neonatal intensive care units of tertiary care hospitals [25].

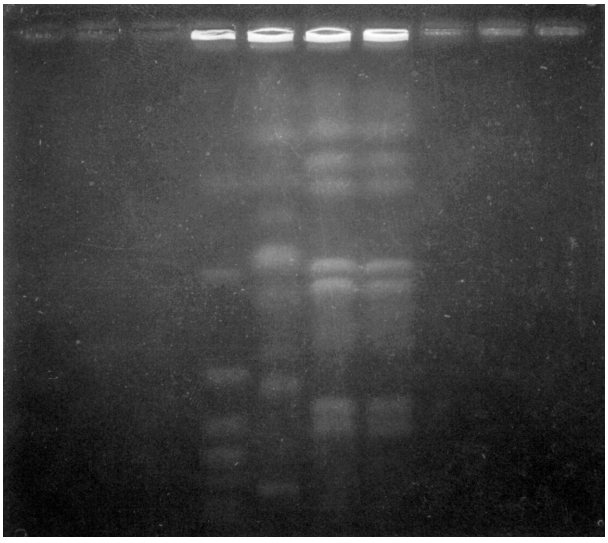
Although *Candida* spp can be a perinatal pathogen, the predominant mode of acquisition in NICU populations is nosocomial transmission, which is even more important with *C. parapsilosis* infections [5, 6]. In Brazil, Levin, et al., have described a cluster of 6 cases of fungemia due to *C. parapsilosis* among adult patients [26]. To our knowledge, the present series represents the first report of a *C. parapsilosis* outbreak in a NICU setting described in this country.

The use of liquid glycerin given as a suppository was identified as a common source of a *C. parapsilosis* outbreak in a NICU [19]. In 2 other *C. parapsilosis* outbreaks, molecular based studies showed similar profiles among patients and hands of health care workers [20, 26]. Unfortunately, in the present report, we were not able to investigate the common source of the *C. parapsilosis* infections. However, the genomic relatedness of the 2 *C. parapsilosis* strains, and the geographic and temporal association are strong arguments for considering this occurrence a definite outbreak. We suggest that nosocomial acquisition through indirect patient contact could have occurred.

Very low birth weight (VLBW - birth weight less than 1,500 g) infants represent a special risk group of patients with increased susceptibility for fungal infections due to their impaired specific and nonspecific defense mechanisms [27]. Rates of fungal colonization among VLBW infants are over 20%, *Candida albicans* and *C. parapsilosis* being the most prevalent species; and 7.7% of the colonized patients later develop systemic *Candida* infections [8-16, 18-20, 28]. The lower weight the baby, the higher the risk. In a prospective study, all infants who developed candidemia, weighed less than 1,000 g and were previously colonized [29]. In addition to baseline conditions, life saving and supporting measures such as broad spectrum antibiotics, mechanical ventilation, parenteral nutrition and central venous catheters are risk factors for fungal infection [7, 30, 31]. In our series, all 3 patients weighed less than 1,000 g at birth and were exposed to these risk factors previous to the fungal infection.

Among adult patients, systemic infections due to C*andida parapsilosis* have been associated with a lower mortality rate compared to other species of

Figure 1. Electrophoretic karyotype of 3 *C. parapsilosis* isolates, exhibiting the genetic relatedness among 2 samples obtained from NICU patients (Lanes 2 and 3). Lane 1 represents an external control of *C. parapsilosis* and *Sc* is the DNA marker (*S. cerevisiae*)



Candida 32-34]. This clinical finding is in accordance with experimental data showing that *C. parapsilosis* isolates exhibit a lesser potential for tissue invasion [35]. In the NICU population, mortality due to *C. parapsilosis* may be considerably higher, especially among the smallest babies [36]. In the largest outbreak of *C. parapsilosis* reported among neonates, 21 of 23 babies with systemic infection had a gestational age of less than 29 weeks, the case-fatality was 39%, and risk of death was 16-fold greater than that of controls [18].

The present cases of candidemia were successfully treated with amphotericin B. As expected, the 2 *C. parapsilosis* isolates were very susceptible to all of the antifungal drugs tested. Of interest, 1 patient continued to have positive blood cultures despite 11 days of high dose amphotericin B therapy. The cultures became negative just after the removal of the CVC catheter.

The issue of removing a central venous catheter in patients with candidemia is a matter of great controversy. In a recent report, we described a cohort of 145 patients with candidemia where catheter retention was recognized as an independent risk factor for death [32]. The case mentioned above illustrates that removing the catheter may influence clinical outcome.

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