

Candida parapsilosis candidaemia in a neonatal unit over 7 years: a case series study

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ABSTRACT

Objective: To evaluate *Candida parapsilosis* candidaemia in a neonatal unit over 7 years.

Design: Case series study.

Setting: A 2000-bed tertiary-care university hospital at São Paulo, Brazil.

Participants: Neonates hospitalised in a 63-bed neonatal unit.

Primary and secondary outcome measures: We evaluated the incidence of *C parapsilosis* fungemia in a neonatal unit from 2002 through 2008 and the main microbiological, clinical and epidemiological aspects of this disease in neonates. During the study period an outbreak occurred, an infection control programme was implemented, and isolates from blood and hand healthcare workers (HCWs) were submitted to molecular typing.

Results: During 7 years, there were 36 cases of *C parapsilosis* fungaemia and annual incidence varied from 0 to 19.7 per 1000 admissions. Evaluating 31 neonates with fungemia, the mean age at diagnosis was 19 days. All children except for one were premature; all had received total parenteral nutrition and all but one had used central venous catheter. Three neonates had received antifungal treatment previously to the diagnosis. Thirty-day mortality was 45%. Only lower birthweight was associated with mortality. *C parapsilosis* species complex was isolated from hand cultures in eight (11%) of the HCWs (one isolate was identified as *C orthopsilosis*). By molecular typing no HCW isolate was similar to any of the blood isolates.

Conclusions: The incidence of *C parapsilosis* fungemia in a neonatal unit varied widely over 7 years. We observed in our series a higher death rate than that reported in European countries and the USA.

INTRODUCTION

Candida species are the leading cause of invasive fungal infections in the neonatal intensive care unit (NICU) and are the third most common blood culture isolates recovered

ARTICLE SUMMARY

Article focus

- *Candida* species are the third most common blood culture isolates recovered from cases of late-onset sepsis in the neonatal intensive care unit.
- *Candida parapsilosis* is probably the species that has had the largest increase in incidence since 1990, becoming the predominant agent of candidaemia in certain centres.
- Systemic infections because of *C parapsilosis* have been associated with significantly better survival rates when compared with infections caused by other species.
- Understand the epidemiology of *C. parapsilosis* candidemia in a Brazilian NICU, given the local situation is distinct from that found in series from developing countries.

Key messages

- In our study, mortality was 45%, higher than expected for *Candida parapsilosis* candidaemia and we describe points that could explain this result.
- We speculate that some points could explain this result: low birth weights, failure to remove catheter and delay to start a treatment.
- One must understand the epidemiology of *C parapsilosis* candidaemia in a Brazilian neonatal intensive care unit, given the local situation is distinct from that found in series from developing countries.
- To our knowledge this is the largest Brazilian clinical and epidemiological description of neonatal *C. parapsilosis* candidemia.
- An outbreak occurred with a polyclonal pattern, suggesting multiple sources. Strengths and limitations.
- Molecular typing methods were performed to investigate the outbreak.
- Only one sample per patient and one per healthcare worker was evaluated during the outbreak, and this was a limitation to the present study.

from cases of late-onset sepsis in the NICU.¹⁻⁵ *C parapsilosis* is probably the species that has had the largest increase in incidence since 1990, becoming the predominant agent of candidaemia in certain centres.⁶⁻⁹ Numerous risk factors for candidaemia have been identified in NICU patients such as immature skin structure,¹⁰ prolonged use of antimicrobials,¹¹ indwelling central venous catheters,¹² hyperalimentation,¹³ mechanical ventilation,¹¹ surgery¹⁴ or pre-existing fungal colonisation.^{8, 13, 15}

In Brazil, there are few data assessing exclusively the epidemiology of *C parapsilosis* candidaemia,^{16, 17} and in the neonatal population only a description of a cluster with three cases of *C parapsilosis* sepsis.¹⁸ So far, no description of the epidemiology of *C parapsilosis* candidaemia was evaluated in the NICU setting.

We conducted a retrospective, observational study involving a NICU in the city of São Paulo, Brazil, to evaluate the incidence rates of *C parapsilosis* candidaemia over a 7-year period in our hospital and to characterise the main microbiological, clinical and epidemiological aspects of this disease in neonates.

METHODS

Hospital das Clínicas is a 2000-bed tertiary-care university hospital affiliated to the University of São Paulo, Brazil. It has a neonatal unit with 63 beds that accepts only babies born in the hospital, usually from high-risk pregnancies. There are five sections within the unit: for normal newborns (23 beds); medium-risk unit (15 beds); high-risk (9 beds); isolation (8 beds) and a neonatal intensive care unit (8 beds).

We evaluated the incidence of fungemia during a 7-year period beginning in 2002 through 2008. Cases were collected prospectively by the infection control nurses during active surveillance at the neonatal unit. A case was defined as a baby hospitalised in the neonatal unit, born in the hospital, who presented with at least one positive blood culture collected from a peripheral vein for *C parapsilosis*. Only the first episode of candidaemia for each newborn was considered. The annual incidence of candidaemia for the neonatal unit was calculated using as a denominator the number of admissions (except for the normal newborn admissions).

Clinical data

We reviewed the hospital records of neonates with candidaemia caused by *C parapsilosis*. The demographic and clinical data evaluated were: sex, birth weight, type of delivery, Apgar score¹⁹ and underlying diseases; post-natal age at the time of infection; use of invasive procedures such as central venous catheter (CVC), total parenteral nutrition (TPN), dialysis, infections caused by other microorganisms, surgery and use of antimicrobial (the days of use of each drug were added), steroids and antifungal drugs during the previous 30 days; and clinical outcome 30 days after diagnosis. Prophylaxis with fluconazole was not used.

C parapsilosis outbreak

During the period from May 2004 through July 2005, an investigation designed to evaluate *Candida* colonisation in candidaemic patients in the main building of the hospital, where the neonatal unit is located,²⁰ was taken. During this study a large number of cases of candidaemia caused by *C parapsilosis* were observed in the neonatal unit. An outbreak of *C parapsilosis* was suspected and an infection control programme was started in the unit. Besides colonisation isolates, *C parapsilosis* blood isolates from these patients were available for further microbiological study. Hand cultures were obtained from healthcare workers (HCW) of the unit, except physicians who refused having their hands sampled.

The healthcare practices in the neonatal unit were evaluated, meetings with the medical and nursing staff were held and visits were made to the Pharmacy where TPN was produced.

The following control measures were implemented starting in April 2005:

- ▶ Changes in the composition of TPN and prohibition of adding components to the solution by the staff of the unit.
- ▶ Decrease in the number of patients admitted, applying strict admission criteria, so as to achieve a monthly occupation rate of 90%.
- ▶ Increase in the availability of supplies such as gowns and antiseptic solutions.

Microbiological methods

The blood cultures had been collected due to clinical indication by the physician of each patient and were performed in the clinical laboratory of the hospital using an automated system (Bactec 9240, Becton Dickinson, USA).

The hands of the HCWs who had direct contact with patients were cultured from March to June 2005, by swabbing the interdigital areas and under their nails, using sterile swabs with Stuart medium.

Specimens were inoculated on Sabouraud dextrose (Difco, Sparks, USA) broth supplemented with chloramphenicol agar (100 mg/ml).²¹ Cultures were considered negative if no growth occurred for 21 days.

The phenotypic identification of the isolates of *C parapsilosis* from the blood and hands of HCWs was done by the morphology of colonies, germ tube test, chromogenic medium (CHROMagar Microbiology, Paris, France) and carbon assimilation test (ID 32C; bioMérieux, Marcy l'Etoile, France).²² Identification was confirmed manually using the test of carbohydrate assimilation.²³

All the available isolates that were identified morphologically and biochemical as *C parapsilosis* had the internal transcribed spacer (ITS) region and the ribosomal RNA gene amplified with the utilisation of the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCG CTTATTGATATGC-3').²⁴ The PCR products were then purified and subjected to automated DNA sequencing. The aligned sequences were then

analysed with BioEdit sequence programme.²⁵ Further analysis was performed by comparison with ITS sequences of *C parapsilosis*, *C metapsilosis* and *C orthopsilosis* included in the BLAST.²⁵

Two molecular typing methods were performed for *C parapsilosis* isolated from the blood of neonates and from hands of HCWs.

1. Pulsed-field gel electrophoresis (PFGE) as described previously^{26, 27} and generated electrophoretic karyotypes (EK).
2. Randomly amplified polymorphic DNA (RAPD) typing was performed as described previously^{28, 29} with minor modifications. Four primers were used: Leg 2 (5'-CTG GCTTCTTCCAGCTTCA-3'), Oligo 1 (5'-TCACGATG CA-3'); Oligo 3 (5'-GCCGCCGAAACGATCTAC-3') and P5 (5'-GTCATGAGGAATCTCGCCTG-3'). Isolates were considered to be different if the banding patterns differed by one, or more than one, readily detectable band. The RAPD analysis was repeated on three separate occasions.

To be considered indistinguishable, isolates had to be similar by both methods used.

Statistical analysis

Patients who died during hospitalisation were compared with patients who survived. Continuous data were compared using the Mann-Whitney U test for nonparametric data, and categorical variables were compared using the χ^2 test or Fisher exact test. All p values were two-tailed; a p value of less than 0.05 was considered to reveal a statistically significant difference. Statistical analysis was conducted with Epi Info, V.3.5.3.

Informed consent was not necessary in our study as cultures were part of our local infection control programme.

RESULTS

The number of cases of *C parapsilosis* fungemia and their incidence in the neonatal unit can be seen in table 1. There was a statistically significant increase in cases from

2002 to 2004 (p: 0.00007), thus an outbreak was identified. From 2002 through 2008 there were 36 cases. The data on this population were available for 31 patients and can be seen in table 2. All data were unavailable for five infants, except for the presence of prematurity and outcome: all were premature and two neonates died. The cases occurred in three sections of the neonatal unit: intensive care unit,²⁷ high-risk (three) and isolation (one).

All children except for one were premature; 12 neonates were born weighing <1000 g. Twenty-eight neonates had a CVC at the moment of candidaemia and the median time between diagnosis and catheter removal was 3.5 days (range: 0–16). The median number of previous antimicrobials used per patient was 4 (range: 2–6), and the mean duration of use was 33 days (SD: 17). Third generation cephalosporins and carbapenems were used, by 16 (52%) and 15 (48%) neonates, respectively. The treatment with amphotericin and fluconazole had been used previously to the diagnosis by two and one neonate, respectively. Infants with candidaemia were treated with amphotericin in 26 cases, fluconazole in 2 and a combination of amphotericin and fluconazole in 1. Two patients were not treated and died. The time elapsed between the episodes of candidaemia and starting of antifungal therapy was more than 48 h in 16 neonates (52%).

The comparison of the clinical features of the cases who died and those who survived can be seen in table 3.

Nine neonates had previous laboratory confirmed bacterial bloodstream infection: five coagulase-negative *Staphylococci* and one each of *Acinetobacter baumannii*, *Serratia marcescens*, *Escherichia coli*, *Streptococcus agalactiae* and *Staphylococcus aureus*.

Outbreak

During the outbreak, the evaluation of the unit and the observation of practices revealed the following:

- ▶ No problems were observed in the Central Pharmacy with TPN preparation; however manipulation of TPN

Table 1 Incidence of cases of *Candida parapsilosis* candidaemia in a neonatal unit from 2002 through 2008 (Hospital das Clínicas, University of São Paulo, Brazil)

Year	Number of admissions to the unit	Number of cases of <i>C parapsilosis</i> fungemia (percentage of total <i>Candida</i> fungemia)	<i>C parapsilosis</i> incidence (per 1000 admissions)	Number of cases of other <i>Candida</i> fungemia	Total incidence of all <i>Candida</i> fungemia (per 1000 admissions)
2002	1470	0 (0)	0	2	1.4
2003	562	1 (6.7)	1.8	14	26.7
2004	550	11 (61)	20*	7	32.7
2005	508	10 (59)	19.7	7	34.5
2006	756	6 (50)	7.9	6	14.0
2007	859	6 (86)	7.0	1	8.1
2008	935	2 (100)	2.1	0	2.1

*p=0.00007 (χ^2 for trend from 2002 to 2004).

Table 2 Characteristics of 31 patients in the neonatal unit with *Candida parapsilosis* fungemia from 2002 through 2008 (Hospital das Clínicas, University of São Paulo, Brazil)

Characteristic	N (%)
Dichotomous variables	
Male gender	13 (42)
Delivery by caesarean section	22 (71)
Prematurity	30 (97)
Use of central venous catheter	30 (97)
Use of total parenteral nutrition	31 (100)
Use of dialysis	1 (3)
Surgery	4 (13)
Previous use of antibacterial drugs	31 (100)
Previous use of antifungal drugs	3 (10)
Use of corticosteroids	0
Death within 30 days of positive culture	14 (45)
Continuous variables	
Birth weight (g)	1288 (731)
Mean (SD)	
Apgar score at 5 min of age	28 (3–10)
Median (range)	
Age at diagnosis of candidaemia (days)	18 (6–37)
Median (range)	
Use of TPN (days)	16 (0–32)
Median (range)	
Use of CVC (days)	14 (0–33)
Median (range)	
Time the CVC remained in place after diagnosis (days)	3.5 (0–16)
Median (range)	

CVC, central venous catheter; TNP, total parenteral nutrition.

solutions by nurses, adding components in the neonatal unit occurred.

- ▶ Overcrowding of the unit was observed with the inclusion of extra beds between the officially registered beds.
- ▶ Shortage of supplies such as gowns and antiseptic solutions occurred.

Recommendations by the Infection Control Department were only fully implemented in 2005. After the interventions the incidence of *C parapsilosis* fungemia decreased gradually as can be seen in table 1.

During the 2004–2005 outbreak, 71 HCWs were submitted to cultures of their hands. These represented 90% of the staff of the unit: 20 nurses, 40 assistant nurses, 2 respiratory therapists, 5 administrative workers and 2 cleaning workers. *Candida* sp. was isolated from hand cultures in 14% of the HCWs, *C parapsilosis* in eight (11%) and *Candida albicans* in two (3%). Other yeasts recovered from HCWs included *Rhodotorula rubra*, *Rhodotorula glutinis* and *Trichosporum* sp. (one from each HCW).

Molecular typing

Twelve blood *C parapsilosis* isolates from 11 newborns obtained from May 2004 through July 2005, during the outbreak, and nine *C parapsilosis* isolates from the hands of eight HCWs underwent molecular typing. The 12 blood isolates from neonates presented eight different EK patterns. The nine hand isolates from HCWs presented seven EK patterns. The RAPD done with primer Leg 2 in 12 blood isolates from 11 neonates yielded only two profiles. Primers Oligo 1, Oligo 3 and P5 did not present a good discriminatory power. The nine hand isolates from HCWs presented five RAPD patterns.

Considering both typing methods, the nine isolates from HCWs differed between each other and no isolates from HCWs were similar to any of the blood isolates.

Only two pairs of indistinguishable isolates were found, all of them from blood isolates from newborns. The infants with identical isolates were not in the NICU concurrently.

All isolates were *C parapsilosis* except one HCW hand isolate which was identified as *C orthopsilosis*.

DISCUSSION

We described here a case series of neonatal patients with *C parapsilosis* candidaemia. To our knowledge this is the

Table 3 Bivariate analysis of variables potentially associated with 30-day mortality in neonates with *Candida parapsilosis* fungemia from 2002 through 2008 (Hospital das Clínicas, University of São Paulo, Brazil)

Variable	Death N (%)	Survival N (%)	OR (95% CI)	p Value
Male gender	5 (36)	8 (47)	1.6 (0.38 to 6.82)	0.50
Delivery by caesarean section	8 (57)	14 (82)	3.5 (0.68 to 17.96)	0.12
Delay to remove CVC after diagnosis ≥ 2 days	9 (64)	11 (65)	0.98 (0.22 to 4.30)	0.64
Time from diagnosis to initiating treatment > 2 days	6 (43)	12 (69)	0.28 (0.06 to 1.32)	0.10
Birth weight (g)	845 (600–4150)	1290 (680–2460)	–	0.01
Median (range)				
Apgar score at 5 min	8 (6–10)	8 (3–10)	–	0.68
Median (range)				
Age at diagnosis of candidaemia (days)	18 (8–37)	19 (6–37)	–	0.54
Median (range)				

CVC, central venous catheter.

largest Brazilian clinical and epidemiological description of neonatal *C parapsilosis* candidaemia.^{6 9 30–32}

Greater than 80% of our cases had a gestational age at birth of 30 weeks or less, and nearly 80% had a birth weight of <1500 g, highlighting the significant burden of this disease among premature infants and those with very low birth weight.

Other neonatal problems resulting in prolonged stay in the NICU may have, also contributed to the occurrence of candidaemia. In our study, most neonates had a CVC at the moment of candidaemia and all had CVC in place and received TPN during the previous 30 days of the diagnosis. Another factor was the long duration of broad-spectrum antibiotic therapy used for all neonates.

Systemic infections due to *C parapsilosis* have been associated with significantly better survival rates when compared with infections caused by other species. In our study, mortality was 45%, higher than expected.^{7 30} We speculate that some points could explain this result. The neonates who died had lower birth weights comparing with the ones who survived.

Studies suggest that failure to remove catheter as soon as candidaemia is detected in neonates is a risk factor for death,^{4 33} in our study this factor was frequent, and 55% of neonates had retention of the catheter for at least 2 days after diagnosis of candidaemia. Also in our study, antifungal therapy was started after 48 h in a large proportion of neonates (52%). Delays of more than 48 h to start a treatment may worsen outcome.³⁴ In our study however there was no difference in the delay to remove catheter between patients who died and survived.

If the gut is the primary source of candidaemia, attempts aimed at reducing gut colonisation, such as the control of exposure to broad-spectrum antimicrobials, and the use of antifungal prophylaxis, may have an impact in reducing the incidence of candidaemia.^{35 36} In contrast, implementation of intensive programmes to maximise compliance with hand hygiene, and adherence to current recommendations for placement and care of CVCs, should be implemented to control exogenous sources.

In 2004, a large number of cases of *C parapsilosis* candidaemia were observed in the neonatal unit. An outbreak of *C parapsilosis* was suspected and the practices of healthcare in the unit were evaluated. The observation of practices revealed the manipulation of TPN solution by nurses in the neonatal unit; overcrowding of the unit with the inclusion of extra beds, and shortage of supplies such as gowns and antiseptic solutions. After recommendations and interventions the incidence of *C parapsilosis* candidaemia decreased gradually, which further reinforced the hypothesis of exogenous infection. This outbreak offered a great opportunity to study the source of *C parapsilosis* neonatal candidaemia. Molecular typing of *C parapsilosis* isolates demonstrated similarity between 12 blood isolates obtained from newborns and 6 catheter tips in a previous study in our

hospital,²⁰ without any similarity between blood isolates and isolates that colonised the skin or gastrointestinal tract. This absence of colonisation at other body sites suggests an exogenous source of *C parapsilosis*. In the present study, no isolates from HCWs were similar to blood isolates, thus no human source could be implicated. This finding was different from prior studies showing possible transmission of yeast strains from HCWs to patients.^{37 38} However, a limitation is that no samples from physicians were obtained.

In the present study a higher discriminatory power was achieved by combining different typing methods: PCR and PFGE-based typing methods. PFGE presented more profiles than RAPD; however, the combination of both methods was more discriminatory than PFGE alone.

As discussed above, HCWs were not a primary source and other possible sources such as contaminated solutions or medications would probably lead to a monoclonal outbreak.

These results suggest that blood strains did not have a common source. Maternal isolates or the environment could be the source of the infants' blood isolates, but they were not evaluated, which is a limitation of our study.

Only one sample per patient and one per HCW was evaluated during the outbreak, and this was a limitation to the present study. Future studies that focus on the daily HCW–patient interactions and include multiple sampling, taking into account factors as hand washing, glove use and use of invasive devices, may help to define the role of these interactions in the transmission of yeasts.

In conclusion, we observed that the incidence rate of *C parapsilosis* in an NICU varied widely. The crude mortality was higher than reported in European countries and the USA. One must understand the epidemiology of *C parapsilosis* candidaemia in a Brazilian NICU, given the local situation is distinct from that found in series from developing countries, and particularly with regard to reduced availability of resources in health. An outbreak occurred with a polyclonal pattern, suggesting multiple sources.

Contributors LNM: analysis and interpretation of data, drafting the article and final approval of the version to be published; ECAR: acquisition of data, drafting the article and final approval of the version to be published; SFC: analysis and interpretation of data, revising the article critically for important intellectual content and final approval of the version to be published; IMH: acquisition of data, revising the article critically for important intellectual content and final approval of the version to be published; KCD: analysis and interpretation of data, drafting the article and final approval of the version to be published; RDL: acquisition of data, drafting the article and final approval of the version to be published; MB: acquisition of data, revising the article critically for important intellectual content and final approval of the version to be published; GFV: acquisition of data, drafting the article and final approval of the version to be published; VLJK: substantial contributions to conception and design, drafting the article and final approval of the version to be published; MABCG: acquisition of data, revising the article critically for important intellectual content and final approval of the version to be published; PRC: analysis and interpretation of data, drafting the article and

final approval of the version to be published; ASL: substantial contributions to conception and design, revising the article critically for important intellectual content and final approval of the version to be published.

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