Two different clones of *Candida pelliculosa* bloodstream infection in a tertiary neonatal intensive care unit

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Abstract

Introduction: Fungemia in preterm infants results in high mortality and morbidity. The genotypes, drug susceptibilities of *Candida pelliculosa* strains, and clinical features of two outbreaks of neonatal candidemia caused by *C. pelliculosa* were analyzed, in order to provide evidence for the outbreaks and characteristics of *C. pelliculosa* neonatal candidemia.

Methodology: The strains were genotyped by pulsed-field gel electrophoresis to investigate their genetic relatedness. The broth microdilution method was used to determine *in vitro* susceptibility of the isolates to antifungal drugs. Clinical features of the infected patients were collected to analyze the risks for *C. pelliculosa* infection.

Results: Fourteen neonates, hospitalized in the neonatal intensive care unit from November 2012 to October 2013, were infected by *C. pelliculosa*. All 14 patients were cured after treatment with fluconazole and discharged without any complications. The *C. pelliculosa* isolates from the 14 patients were clustered into two groups, indicating that the outbreaks were caused by two types of strains. Eight of nine strains isolated from the 2013 outbreak were clustered into the same group, while one isolate was grouped together with five isolates from the 2012 outbreak. *In vitro* experiments demonstrated high antifungal activity of fluconazole, voriconazole, amphotericin B, and 5-fluorocytosine to *C. pelliculosa*. The common symptoms of *C. pelliculosa* candidaemia were fever, cyanosis, polypnea, hypoactivity, and apnea.

Conclusions: The current study revealed high *in vitro* susceptibility of *C. pelliculosa* to antifungals. As *C. pelliculosa* candidaemia cannot be characterized by clinical symptoms and routine blood testing alone, monitoring unusual strains isolated from immunodeficient hosts is very important to prevent possible outbreaks.

Key words: Candida pelliculosa; genotyping; fungemia; outbreak; genetic relatedness.

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Introduction

Candidemia is caused by *Candida* species and has severe complications in premature and low-birthweight infants; it is the third most common nosocomial bloodstream infection during late-onset neonatal sepsis [1], with an overall incidence of 0.3–9.9% and a mortality of 30–60% in the neonatal intensive care unit (NICU) [2-4]. *Candida albicans*, *C. parapsilosis*, and *C. tropicalis* are the most common species responsible for candidaemia [5]. *Candida parapsilosis* has recently become the most frequently isolated species in neonates [6], although *C. albicans* was the most prevalent yeast pathogen in the past decades [7]. *Candida pelliculosa* (former names: *Pichia anomala*, *Hansenula anomala*) is mainly found in plants and oil, and was first reported as the cause of an NICU outbreak of fungemia in 1986 [8] that infected immunocompromised patients, especially those with acquired immunodeficiency syndrome (AIDS) and cancer [9,10]. Outbreaks of candidemia caused by *C. pelliculosa* among neonates hospitalized in the NICU [11-13] have been reported, although this species rarely causes fungemia.

Early clinical manifestations of neonatal fungal infection are atypical and insidious, resulting in difficulties for early precursive diagnosis and timely treatment. With the increasing prevalence of drug-resistant strains, effective and quick characterization of *Candida* spp. causing candidemia in the NICU is particularly important. Pulsed-field gel electrophoresis (PFGE) is considered the gold standard for microbial species identification and epidemiologic studies of *Candida* spp. [12,14,15]. Herein, we report two outbreaks of neonatal candidemia caused by *C. pelliculosa*. We analyzed the clinical features of the
patients, the in vitro antifungal activity, as well as the genotypes of the isolated C. pelliculosa strains, using PFGE for the first time for this Candida species. The findings provide evidence for the outbreaks and characteristics of C. pelliculosa neonatal candidemia.

Methodology

Definitions

All patients included in this study had either a positive culture of C. pelliculosa in their blood or peripherally inserted central catheter (PICC). Clinical data of the patients were collected retrospectively to analyze the potential risk factors of C. pelliculosa infection, including age, gestational age, birth weight, mechanical ventilation, PICC, and previous use of broad-spectrum antibiotics. Fluconzole was administered intravenously at a preventive dose of 6 mg/kg for preterms as per standard of care in neonatal ICU, and at a therapeutic dose of 12 mg/kg for infected infants, and the usage time was recorded.

Microbiological and molecular investigation

All C. pelliculosa strains were isolated from 14 patients hospitalized for 11 days to one month in the NICU from November 2012 to October 2013. The Candida spp. strains were identified by the Vitek 2 yeast identification (bioMérieux, Durham, USA) card and genotyped by PFGE (CHEF DR-II; BiORAD, Hercules, USA), as described previously [16,17].

The strains were subcultured from stocks with Sabouraud dextrose broth agar (SDA) (Difco; BD Bioscience, Becton, USA) and incubated for 48 hours at 25 °C, from which a single colony was transferred to 2 mL of cell suspension buffer (CSB) (mixed by 100 mM Tris-HCl, 100 mM ethylenediamine tetraacetic acid (EDTA), pH 8.0). The concentrations of the C. pelliculosa strains were measured by a BioPhotometer Plus (Eppendorf AG, Hamburg, Germany) colorimeter at 600 nm and adjusted to a high concentration of CSB. A 100-μL aliquot of the cell suspension was centrifuged at 12,000 rpm for 3 min. The supernatant was discarded, the precipitate was resuspended in 100 μL of CSB, and 5 μL of lyticase (Sigma-Aldrich, France, 5000 U/mL) was added and mixed. The cell suspensions were incubated at 37 °C for 30 minutes in a water bath. A 100-μL sample of cell suspension was mixed with 100 μL of 1% SeaKem gold agarose (SKG) (Lonza, Basel, Switzerland)/1% sodium dodecyl sulfate (SDS) that was preserved at 55 °C in a water bath; this mixture was applied immediately to produce fungal plugs. The plugs were placed at 4 °C for 5 minutes; each group of plugs was extruded into a single 15-mL tube with 5 mL of prepared cell lysis buffer (CLB) (100 mM Tris-HCl, 500 mM EDTA, pH 8.0, 1% N-lauroyl-sarcosine, sodium salt -Sarcosyl-) and 250 μL of proteinase K (QIAGen, Courtaboeuf, France) (20 mg/mL). The tubes were placed horizontally in a water bath at 50 °C and shaken at a speed of approximately 170 rpm for 16 hours. The plugs were washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 50 °C with shaking in a water bath and used immediately or stored in 10 mL of TE buffer at 4 °C.

For BssIII (New England BioLabs, Ipswich, MA, USA) digestion, the plug was cut into a 2-mm-wide piece, added to 200 μL of buffer 1 (180 μL of distilled water and 20 μL of CutSmart buffer), and incubated at 50 °C for 1 hour. After removal of buffer 1, the plug was digested by 200 μL of buffer 2 (10 μL of 5000 U/mL BssIII, 20 μL of CutSmart buffer, and 170 μL of distilled water) and incubated at 50 °C for 24 hours. Each plug was soaked in 200 μL of 0.5× Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer (10× TBE: 0.9 M Tris base, 0.9 M boric acid, and 0.02 M EDTA) three times for 0.5 hours at room temperature for removal of buffer salts. The fungal plugs were embedded with 100 mL of melted 1% SKG (1 g of SKG, 100 mL of 0.5× TBE) to form the gel for electrophoresis in a contour-clamped homogenous electric field apparatus (CHEF DR-II; BiORAD, Hercules, USA) for 18 hour (switch time of 6–45 seconds, temperature of 14 °C, 120 V, and 6 V/cm) [17-19]. After electrophoresis, the gels were stained with ethidium bromide solution for 30 min and destained with distilled water. The bands were photographed using AlphaEase FC and Alphalmager. The band profiles and clinical data were analyzed using BioNumerics 6.5 software (Applied Maths NV, Sint-Martens-Latem, Belgium) and SPSS 13.0.

Ethical approval

Informed written consent was obtained from the patient’s guardians for publication of this manuscript and accompanying data. We are complying with the specific requirements of China.

Results

The clinical characteristics of the patients are shown in Table 1. A total of 14 patients with C. pelliculosa candidaemia were enrolled. Five patients were admitted within 17 days in November 2012, and nine patients were diagnosed with C. pelliculosa fungemia from July 2013 to October 2013, among which seven patients were admitted within four days. All of the patients were
premature infants with a gestational age of 28–35 weeks and hospitalized immediately after birth. Besides, several candidaemia cases were infected by other Candida species during that period, including *C. parapsilosis*, *C. albicans*, *C. himulaei*, and *C. glabrata*, among which *C. pelliculosa* was the predominant.

As shown in Tables 2 and 3, all of the patients with *C. pelliculosa* candidemia were administered fluconazole for precaution against fungal infection, but no obvious difference was observed in the routine blood tests. Logistic analysis showed no difference between catheter-related bloodstream infections (CRBSI) and bloodstream infections (BSI). The common symptoms of *C. pelliculosa* candidaemia were fever, cyanosis, polypnea, hypoactivity, and apnea.

### Table 1. Clinical characteristics of the 14 patients with *C. pelliculosa* candidemia.

| Patient | GA (wk)/gender | BW (g) | Diagnosis of blood infection | Date of blood culture sampling (y/m/d) | Age at the time of fungemia detected (d) | Mode of mechanical ventilation/time (h) | ETT (time) | Time of broad-spectrum antibiotic* use before fungemia (d) | PICC/time (d) |
|---------|----------------|--------|-----------------------------|----------------------------------------|------------------------------------------|-----------------------------------------|------------|-------------------------------------------------------------|--------------|
| 1       | 32/F           | 1430   | BSI                         | 2012/11/04                             | 29                                       | NCPAP/56                                | No         | 25                                                          | Yes/14       |
| 2       | 32/M           | 1760   | BSI                         | 2012/11/06                             | 13                                       | NCPAP/69                                | No         | 8                                                           | No           |
| 3       | 28/M           | 1090   | BSI                         | 2012/11/06                             | 46                                       | HFN/82                                  | Yes        | 25                                                          | Yes/42       |
| 4       | 32/M           | 1085   | CR-BSI                      | 2012/11/21                             | 25                                       | NCPAP/96                                | Yes        | 16                                                          | Yes/24       |
| 5       | 30/F           | 1200   | CR-BSI                      | 2012/11/22                             | 35                                       | NCPAP/52                                | No         | 22                                                          | Yes/34       |
| 6       | 30/M           | 1400   | SCR-BSI                     | 2013/07/03                             | 13                                       | NCPAP/57                                | Yes        | 11                                                          | Yes/12       |
| 7       | 29/M           | 1340   | CR-BSI                      | 2013/09/02                             | 19                                       | No                                      | Yes        | 10                                                          | Yes/16       |
| 8       | 32/F           | 1720   | BSI                         | 2013/10/04                             | 24                                       | No                                      | No         | 10                                                          | No           |
| 9       | 30/M           | 1320   | BSI                         | 2013/10/04                             | 32                                       | NCPAP/46                                | Yes        | 11                                                          | Yes/30       |
| 10      | 35/F           | 1220   | CR-BSI                      | 2013/10/05                             | 22                                       | SIMV+/NCPAP/215                         | Yes        | 14                                                          | Yes/22       |
| 11      | 31/F           | 1690   | BSI                         | 2013/10/06                             | 12                                       | No                                      | No         | 7                                                           | No           |
| 12      | 31/F           | 1340   | SCR-BSI                     | 2013/10/06                             | 24                                       | No                                      | No         | 7                                                           | Yes/21       |
| 13      | 30/M           | 1630   | BSI                         | 2013/10/06                             | 11                                       | HFN/37                                  | No         | 10                                                          | No           |
| 14      | 29/F           | 1130   | BSI                         | 2013/10/08                             | 25                                       | NCPAP/68                                | Yes        | 10                                                          | Yes/15       |

GA: gestational age; BW: birth weight; ETT: endotracheal tube; PICC: peripherally inserted central catheter; BSI: bloodstream infection; CR-BSI: catheter-related bloodstream infection; SCR-BSI: suspicious catheter-related bloodstream infection; NCPAP: noninvasive continuous positive airway pressure; HFN: high-flow nasal cannula; SIMV: synchronized intermittent mandatory ventilation; * broad-spectrum antibiotics used: latamoxef: cefoperazone sodium and sulbactam sodium and meropenem, and imipenem.

### Table 2. Prophylaxis and routine blood testing results of the 14 patients with *C. pelliculosa* candidemia.

| Patient | Fluconazole dose (mg/kg)/time (d) | Routine blood test results before fungemia | Interval time between two blood tests (d) | First routine blood test results after diagnosis of fungemia |
|---------|----------------------------------|-------------------------------------------|-------------------------------------------|----------------------------------------------------------|
|         |                                  | WBC (>109/L) | HGB (g/L) | PLT (>109/L) | WBC (>109/L) | HGB (g/L) | PLT (>109/L) |
| 1       | 6/24                             | 8.47          | 84        | 297          | 2            | 2.2        | 133         | 178          |
| 2       | 6/12                             | 13.65         | 103       | 712          | 3            | 7.54       | 149         | 367          |
| 3       | 6/38                             | 6.56          | 94        | 292          | 6            | 5.05       | 144         | 191          |
| 4       | 6/22                             | 11.8          | 105       | 265          | 3            | 4.95       | 111         | 208          |
| 5       | 6/30                             | 3.75          | 107       | 396          | 7            | 5.55       | 95          | 194          |
| 6       | 6/11                             | 15.58         | 125       | 336          | 5            | 3.3        | 116         | 220          |
| 7       | 6/12                             | 3.26          | 141       | 265          | 5            | 6.5        | 95          | 158          |
| 8       | 6/20                             | 13.06         | 129       | 529          | 3            | 6.73       | 137         | 264          |
| 9       | 6/26                             | 13.7          | 97        | 409          | 4            | 10.53      | 146         | 281          |
| 10      | 6/18                             | 5.04          | 120       | 234          | 1            | 2.36       | 124         | 234          |
| 11      | 6/4                              | 7.73          | 120       | 326          | 4            | 10.04      | 105         | 239          |
| 12      | 6/20                             | 5.23          | 123       | 249          | 5            | 8.63       | 113         | 235          |
| 13      | 6/9                              | 13.64         | 132       | 470          | 3            | 12.04      | 129         | 355          |
| 14      | 6/14                             | 5.56          | 148       | 270          | 4            | 3.04       | 131         | 93           |

WBC: white blood cell; HGB: hemoglobin; PLT: platelets; all patients were transfused with red blood cells, except for patient 2; almost all of the patients were transfused with plasma, except for patients 1, 2, 3, 7, and 9.
Two distinct *C. pelliculosa* clones were detected from two outbreaks in the NICU. The PFGE revealed that all of the *C. pelliculosa* isolates from November 2012 were in a cluster, with a similarity of 92.6–100%. Nine of the ten *C. pelliculosa* isolates from July 2013 to October 2013 were in a group with a similarity of 85.7–98.4%; the remaining isolate C2813 was similar to the isolates from November 2012. In the cluster of the isolates from July 2013 to October 2013, the similarity between C2690 and C2752 was 91.8%, whereas the similarities among the other isolates ranged from 93.8% to 98.4% (Figure 1 and Table 4). All of the *C. pelliculosa* strains demonstrated high *in vitro* susceptibility to fluconazole, itraconazole, voriconazole, amphotericin B, and 5-fluorocytosine.

| Patient | Clinical feature | Antifungal drug | Dose (mg/kg) | Time (d) | Outcome |
|---------|-----------------|----------------|-------------|----------|---------|
| 1       | Fever, apnea, cyanosis | Fluconazole | 12 | 17 | Cured |
| 2       | Fever, increased heart rate | Fluconazole | 12 | 15 | Cured |
| 3       | Fever (Transient#) | Fluconazole | 12 | 14 | Cured |
| 4       | Fever, cyanosis, apnea | Fluconazole | 12 | 21 | Discharged* |
| 5       | Fever, increased heart rate | Fluconazole | 12 | 14 | Cured |
| 6       | Fever, cyanosis, hypoactivity, polynepra | Fluconazole | 12 | 27 | Cured |
| 7       | Fever, cyanosis, apnea, polynepra | Fluconazole | 12 | 28 | Cured |
| 8       | Fever, hypoactivity, poor sucking | Fluconazole | 12 | 17 | Cured |
| 9       | - | - | - | - | Cured |
| 10      | Fever, cyanosis, polynepra, hypoactivity, increased heart rate | Fluconazole | 12 | 28 | Cured |
| 11      | Fever, cyanosis, polynepra, hypoactivity, apnea | Fluconazole | 12 | 14 | Cured |
| 12      | Fever, polynepra, increased heart rate | Fluconazole | 12 | 21 | Cured |
| 13      | Fever, polynepra, apnea | Fluconazole | 12 | 21 | Cured |
| 14      | Fever, polynepra | Fluconazole | 12 | 21 | Cured |

# Caused by high incubation temperature; * The patient’s symptoms were improved, and her parents requested discharge without achieving total enteral feeding.

**Table 4.** Results of PFGE DNA pattern similarity of *C. pelliculosa* isolated analyzed by UPGMA dendrogram.

| No. | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 |
|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1   | 100 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2   | 100 | 100 |    |    |    |    |    |    |    |    |    |    |    |    |
| 3   | 100 | 100 | 100 |    |    |    |    |    |    |    |    |    |    |    |
| 4   | 94.3 | 94.3 | 94.3 | 100 |    |    |    |    |    |    |    |    |    |    |
| 5   | 96.2 | 96.2 | 96.2 | 94.3 | 100 |    |    |    |    |    |    |    |    |    |
| 6   | 70.2 | 70.2 | 63.2 | 67.9 | 65.5 | 100 |    |    |    |    |    |    |    |    |
| 7   | 65.5 | 62.1 | 58.6 | 66.7 | 64.3 | 91.8 | 100 |    |    |    |    |    |    |    |
| 8   | 65.5 | 62.1 | 55.2 | 66.7 | 60.7 | 91.8 | 93.6 | 100 |    |    |    |    |    |    |
| 9   | 70.2 | 59.2 | 52.6 | 64.3 | 61.8 | 96.7 | 95.1 | 98.4 | 100 |    |    |    |    |    |
| 10  | 69.0 | 62.1 | 58.6 | 70.2 | 64.3 | 91.8 | 90.3 | 96.8 | 98.4 | 100 |    |    |    |    |
| 11  | 70.2 | 70.2 | 59.7 | 71.4 | 69.1 | 90.0 | 91.8 | 95.1 | 96.7 | 98.4 | 100 |    |    |    |
| 12  | 72.4 | 69.0 | 58.6 | 66.7 | 60.7 | 88.5 | 90.3 | 96.8 | 98.4 | 96.8 | 95.1 | 100 |    |    |
| 13  | 70.0 | 64.4 | 61.0 | 67.8 | 58.6 | 85.7 | 87.5 | 93.8 | 95.2 | 96.9 | 95.2 | 96.9 | 100 |    |
| 14  | 94.5 | 94.5 | 94.5 | 92.6 | 94.3 | 72.4 | 71.2 | 64.4 | 65.3 | 71.2 | 75.9 | 71.2 | 65.6 | 100 |

Groups were determined by a DNA pattern similarity > 90%.
The in vitro antifungal susceptibilities of the two types of strains were similar (Table 5).

**Discussion**

This report highlights the clinical importance of emergent *C. pelliculosa* in the NICU. Neonatal candidemia is mainly caused by immature innate and adaptive immune systems, and many invasive operations including prolonged indwelling medical devices, especially in very premature infants [20]. There are several reports of nosocomial cross-infections due to Candida spp. in NICUs [12,21,22]. *Candida albicans* is the dominant species of candidaemia in Europe and the USA, while non-albicans Candida species, including *C. parapsilosis, C. tropicalis,* and *C. guilliermondii* (newly named as Meyerozyma guilliermondii) are predominant in Asia [1] and Africa [13,23,24]. Several outbreaks of *C. pelliculosa* fungemia have been reported in NICUs [11,12,24], although *C. pelliculosa* is rare in neonatal candidaemia [12]. Kalkanci et al. [24] reported an outbreak of *C. pelliculosa* candidaemia among two preterm and two term newborns hospitalized in the same room of the NICU. More recently, da Silva et al. [11] reported an outbreak of *C. pelliculosa* fungemia among four preterm newborns and one term newborn hospitalized in the same room of the NICU. In this report, we identified two different types of outbreaks from 14 patients with *C. pelliculosa* candidaemia within one year in the NICU using PFGE. *Candida pelliculosa* is a nonpathogenic fungus found in plants and soil, and it is indigenous in the alimentary and respiratory tracts of animals and humans [25]. Recently, an increased incidence of *C. pelliculosa* candidaemia [12,26] suggests that *C. pelliculosa* may be an opportunistic fungus for fungemia by infecting premature infants as well as immunodeficient patients with AIDS or cancer [27]. Therefore, further studies are urgently needed to analyze strains, risk factors, and susceptibility of *C. pelliculosa* to antifungal drugs.

The common clinical symptoms of *C. pelliculosa* fungemia in the two outbreaks of our study included fever, cyanosis, polypnea, hypoactivity, and apnea, which are similar to those of bacterial infection and other types of fungemia [12,26]. Therefore, it is difficult to use clinical symptoms for the diagnosis of *C. pelliculosa* candidemia. Lin et al. [12] have suggested that thrombocytopenia or a blood platelet count less than 150 × 10^9/L is an early laboratory indicator of fungal infection [12]. We found no obvious difference in the routine blood test results between one week before fungemia and upon the first diagnosis of *C. pelliculosa* candidemia (Table 2), consistent with a previous report [26]. Therefore, routine blood testing is unable to characterize *C. pelliculosa* candidemia.

All *C. pelliculosa* strains from our patients demonstrated good in vitro susceptibility to fluconazole, itraconazole, voriconazole, amphotericin B, and 5-fluorocytosine (Table 5), with no significant difference between the two pulso-types of strains. *Candida pelliculosa* is a less common species of *Candida* with a lower susceptibility to flucytosine [28]. However, previous studies [28,29] have revealed no obvious differences in the susceptibility of *C. pelliculosa* to fluconazole or ravuconazole, compared with other *Candida* species. Previous studies have demonstrated that most patients with *C. pelliculosa* candidemia are cured with amphotericin B combined or not with fluconazole and flucytosine [12,24,27-29]. Fluconazole is a safe antifungal drug for neonates, with

| No. | Isolated Time | Specimen | 5-FC | AMB | FCA | ITR | VRC |
|-----|--------------|----------|------|-----|-----|-----|-----|
| 1   | 2012/11/4    | PB       | -    | -   | -   | -   | -   |
| 2   | 2012/11/6    | PB       | ≤4   | ≤0.5| ≤1  | ≤0.125| ≤0.06|
| 3   | 2012/11/20   | CB       | ≤4   | ≤0.5| ≤1  | ≤0.125| ≤0.06|
| 4   | 2012/11/21   | PB       | ≤4   | ≤0.5| ≤1  | ≤0.125| ≤0.06|
| 5   | 2012/11/22   | PB       | ≤4   | ≤0.5| ≤1  | ≤0.125| ≤0.06|
| 6   | 2013/7/4     | PB       | ≤4   | ≤0.5| 2   | 0.125  | 0.125|
| 7   | 2013/9/2     | PB       | ≤4   | ≤0.5| 2   | 0.25  | 0.125|
| 8   | 2013/10/4    | PB       | ≤4   | ≤0.5| 2   | 0.25  | 0.125|
| 9   | 2013/10/4    | PICC     | ≤4   | ≤0.5| 2   | 0.25  | 0.125|
| 10  | 2013/10/5    | PB       | ≤4   | ≤0.5| 2   | 0.25  | 0.06 |
| 11  | 2013/10/6    | PB       | ≤4   | ≤0.5| 2   | 0.25  | 0.125|
| 12  | 2013/10/6    | PB       | ≤4   | ≤0.5| 2   | 0.25  | 0.125|
| 13  | 2013/10/6    | PB       | ≤4   | ≤0.5| 2   | 0.25  | 0.125|
| 14  | 2013/10/8    | PB       | ≤4   | ≤0.5| 2   | 0.25  | 0.06 |

PB: peripheral blood; PICC: peripherally inserted central catheter; CB: catheter blood from PICC. 5-FC: 5-fluorocytosine; AMB: amphotericin B; FCA: fluconazole; ITR: itraconazole; VRC: voriconazole.
few side effects. In the present study, the patients in the two outbreaks of *C. pelliculosa* blood infections were all treated with fluconazole at a dose of 12 mg/kg, cured, and discharged without any complications. In addition, before applying antifungal drugs, the symptoms of many patients were improved after quitting or removing the PICC. In particular, one patient improved without a therapeutic dose of antifungal drugs, suggesting the lower virulence of *C. pelliculosa*. Furthermore, a few courses of treatment of fluconazole for fungemia were reported. In the present study, fluconazole administration was stopped when the last two blood cultures were negative. Therefore, a total of 14–28 days of fluconazole administration was required.

Genotyping of *Candida* isolates is important to determine adequate measures for the interruption of transmission of this yeast. The PFGE analysis clustered the 14 *C. pelliculosa* isolates from two outbreaks of *C. pelliculosa* candidemia into two clones using a DNA pattern similarity of >90% as the cutoff criterion. PFGE is considered as the gold standard method to discriminate isolates belonging to the same species and to detect the sources of infection [24,30]. Our study confirmed that all of the *C. pelliculosa* strains isolated from the same outbreak in a one-week period belonged to the same clone, except for the isolate C2813. To the best of our knowledge, this report is the first to successfully discriminate different *C. pelliculosa* isolates into clusters with PFGE. Although PFGE is time-consuming and requires expensive equipments and expertise, it still essential for the investigation of possible clinical or environmental sources of infection with *C. pelliculosa*.

All of the patients infected with either of the two different clones of *C. pelliculosa* fungemia were premature infants with a low birth weight, supporting the previous results that premature infants are more susceptible to *Candida* infections. In addition to their immature immune system and immature skin that is not an efficient barrier to *Candida* spp. [21], the premature infants in the NICU were also exposed to reported risk factors, including mechanical ventilation, PICC, and endotracheal intubation [21,31]. In the two outbreaks of this study, 71% (10/14) of the patients were subjected to invasive/noninvasive mechanical ventilation due to an immature respiratory system or lung disease. Previous studies have revealed that PICC or central venous catheters are risk factors for candidemia, and fungi are common pathogens of CRBSI [32]. Among the 71% (10/14) of *C. pelliculosa* bloodstream infection patients who received PICC in the two outbreaks, four were proved to have CRBSI, two were suspicious of CRBSI, and for eight the infecting source was unknown. Therefore, PICC appears to be an important risk factor for *C. pelliculosa* infection. When an unusual pathogen is isolated from patients, particularly from immunodeficient subjects, specific attention should be paid to monitor the possibility of an outbreak.

**Author’s contributions**

Yulan Yang and Weiyuan Wu: designed the study, performed research, analyzed data, contributed new methods or models, and wrote the paper. They contributed equally to this work. Lu Ding: performed research, analyzed data, and contributed new methods or models. Lin Yang: performed research, contributed new methods or models, and wrote the paper. Jinzhen Su: performed research and contributed new methods or models. Benqing Wu: designed the study, analyzed data, contributed new methods or models, and wrote the paper.

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