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Original Article

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-birth-weight 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 (hydroxymethyl) aminomethane hydrochloride (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 BssHII (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 BssHII, 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 AlphaImager. 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	2012/11/20	46	HFNC/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	2013/07/04	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/M	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	HFNC/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; HFNC: high-flow nasal cannula; SIMV: synchronized intermittent mandatory ventilation; * broad-spectrum antibiotics used: latamoxef: cefoperazone sodium and sulbactam sodium, meropenem, and imipenem.

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

Patient	Fluconzole 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	13.68	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.

Figure 1. Electropherogram of the 14 *C. pelliculosa* isolates digested by BssHII. Groups 1-14 are the 14 *C. pelliculosa* isolates isolated by 14 patients respectively. Group 15-16 are isolated form adult intensive care unit.

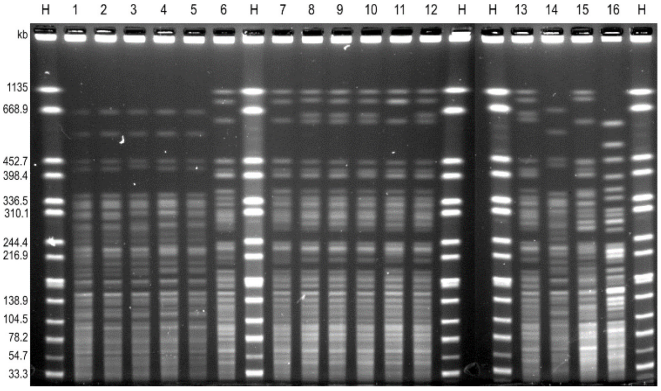

Table 3. Clinical features, treatments, and outcomes of the 14 patients with *C. pelliculosa* candidemia.

Patient	Clinical feature	Treatment			Outcome
		Antifungal drug	Dose (mg/kg)	Time (d)	
1	Fever, apnea, cyanosis	Fluconzole	12	17	Cured
2	Fever, increased heart rate	Fluconzole	12	15	Cured
3	Fever (Transient#)	Fluconzole	12	14	Cured
4	Fever, cyanosis, apnea	Fluconzole	12	21	Discharged*
5	Fever, increased heart rate	Fluconzole	12	14	Cured
6	Fever, cyanosis, hypoactivity, polypnea	Fluconzole	12	27	Cured
7	Fever, cyanosis, apnea, polypnea	Fluconzole	12	28	Cured
8	Fever, hypoactivity, poor sucking	Fluconzole	12	17	Cured
9	-	-	-	-	Cured
10	Fever, cyanosis, polypnea, hypoactivity, increased heart rate	Fluconzole	12	28	Cured
11	Fever, cyanosis, polypnea, hypoactivity, apnea	Fluconzole	12	14	Cured
12	Fever, polypnea, increased heart rate	Fluconzole	12	21	Cured
13	Fever, polypnea, apnea	Fluconzole	12	21	Cured
14	Fever, polypnea	Fluconzole	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	65.5	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* candidemia [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 indictor 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

Table 5. The results of antifungal drug susceptibility testing of the 14 isolates.

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.

References

1. Makhoul IR, Sujov P, Smolkin T, Lusky A, Reichman B (2002) Epidemiological, clinical, and microbiological characteristics of late-onset sepsis among very low birth weight infants in Israel: a national survey. Pediatrics 109: 34-39.
2. Fu J, Ding Y, Jiang Y, Mo S, Xu S, Qin P (2018) Persistent candidemia in very low birth weight neonates: risk factors and clinical significance. BMC Infect Dis 18: 558.
3. Walsh TJ, Katragkou A, Chen T, Salvatore CM, Roilides E (2019) Invasive candidiasis in infants and children: recent advances in epidemiology, diagnosis, and treatment. J Fungi (Basel) 5: 11.
4. Caggiano G, Lovero G, Giglio OD, Barbuti G, Montagna O, Laforgia N, Montagna MT (2017) Candidemia in the neonatal intensive care unit: a retrospective, observational survey and analysis of literature data. Biomed Res Internat 2017: 7901763.
5. Wang H, Xu YC, Hsueh PR (2016) Epidemiology of candidemia and antifungal susceptibility in invasive *Candida* species in the Asia-Pacific region. Future Microbiol 11: 1461-1477.
6. Sular FL, Szekely E, Cristea VC, Dobreanu M (2018) Invasive fungal infection in Romania: changing incidence and epidemiology during six years of surveillance in a tertiary hospital. Mycopathologia 183: 967-972.
7. Luzzati R, Amalfitano G, Lazzarini L, Soldani F, Bellino S, Solbiati M, Danzi MC, Vento S, Todeschini G, Vivenza C, Concia E (2000) Nosocomial candidemia in non-neutropenic patients at an Italian tertiary care hospital. Eur J Clin Microbiol Infect Dis 19: 602-607.
8. Murphy N, Buchanan CR, Damjanovic V, Whitaker R, Hart CA, Cooke RW (1986) Infection and colonisation of neonates by *Hansenula anomala*. Lancet 1: 291-293.
9. Lopez F, Martin G, Paz ML, Sanz MA (1990) *Hansenula anomala* infection in acute leukemia. Enferm Infecc Microbiol Clin 8: 363-364. [Article in Spanish]
10. Paula CR, Krebs VL, Auler ME, Ruiz LS, Matsumoto FE, Silva EH, Diniz EM, Vaz FA (2006) Nosocomial infection in newborns by *Pichia anomala* in a Brazilian intensive care unit. Med Mycol 44: 479-484.

11. da Silva CM, de Carvalho Parahym AM, Leao MP, de Oliveira NT, de Jesus Machado Amorim R, Neves RP (2013) Fungemia by *Candida pelliculosa* (*Pichia anomala*) in a neonatal intensive care unit: a possible clonal origin. Mycopathologia 175: 175-179.
12. Lin HC, Lin HY, Su BH, Ho MW, Ho CM, Lee CY, Lin MH, Hsieh HY, Lin HC, Li TC, Hwang KP, Lu JJ (2013) Reporting an outbreak of *Candida pelliculosa* fungemia in a neonatal intensive care unit. J Microbiol Immunol Infect 46: 456-462.
13. van Schalkwyk E, Iyaloo S, Naicker SD, Maphanga TG, Mpembe RS, Zulu TG, Mhlanga M, Mahlangu S, Maloba MB, Ntlemo G, Sanyane K, Mawela D, Govender NP (2018) Large outbreaks of fungal and bacterial bloodstream infections in a neonatal unit, South Africa, 2012-2016. Emerg Infect Dis 24: 1204-1212.
14. Chan AW, Cartwright EJ, Reddy SC, Kraft CS, Wang YF (2013) *Pichia anomala* (*Candida pelliculosa*) fungemia in a patient with sickle cell disease. Mycopathologia 176: 273-277.
15. Pulcrano G, Roscetto E, Iula VD, Panellis D, Rossano F, Catania MR (2012) MALDI-TOF mass spectrometry and microsatellite markers to evaluate *Candida parapsilosis* transmission in neonatal intensive care units. Eur J Clin Microbiol Infect Dis 31: 2919-2928.
16. Chen KW, Chen YC, Lin YH, Chou HH, Li SY (2009) The molecular epidemiology of serial *Candida tropicalis* isolates from ICU patients as revealed by multilocus sequence typing and pulsed-field gel electrophoresis. Infect Genet Evol 9: 912-920.
17. Song ES, Shin JH, Jang HC, Choi MJ, Kim SH, Bougnoux ME, d'Enfert C, Choi YY (2014) Multilocus sequence typing for the analysis of clonality among *Candida albicans* strains from a neonatal intensive care unit. Med Mycol 52: 653-658.
18. Chou HH, Chiou CS, Chen KW, Li SY (2007) Suitable restriction enzymes for pulsed-field gel electrophoresis analysis of Candida tropicalis. Diagn Microbiol Infect Dis 57: 451-454.
19. Heo SM, Sung RS, Scannapieco FA, Haase EM (2011) Genetic relationships between *Candida albicans* strains isolated from dental plaque, trachea, and bronchoalveolar lavage fluid from mechanically ventilated intensive care unit patients. J Oral Microbiol 3: 6362.
20. Wynn JL, Wong HR (2017) Pathophysiology of neonatal sepsis. In Polin RA, Abman SH, Rowitch DH, Benitz WE, Fox WW, editors. Fetal and neonatal physiology. Fifth edition, Philadelphina, PA: Elsevier. 1536–1552.
21. Jang SJ, Han HL, Lee SH, Ryu SY, Chaulagain BP, Moon YL, Kim DH, Jeong OY, Shin JH, Moon DS, Park YJ (2005) PFGE-based epidemiological study of an outbreak of *Candida tropicalis candiduria*: the importance of medical waste as a reservoir of nosocomial infection. Jpn J Infect Dis 58: 263-267.
22. Qi L, Fan W, Xia X, Yao L, Liu L, Zhao H, Kong X, Liu J (2018) Nosocomial outbreak of *Candida parapsilosis sensu stricto* fungaemia in a neonatal intensive care unit in China. J Hosp Infect 100: e 246-e252.
23. Govender NP, Patel J, Magobo RE, Naicker S, Wadula J, Whitelaw A, Coovadia Y, Kularatne R, Govind C, Lockhart SR (2016) Emergence of azole-resistant *Candida parapsilosis* causing bloodstream infection: results from laboratory-based sentinel surveillance in South Africa. J Antimicrob Chemother 71: 1994-2004.
24. Ben Abdeljelil J, Saghrouni F, Khammari I, Gheith S, Fathallah A, Ben Said M, Boukadida J (2012) Investigation of a cluster of Candida albicans invasive Candidiasis in a neonatal intensive care unit by pulsed-field gel electrophoresis. Scientific World Journal 2012: 138989.
25. Mujdeci G, Arevalo-Villena M (2018) Yeast identification during fermentation of turkish gemlik olives. J Food Sci 83: 1321-1325.
26. Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ, Jones RN (2004) In vitro susceptibilities of rare *Candida* bloodstream isolates to ravuconazole and three comparative antifungal agents. Diagn Microbiol Infect Dis 48: 101-105.
27. Pfaller MA, Castanheira M, Messer SA, Jones RN (2015) In vitro antifungal susceptibilities of isolates of *Candida* spp. and *Aspergillus* spp. from China to nine systemically active antifungal agents: data from the SENTRY antifungal surveillance program, 2010 through 2012. Mycoses 58: 209-214.
28. Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ, Jones RN (2003) In vitro activities of voriconazole, posaconazole, and four licensed systemic antifungal agents against *Candida* species infrequently isolated from blood. J Clin Microbiol 41: 78-83.
29. Citak S, Ozcelik B, Cesur S, Abbasoglu U (2005) In vitro susceptibility of *Candida* species isolated from blood culture to some antifungal agents. Jpn J Infect Dis 58: 44-46.
30. Barchiesi F, Tortorano AM, Di Francesco LF, Rigoni A, Giacometti A, Spreghini E, Scalise G, Viviani MA (2005) Genotypic variation and antifungal susceptibilities of *Candida pelliculosa* clinical isolates. J Med Microbiol 54: 279-285.
31. Kliasova GA, Blokhina EV, Gracheva AN, Kravchenko SK, Parovichnikova EN, Galstian GM (2013) Results of treatment for candidemia in patients with blood system tumors. Ter Arkh 85: 47-53. [Article in Russian]
32. Inigo M, Del Pozo JL (2018) Fungal biofilms: from bench to bedside. Rev Esp Quimioter 31 Suppl 1: 35-38.

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