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ORIGINAL ARTICLE

Reporting an outbreak of *Candida pelliculosa* fungemia in a neonatal intensive care unit

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Background: Fungemia in preterm infants is associated with high mortality and morbidity. This study reports an outbreak of unusual fungemia in a tertiary neonatal intensive care unit (NICU).
Methods: Ten *Candida pelliculosa* bloodstream isolates were identified from six infants hospitalized in the NICU from February to March 2009. Environmental study was performed, and genetic relatedness among the 10 clinical isolates of *C pelliculosa* and six control *C pelliculosa* strains was characterized by randomly amplified polymorphic DNA assay. *In vitro*

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susceptibility of isolates to six antifungal agents was analyzed by broth microdilution method. Amphotericin B was given to infected infants and prophylactic fluconazole was prescribed to the other noninfected extremely low birth weight infants during the outbreak.
Results: Thrombocytopenia (platelet counts $<100 \times 10^9$/L) was the early laboratory finding in four infants. One of six patients died, making overall mortality 17%. Fluconazole, voriconazole, amphotericin B, and micafungin provided good antifungal activity. Cultures from the environment and hands of caregivers were all negative. Molecular studies indicated the outbreak as caused by a single strain. The outbreak was controlled by strict hand washing, cohort infected patients, confined physicians and nurses to take care of patients, prophylactic fluconazole to uninfected neonates, and proper management of human milk.
Conclusion: The study demonstrated the clinical importance of emerged non-*albicans Candida* species in NICU. For unusual pathogen isolated from immunocompromised hosts, more attention should be paid to monitor the possibility of an outbreak.

Introduction

Invasive fungal infection is the third most common cause of late-onset sepsis in very low birth weight (VLBW) infants,[1] a critical issue in neonatology over the last 20 years. *Candida* spp. account for most invasive fungal infection in VLBW infants in the neonatal intensive care unit (NICU) and are associated with high mortality rate, significant morbidity, prolonged hospital course, and neurodevelopmental impairment in later life.[1–3] Risk factors related to invasive *Candida* infection include low birth weight and gestational age, broad-spectrum antibiotics, presence of central vascular access, prolonged total parenteral nutrition, and colonization of *Candida* spp. on skin and mucosal surfaces.[2,3] *Candida albicans* and *C parapsilosis* account for most of invasive fungal infection[4,5]; recent studies in this area aim at preventing colonization by *Candida* species, using prophylactic antimicrobials in VLBW infants.[6]

Increasing numbers of reports associate nosocomial fungal infections with unusual *Candida* species. *Candida pelliculosa*, also known as *Hansenula anomala*, a yeast mainly found in plants, fruits, and oil, was first described as causative in an NICU outbreak of fungemia in 1986,[7] with sporadic events related to immunocompromised patients[8–11] and outbreaks[12–16] reported since. This study probes such an outbreak caused by this rare pathogen, *C pelliculosa*, in a tertiary NICU of China Medical University Hospital (CMUH) in Taiwan.

Methods

Hospital

CMUH is a 1700-bed tertiary-care teaching hospital located in central Taiwan. Its NICU, with a capacity of 30 beds, provides tertiary care for critical newborns; most are premature infants or those with respiratory distress.

Definitions and study design

Cases were defined as those patients with at least one blood culture positive for *C pelliculosa*. Neonates were defined as those patients aged 28 days or younger at the onset of fungemia. Preterm birth was defined as birth that occurred before 37 weeks of gestation. Medical records were reviewed regarding demographic and clinical data for assessing potential risk factors of fungus infection: e.g., younger gestational age, low birth weight, previous use of broad-spectrum antibiotics, peripherally inserted central catheter (PICC), endotracheal tube (ETT), nasal-continuous positive airway pressure (N-CPAP), usage of total parenteral nutrition (TPN). Amphotericin B was given intravenously to all infected infants, with prophylactic fluconazole given intravenously to the remaining noninfected extremely low birth weight infants during this outbreak.

Outbreak investigation

From February 4 to March 19, 2009, six preterm infants had documented *C pelliculosa* fungemia. Time span between identification of first and second patients was 24 days. Epidemiological investigation proceeded immediately on March 14, 2009, when a third case was confirmed 33 days after the first. Multiple surveillance surface cultures were performed on infected and noninfected neonates. We collected one of each culture sample from the mouth and anus of VLBW infants hospitalized from March 14 to March 31, 2009, during the outbreak. Environmental samples were obtained from patients' bedding, incubators, sinks, bottle warmers, tops of trolleys, air ducts, respiratory care equipment, Hibisol used for cleaning hands in the NICU, alcohol used for sterile performance, outer and inner surface of the refrigerator for storing breast milk, and 14 samples from breast milk fed to infants during this period. A total of 62 swabs were collected from inanimate environmental sources. Hand samples of NICU staff members (three physicians and 26 nurses) were obtained for culture.

Microbiological investigation

During the outbreak, all patients with sepsis had at least one blood culture obtained for examination. Blood samples were drawn from a peripheral vein using aseptic techniques and cultures were performed with automated Bactec 9240

(Becton Dickinson, Cockeysville, MD, USA). All 10 strains of *C pelliculosa* from six cases were confirmed according to API-32C and Mini API system.

Antifungal susceptibility testing

Antifungal agents tested were fluconazole (Pfizer, Inc., New York, NY, USA), itraconazole (Janssen Pharmaceuticals, Titusville, NJ, USA), voriconazole (Pfizer, Inc.), amphotericin B (Sigma, St Louis, MO, USA), caspofungin (Merck & Co., Inc., Whitehouse Station, NJ, USA) and micafungin (Astellas Pharma, Inc., Deerfield, IL, USA). Susceptibility testing of all clinical isolates was conducted via broth microdilution method described by the Clinical and Laboratory Standards Institute (CLSI).[17] All minimum inhibitory concentrations (MICs) were determined spectrophotometrically (530 nm) after incubation for 48 hours. To categorize isolates as susceptible, CLSI interpretive criteria were employed for fluconazole (MICs of 8 μg/mL), itraconazole (MICs of 0.125 μg/mL), voriconazole (MICs of 1 μg/mL), caspofungin (MICs of 2 μg/mL), and micafungin (MICs of 2 μg/mL). A breakpoint of MICs 1 μg/mL was assumed for amphotericin B based on pharmacokinetic data.[18,19] *Candida krusei* ATCC 6258, *C parapsilosis* ATCC 22019 and *C albicans* ATCC 90028 served as quality control strains.[20]

Molecular investigation

Genetic relatedness among 10 clinical isolates from six patients obtained during the outbreak, as well as control organisms (six *C pelliculosa* strains obtained from six patients with fungemia from the burn unit and emergency room during 2008), were characterized by randomly amplified polymorphic DNA (RAPD) assay.[21]

DNA extraction

Isolates were grown on BAP agar plates (BBL Microbiology Systems, Becton Dickinson). Subsequently, 3 to 5 colony growth loops were suspended in 600 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), then microcentrifuged briefly. DNA was extracted with Genomic DNA Mini Kits (Geneaid, Shijr City, Taiwan).

RAPD

This method has been described previously.[21] The amplification reaction contained 1 U of *Taq* polymerase (Finnzyme, Espoo, Finland), 200 mM (each) deoxynucleoside triphosphates (Viogene, Taiwan), and 0.2 mM (each) primer in reaction buffer (1.5 mM $MgCl_2$ and 50 mM KCl in 10 mM Tris-HCl, pH 8.3, 20 μL reaction volume). Five μL of pure extraction DNA were added to 70-μL aliquots of PCR mixture. Sequences of the RAPD primer were 5′-GCA-TATTCTCAG-3′. After initial denaturation at 94°C for 5 minutes, reaction mixture was run through 5 cycles of denaturation at 95°C for 45 seconds, annealing at 25°C for 45 seconds, and extension at 72°C for 1 minute, and 35 cycles of denaturation at 95°C for 45 seconds, annealing at 45°C for 45 seconds, and extension at 72°C for 1 minute. Reaction mixture was heated at 72°C for 3 minutes, then cooled to 4°C. Five μL of each PCR product mixture were analyzed by gel electrophoresis on a 1% (wt/vol) agarose I gel (BBI Biotech Research Laboratories, Inc., Gaithersburg, MD, USA) in TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA electrophoresis buffer, pH 8.0) at 100 V for 30 minutes. Gels contained 50 ng of ethidium bromide per mL.

Results

Description of epidemic and characteristics of patients

The first case of *C pelliculos*a fungemia was a 26-day-old premature infant (Table 1). Hospital course before fungemia was complicated with *Klebsiella pneumoniae* sepsis; the patient received broad spectrum antibiotics (piperacillin/tazobactam and teicoplanin) for two weeks. Three days later, the patient was noted having increased frequency of apneic episode on his 26th day of life when using N-CPAP for apnea of prematurity and treated with meropenem trihydrate initial (sepsis was suspected). The antibiotic was later switched to amphotericin B after a blood culture, available seven days after onset of frequent apnea, yielded *C pelliculosa*. Upon these findings, PICC was removed immediately.

Time span between identification of first and second patients was 24 days. An epidemiological investigation was conducted immediately after the third patient was identified, 33 days after the first. The total number of patients admitted during this period (February 4 to March 19) and treated in NICU for at least 48 hours was 54. Six patients were diagnosed with *C pelliculosa* fungemia within 6 weeks, five of them in the last 3 weeks of the outbreak. Table 1 records their demographic data and characteristics. Median (range) gestational age and birth weight of these infants were 28 (23 to 31) weeks and 1180 (700 to 1600) g, respectively. Age at onset of fungemia ranged from 11 to 36 days. All six infants presented nonspecific sign as increased frequency of apneic episodes; thrombocytopenia (platelet counts $<100 \times 10^9$/L) was the early laboratory finding in four. Precipitating risk factors of fungemia included PICC, N-CPAP, use of broad-spectrum antibiotics and TPN. These patients received amphotericin B (1 mg/kg/day) treatment for at least 14 days. Concomitant bacteremia occurred in two patients, one due to *K pneumoniae* and the other attributed to *Burkholderia cepacia*. Both survived. A patient with gestational age of 23 weeks (Table 1) died of septic shock and pulmonary interstitial emphysema, making overall mortality rate 17%. The rest had no complications upon ophthalmologic or central nervous system assessments.

Environmental investigation

Surveillance cultures from the environment (62 samples) and hands of caregivers (29 samples) were all negative; while one culture from a patient's maternal breast milk stored in the refrigerator yielded *Candida* spp. Cultures from mouth and anus of neonates with or without fungemia yielded no *C pelliculosa* during this period. Blood cultures

Table 1 Demographic and clinical characteristics of six premature patients with *Candida pelliculosa* fungemia

Patient	GA (wk)/sex	BW (g)	Date of blood culture sampling (d/mo/y)	Age at the time of fungemia detected (d)	Presence of thrombocytopenia[c] at the time of fungemia	Broad spectrum antibiotics use before fungemia	Invasive procedures	Treatment	Concomitant bacteremia	Outcome
1[a]	29/M	1200	04/02/09 11/02/09	27	Yes	Yes[d]	PICC, N-CPAP	AmB	*Klebsiella pneumoniae*	Cured
2	28/F	1160	28/02/09 03/03/09 11/03/09	11	Yes	No	PICC	AmB		Cured
3[b]	23/M	700	01/03/09 12/03/09	36	Yes	Yes[e]	PICC, ETT	Fluconazole, AmB		Died
4	25/M	1020	09/03/09 14/03/09	12	No	No	N-CPAP	AmB	*Burkholderia cepacia*	Cured
5	28/F	1295	18/03/09	12	Yes	No	N-CPAP	AmB		Cured
6	31/F	1600	19/03/09 22/03/09	15	No	No	N-CPAP	AmB		Cured

[a] Other risk factor: intravenous amino acid use.
[b] Other risk factors: skin wound and intravenous lipid use.
[c] Thrombocytopenia, defined as platelet count $<100 \times 10^9$/L.
[d] Piperacillin/tazobactam, teicoplanin, meropenem trihydrate were used previously for *Klebsiella pneumoniae* sepsis.
[e] Piperacillin/tazobactam and teicoplanin were used for previously clinical sepsis.
AmB = amphotericin B; BW = birth weight; ETT = endotracheal tube; F = female; GA = gestational age; M = male; N-CPAP = nasal-continuous positive airway pressure; PICC = peripherally inserted central catheter.

Table 2 Results of antifungal drug susceptibility testing of *C pelliculosa* isolates from six patients

Strain from patient	Fluconazole	Itraconazole	Voriconazole	Amphotericin B	Caspofungin	Micafungin
1	2	2	0.125	2	2	0.5
2	2	2	0.125	1	2	0.25
3	2	2	0.125	1	2	0.5
4-1	2	2	0.125	1	2	0.25
4-2	2	2	0.125	1	2	0.5
5	4	2	0.125	1	2	0.25
6-1	2	2	0.125	1	2	0.25
6-2	2	2	0.125	2	2	0.5

Minimum inhibitory concentrations (MICs, mg/L) of different drugs after 48 hours incubation at 35°C.

from other infants in NICU who received prophylactic fluconazole during the outbreak were all negative.

Intervention

In order to prevent spread of infection, we isolated all infected patients together in one section of the facility; these were cared for by specified medical staffs (nurses and one resident). Following the positive culture of *Candida spp*. from one sample of human milk stored in our refrigerator (though not *C pelliculosa*) all human milk was discarded and no longer provided to patients. Furthermore, we modified the standard operating procedures of handling human milk provided to patients. All medical staffers were reminded of the importance of strict hand washing. There have been no new cases of *Candida* infection since March 24, 2009.

Antifungal susceptibility testing

Table 2 plots *in vitro* susceptibility. Amphotericin B MICs of six *C pelliculosa* isolates were 1 μg/mL; two isolates were considered resistant to the drug with MIC of 2 μg/mL. Fluconazole, voriconazole, and micafungin showed good antifungal activity with MICs of 2 to 4 μg/mL, <0.125 μg/mL, and 0.25 to 0.5 μg/mL, respectively. However, low susceptibility to itraconazole (MICs 2 μg/mL) was observed.

Molecular studies

Genetic relatedness among clinical isolates was analyzed by RAPD assay. Fig. 1 illustrates RAPD banding patterns of 16 clinical isolates of *C pelliculosa*: 10 clinical strains taken during the outbreak (all from blood cultures of different patients) and six control strains acquired from patients visiting the emergency room (five strains) and burn unit (one strain) in 2008. RAPD patterns of 10 isolates from the six patients were identical to each other but different from controls (Fig. 1).

Discussion

Despite significant improvement in neonatal care, nosocomial fungemia due to *Candida* spp. carries high mortality (10.2% to 43%) among VLBW infants.[3,22,23] Beyond common well-known *Candida* spp. that cause invasive fungal infection, such as *C albicans* and *C parapsilosis*, the clinical importance of unusual pathogens is rising, as reported in recent publications.[24] *Candida pelliculosa*, also named *Hansenula anomala* or *Pichia anomala* in former reports, is a rare pathogen causing fungemia recently gaining importance as an opportunistic pathogen.[7,12,14,15] *C pelliculosa* usually grows in contaminants from industrial or pilot plant fermentations, soil, stored grain, lakes or streams, and fermenting fruit; neonatal and surgical intensive care units

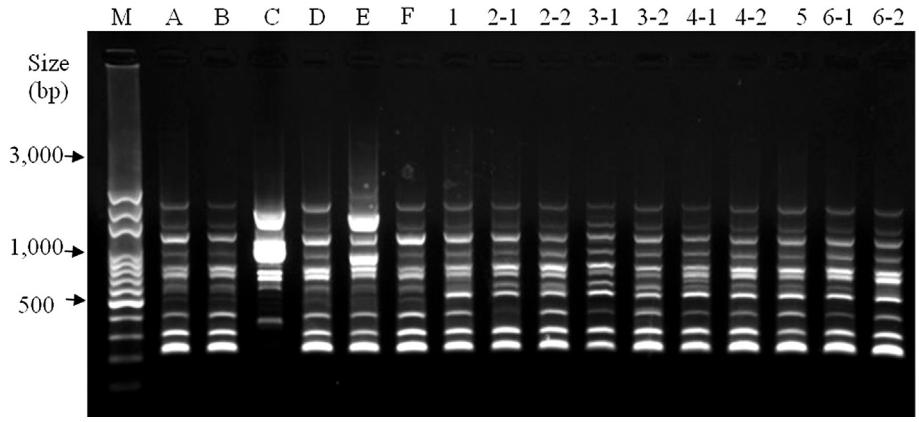

Figure 1. Agarose gel shows amplified DNA products generating in randomly amplified polymorphic DNA (RAPD) analysis of *Candida pelliculosa* strains. Profiles in Lanes A—F are control strains; Lanes 1 to 6-2 correspond to strains isolated during the outbreak. Lane M represents molecular weight size reference markers (100-bp ladder). RAPD patterns of ten isolates from six patients were identical to each other but different from most control strains.

have seen prior outbreaks.[12,14–16] Published reports aim at identifying different strains and susceptibility to antifungal drugs of this yeast due to its rising clinical importance.[25,26]

The first identified patient in this outbreak was a 26-day-old premature male noted as having increased frequency in apneic episodes. He was placed on N-CPAP for respiratory support and PICC. It was thought a sporadic event; no investigation was initiated until the third case was identified 33 days after the first. All affected patients were VLBW infants with common risk factors; one received an ETT with ventilator and three PICC when *C pelliculosa* fungemia was confirmed (Table 1). Two cases used broad-spectrum antibiotics before fungemia developed.

Despite thorough investigation, we failed to trace the source of infection. Most literature reports were not able to identify the source of the infection,[14–16,27] except for one in which *C pelliculosa* was isolated from a physician's hands.[12] Earlier reports identified *C pelliculosa* fungemia in patients with severe diarrhea,[28] acute pancreatitis,[29] receiving an operation for duodenal perforation,[30] as well as infants undergoing abdominal surgery due to congenital anomaly like omphalocele[31] and gastric defect.[13] Therefore, route of infection might be via the gastrointestinal tract through contaminated food.

Patients 4, 5, and 6 (Table 1) had risk factors for invasive fungal infection (VLBW infant with N-CPAP), yet no intravenous route of infection when fungemia was noted. Hence we suggest that patients acquired *C pelliculosa* fungemia through damaged mucosa (e.g., N-CPAP and ETT). The six patients were separated into four different sections of the NICU once fungemia was identified. This indicates that the route of transmission involves most healthcare givers and something that might affect most NICU patients. A long period (33 days) between the first and third cases also suggests the possibility that *C pelliculosa* had emerged from a common source in NICU.

Murphy et al reported a *C pelliculosa* fungemia outbreak in Liverpool that was not eliminated until oral nystatin prophylaxis and topical iodophor to the venipuncture site were implemented.[7] Antifungal prophylaxis with intravenous fluconazole (6 mg/kg/day) has been proven to reduce *Candida* colonization and the rate of invasive candidiasis in VLBW infants.[3] During this outbreak, we prescribed prophylactic fluconazole (6 mg/kg/dose, intravenous, every other day), as suggested by previous reports to every VLBW infant admitting to NICU; this seemed to be an effective method when dealing with such an unusual pathogen. After prophylactic fluconazole use, no further *C pelliculosa* fungemia or colonization were detected among other noninfected patients during the outbreak.

All *C pelliculosa* strains from our patients showed good susceptibility to fluconazole, voriconazole, and micafungin, but not to itraconazole. These results are similar to those of Ribeiro da Matta et al,[25] who reported *in vitro* susceptibility of 58 *P anomala* isolates to five antifungal drugs, plus low susceptibility to itraconazole. Fluconazole, voriconazole, amphotericin B, and caspofungin showed favorable antifungal activity,[25] most *C pelliculosa* fungemia patients treated effectively with amphotericin B with or without 5-flucytosine or fluconazole.[10–13,16] Barchiesi et al reported 46 *C pelliculosa* strains isolated from 37 patients as susceptible *in vitro* to amphotericin B[32]; all but one of our patients were also treated successfully with it. The fatal patient's isolate was sensitive to amphotericin B with MIC of 1 μg/mL; those with MIC 2 μg/mL isolates survived. There is still no consensus on breakpoints for amphotericin B therapy in *C pelliculosa* fungemia, although amphotericin B seems optimal for such infections.[32] Breakthrough fungemias have been reported in immunocompromised patients receiving prophylaxis with fluconazole.[33] With limited data on voriconazole and micafungin treatment of *C pelliculosa* infection, our study revealed promising results with these agents in treating *C pelliculosa* fungemia in neonates. A recent study showed that micafungin is tolerated in young infants with adequate antimicrobial coverage of central nervous system caused by candida infection.[34]

This report highlights clinical importance of emerging non-*albicans Candida* species in the NICU. When dealing with this unusual pathogen, unfamiliarity with it impedes identification of the source and control of transmission. Fungal infections in neonates presented various symptoms; we were forced to treat fungemia empirically, based on clinical suspicion and risk factors. All six infants manifested nonspecific clinical signs, particularly apnea; thrombocytopenia was the early laboratory finding in four of the infected infants. In VLBW infants, fungal and Gram-negative pathogens are associated with lower platelet count and prolonged thrombocytopenia compared with Gram-positive pathogens.[35]

One of the six infected infants died 12 days after onset of fungemia, and the other five had normal neurologic findings at discharge. Murphy et al reported the first outbreak in 1986, and two of eight preterm babies with gestational age 24 to 31 weeks died.[7] An outbreak in a nursery reported four neonates with birth weight 1300 to 4010 g, all improving after amphotericin B treatment with or without flucytosine.[15] A mortality rate of 41.2% was reported in an outbreak at a Brazilian pediatric intensive care unit.[16] This opportunistic pathogen usually infects immunocompromised patients (including preterm infants) and might cause high mortality without prompt treatment.

Tracing the outbreak history, the first episode of *C pelliculosa* fungemia occurred 24 and 25 days before the second and third cases. Our probe started after the third case appeared. Molecular study (RAPD) shows the same pattern, hinting that it could originate from the same pathogen. The limitation of this study was that we could not determine infection source, although one human milk sample yielded *Candida* spp. This outbreak was controlled after applying infection control measures: strict hand washing, prompt handling of human milk, and isolating infected patients. Additionally, reassigning physician and nurses caring for these patients is critical to outbreak control. When an unusual pathogen was isolated from inpatients, especially from immunocompromised hosts, more attention should be paid to monitor the possibility of an outbreak.

Conflicts of interest

All authors declare that they have no conflicts of interest relevant to this article.

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