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Clinical and molecular characteristics of bloodstream infections caused by *Candida albicans* in children from 2003 to 2011

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Abstract

We investigated the clinical and molecular characteristics of *Candida albicans* bloodstream infection (BSI) in children from a tertiary-level medical centre in Taiwan over a 9-year period from January 2003 to December 2011. We performed multilocus sequence typing (MLST) to investigate the genetic relatedness of these *C. albicans* BSI isolates. A total of 79 episodes of *C. albicans* BSI in 76 paediatric patients were identified, including 41 (51.9%) from the paediatric intensive care unit, 24 (30.4%) from the neonatal intensive care unit and 14 (17.7%) from general wards. More than half (59.5%) of these patients had underlying chronic co-morbidities, and the majority (94.9%) had a catheter or some other artificial device. All the isolates were susceptible to the antifungal agents tested. Only 32.9% (26/79) received effective antifungal agents within 24 h of onset of candidaemia. Twenty-five (31.6%) patients had persistent candidaemia (>3 days after the start of antifungal treatment) and candidaemia-attributable mortality rate was 22.8% (18/79). The 72 isolates available for MLST yielded 53 unique diploid sequence types (DSTs). Forty-five DSTs were singletons and eight DSTs were shared by 27 (37.5%) isolates. Seventy-one (98.6%) isolates were clustered within previously known clades. Based on the definition of two or more strains with shared DST occurring within a period of 90 days, 10.1% of the infections were categorized as nosocomial clusters, most commonly identified in the intensive care units. Although cluster-associated candidaemia was not associated with a higher mortality rate, none of the clusters were identified by the hospital infection control team.

Keywords: Antifungal resistance, bloodstream infection, *Candida albicans*, candidaemia, children, mortality, multilocus sequence typing
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Introduction

Candida species are opportunistic pathogens that can cause nosocomial infections in immunocompromised patients. The mortality rate attributable to invasive candidiasis ranges from 19.3% to 40% [1–3]. Candidaemia has emerged as an important infection control issue [3,4], because it contributes significantly to morbidity, and increases length of hospital stay and medical costs [5–7]. In children, bloodstream infections (BSIs) caused

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by *Candida* mainly occur in paediatric and neonatal intensive care units (PICUs and NICUs, respectively) or in patients with underlying haematological/oncological malignancies, with *Candida albicans* being the most common cause of *Candida* infections [8–10]. Risk factors for children with candidaemia include intravenous catheter placement with parenteral nutrition, abdominal surgery, steroid usage and exposure to broad-spectrum antibiotics [11–15].

Although *C. albicans* is less likely to have antifungal resistance when compared with non-albicans *Candida* spp. [15,16], it has a comparable mortality rate [2] and is occasionally associated with clusters of nosocomial infection [17,18]. Identification of relatedness between *Candida* species by using molecular techniques with high discriminatory power is essential for setting up infection control measures to reduce nosocomial candidiasis [19,20]. However, information focused on *C. albicans* BSIs in children, particularly regarding the clinical data and molecular epidemiology, is limited. The recently developed multilocus sequence typing (MLST) method for *C. albicans* has proved to be a highly discriminatory tool for molecular epidemiology [21]. In this study, we aimed to characterize the clinical features and performed MLST to analyse the genetic relatedness among paediatric BSI isolates of *C. albicans* from a teaching hospital in Taiwan over a 9-year period.

Materials and methods

Setting and patient population

This study was conducted in the paediatric department of Chang Gung Memorial Hospital (CGMH), a tertiary-level university-affiliated hospital in northern Taiwan. The paediatric department of CGMH is in a separate, 12-storey building with four NICUs, two PICUs, and seven general wards. One of them is specialized as a paediatric haematology/oncology division (sixth floor), and there is a paediatric stem cell/bone marrow transplantation unit. The total capacity of the paediatric department of CGMH is 353 beds. All paediatric and neonatal patients with BSIs caused by *C. albicans* that occurred between January 2003 and December 2011 were enrolled in this study. Except for multiple hospitalizations, each case patient was included in the study only once, at the time of the first positive blood culture for *C. albicans*. During the study period, no nosocomial clusters of candidaemia were reported by the hospital infection control team in any of the wards or ICUs in CGMH. This study was approved by the institutional review board of CGMH, with a waiver of informed consent because all the patient records and information were anonymized and de-identified before analysis.

Data collection and definitions

The clinical information was from review of medical charts and included age, sex, the length of the hospital stay before candidaemia, underlying diseases, history of immunosuppressive therapy, exposure to antimicrobial agents, and surgery within the previous 30 days, underlying diseases, neutropenia and the presence of an intravenous catheter or any other artificial device at the time of candidaemia. The clinical manifestations at the time of blood culture collection, ICU admission and the antimicrobial regimens used were also collected.

An episode of *C. albicans* candidaemia was defined as at least one blood culture positive for *C. albicans* obtained from patients with compatible clinical signs or symptoms [22]. The empirical therapy was considered to be appropriate if an active antimicrobial agent *in vitro* was administered at the usual recommended doses within the first 24 h of blood culture obtained. Persistent candidaemia was defined as repeated positive blood cultures for *C. albicans* for more than 3 days of antifungal agents. Candidaemia-attributable mortality was defined as patients who died within 7 days after onset of candidaemia or in the presence of persistent clinical sepsis or persistent candidaemia, or those who died of candidaemia-associated complications [23,24]. Breakthrough candidaemia was defined as new occurrence of candidaemia while the patient was on antifungal prophylaxis [25]. To evaluate the proportion of infections caused by clustered isolates, a nosocomial cluster was defined as identification of genetically closely related isolates from two or more patients within a period of 90 days.

Candida albicans BSI isolate collection and MLST analysis

In the Department of Paediatrics of CGMH, a fungus culture was obtained from a peripheral blood vessel, or through central venous access in the PICU when patients had clinical symptoms/signs of sepsis and clinical fungaemia was suspected by the attending physicians. The identification of isolates to species level was based on colony morphology on CHROMagar Candida (BBL, Becton Dickinson, Sparks, MD) at 35°C, microscopic morphology on cornmeal-Tween 80 agar, and a commercially available biochemical identification system (API 20C (bioMérieux, Marcy L'Etoile, France) or the Vitek 2 system Vitek 2 ID-YST (bioMérieux)). All the *C. albicans* BSI isolates were submitted to genotyping.

Multilocus sequence typing for *C. albicans* was performed according to the method developed by Bougnoux et al. [26,27], which was based on the variations of seven housekeeping gene loci, including *AAT1a*, *ACC1*, *VPS13*, *MPIb*, *ADP1*, *ZWF1b* and *SYA1*. The internal regions of these genes were amplified by PCR and sequenced. Each allele's nucleotide sequence was assigned an allele number on the MLST website (http://

calbicans.mlst.net/), and then the combination of the seven allele numbers defined a unique diploid sequence type (DST), which represents its genotype. A dendrogram was constructed with the use of Un-weighted Pair Group Method with Arithmetic mean (UPGMA) by Molecular Evolutionary Genetics Analysis version 6.0 (MEGA6). The numbered clades of *C. albicans* were described as previously [28]. Numbers at nodal points indicate bootstrap values (%) for 1000 replications.

Putative relatedness between isolates was determined by eBURST (http://eburst.mlst.net/). The eBURST algorithm was used for not only placing all related isolates into clonal complexes but also for predicting the ancestral DST of each complex. The results of eBURST displayed the most parsimonious patterns of each descent from the ancestral DST type.

Antifungal susceptibility testing

Susceptibility of the *C. albicans* BSI isolates to nine antifungal agents was determined by a broth microdilution method using a Sensititre YeastOne system (Trek Diagnostic Systems Ltd., East Grinstead, UK) according to the manufacturer's instructions [29,30]. The MIC was recorded as the highest concentration of antifungal agent resulting in the development of a blue colour. All Sensititre® plates include positive control wells, and *Candida krusei** ATCC® 6258 and *Candida parapsilosis* ATCC® 22019 were used as the quality control strains. Fluconazole, itraconazole, voriconazole, posaconazole, micafungin, caspofungin, anidulafungin, 5-flucytosine and amphotericin B were prepared according to the CLSI methods. The criteria for susceptibility of *C. albicans* BSI isolates to nine antifungal agents were based on MIC breakpoints of *Candida* spp. recommended by the CLSI [31].

Statistical analysis

For clinical characteristics, we described the differences between non-neonatal candidaemia in children and neonatal candidaemia. Statistical significance for unadjusted comparisons was determined by the chi-squared or Fisher exact test for categorical data, and Student's *t* test or the Wilcoxon/Mann–Whitney test for continuous variables. Tests were two-sided and a p value < 0.05 was considered significant. All statistics were performed using the commercially available software SPSS 16.0 for Windows (SPSS®, Chicago, IL, USA).

Results

Clinical characteristics of *C. albicans* BSIs

During the study period, a total of 79 episodes of *C. albicans* BSI in 76 patients were identified (three patients had two episodes of *C. albicans* BSI in two hospitalizations), including 24 (30.4%) from NICUs, 41 (51.9%) from PICUs and 14 (17.7%) from general wards. Table 1 lists the demographics, clinical manifestations and outcomes for all patients with *C. albicans* BSIs. Paediatric and neonatal patients had a length of hospital stay before *C. albicans* BSI with a median (interquartile range) of 26.5 (14.5–47.0) and 23.0 (14.5–45.5) days, respectively. However, there were two episodes of early-onset sepsis within 3 days of life in the NICU.

More than half of the case patients had underlying chronic disorders (47/79, 59.5%) (Table 1). About 40% (27 children, five neonatal patients) had multiple chronic conditions. Almost all the patients had previous exposure to antibiotics (94.9%) or a catheter or artificial device (94.9%). The majority of patients had total parenteral nutrition and/or intrafat infusion (69.6%) at onset of candidaemia, but very few cases had neutropenia (6.3%) or recent immunosuppressive therapy (7.6%) at onset of candidaemia.

Although all *C. albicans* BSI isolates were susceptible to all antifungal agents tested, only 32.9% (26/79) received effective antifungal agents within 24 h of onset of candidaemia. There were four episodes of breakthrough candidaemia for patients with antifungal prophylaxis, and 31.6% (25 episodes) had persistent candidaemia even after effective antifungal therapy. The most common antifungal agents used were fluconazole (70.8% for neonates and 65.5% for children) and amphotericin-B (29.2% and 30.9%, respectively). One patient had fulminant sepsis and died before any antifungal agent was prescribed. With antifungal therapy, 43 (54.4%) episodes responded well and all symptoms and signs resolved within 3 days; ten (12.7%) resolved at 3–7 days; and 36.7% had modification of antifungal therapy. A total of 26 (34.2%) patients died in the hospital, including six early deaths within 3 days of candidaemia and 12 deaths with progressive multi-organ failure after candidaemia. The candidaemia-attributable mortality rate was 22.8% (18/79).

Antifungal susceptibility testing

The MIC distributions and *in vitro* susceptibilities of 73 *C. albicans* isolates are summarized in Table 2. In terms of MIC_{90} values (MIC required to inhibit 90% of the isolates), micafungin and voriconazole had the lowest level of 0.008 mg/L. All isolates had an MIC of ≤ 1 mg/L to all antimicrobial agents and were considered susceptible according to the CLSI breakpoints [31].

Molecular epidemiology

A total of 72 *C. albicans* BSI isolates from 71 patients were available for MLST analysis, and 53 DSTs were identified. Twenty-six isolates (36.1%) belonged to 26 novel DSTs first identified (Table 3). Of these 53 DSTs, 45 (62.5%) were singletons, and eight were shared by 27 (37.5%) isolates. DST693 and DST1849 were the two major types with seven and five

TABLE 1. The clinical characteristics of 79 episodes of *Candida albicans* bloodstream infections in 76 paediatric patients

Characteristics	PICU (total *n* = 41)	NICU (total *n* = 24)	General wards (total *n* = 14)
Age (years for PICU and wards, and days for NICU), median (IQR)	4.0 (0.8–7.3)	21.5 (15.0–54.0)	4.0 (1.0–13.0)
Gender (male/female)	20 (48.8)/21 (51.2)	12 (50)/12 (50)	7 (50)/7 (50)
Length of hospital stay before candidaemia (days), median (IQR)	29.0 (15.5-53.0)	23.0 (14.5–45.5)	24.0 (13.0–41.0)
Underlying chronic conditions[a]			
Haematological/Oncological malignancy	2 (4.9)	0 (0)	4 (28.6)
Cardiopulmonary disorder	6 (14.6)	3 (12.5)	0 (0)
Neurological disorder, congenital or acquired	15 (36.6)	2 (8.3)	2 (14.3)
Gastrointestinal disorder	16 (39.0)	5 (20.8)	6 (42.9)
Metabolic	8 (19.5)	1 (4.2)	2 (14.3)
Congenital anomalies	3 (7.3)	0 (0)	1 (7.1)
Neutropenia	3 (7.3)	0 (0)	2 (14.3)
Previous antibiotics exposure[b]	38 (92.7)	23 (95.8)	14 (100)
Previous bacteraemia[b]	7 (17.1)	7 (29.2)	6 (42.9)
Use of immunosuppressive agents[b]	3 (7.3)	0 (0)	3 (21.4)
Under antifungal prophylaxis	2 (4.9)	0 (0)	2 (14.3)
Surgery[b]	9 (22.0)	4 (16.7)	2 (14.3)
Intravascular catheterization	33 (80.5)	22 (91.7)	11 (78.6)
Artificial devices other than intravascular catheterization	13 (31.7)	14 (58.3)	6 (42.9)
Use of total parenteral nutrition and/or intrafat	26 (63.4)	20 (83.3)	9 (64.3)
Clinical manifestations			
Severe sepsis and/or septic shock	13 (31.7)	4 (16.7)	2 (14.3)
Disseminated intravascular coagulopathy	7 (17.1)	3 (12.5)	1 (7.1)
Breakthrough candidaemia/persistent candidaemia	2 (4.9)/15 (36.6)	0 (0)/6 (25.0)	2 (14.3)/4 (28.6)
Treatment and outcomes			
Appropriate empirical antifungal therapy within 24 hours	10 (24.4)	8 (33.3)	8 (57.1)
Duration of candidaemia (days), median (IQR)[c]	1.0 (1.0-6.0)	1.0 (1.0-3.0)	1.0 (1.0-4.0)
Complete response within 72 h	22 (53.7)	14 (58.3)	7 (50.0)
Complete response at 3–7 days after antifungal treatment	4 (9.8)	3 (12.5)	3 (21.4)
Candidaemia-attributable mortality	8 (19.5)	6 (25.0)	4 (28.6)
In-hospital mortality	12/38 (31.6)	8/24 (33.3)	6/14 (42.9)

All data were expressed as number (percentage, %), unless indicated otherwise.
IQR, interquartile range; NICU, neonatal intensive care unit; PICU, paediatric intensive care unit.
[a]Indicating the presence at onset of candidaemia; some patients had more than one underlying chronic condition or co-morbidity.
[b]Within 30 days before onset of candidaemia.
[c]After appropriate antifungal treatment.

isolates, respectively (Table 3). To assign these isolates to existing clades, we performed an analysis of these isolates together with 996 DSTs (from ST1 to ST999) retrieved from the MLST database (57.8% of all DSTs currently available in the MLST database: http://calbicans.mlst.net). Of these 72 BSI isolates, 71 (98.6%) isolates clustered within previously recognized clades (clades 1 to 18), but one (P054) (1.4%) was a singlet [19,26]. Clade 1 (22.2%) constituted the greatest proportion of the isolates, followed by clade 4 (13.9%) and clade 3 (9.7%). Table 3 presents the clade distribution of the 72 isolates stratified by the wards in CGMH over the study period. Within individual wards, one to 13 different clades were identified.

Fig. 1 shows the phylogenetic relatedness between 72 *C. albicans* BSI isolates from the paediatric department of CGMH and 996 strains retrieved from the MLST database. The 72 isolates were distributed over all clades, except clades 2 and 13, which were geographically clustered in Europe and Africa [26]. The UPGMA clades 1, 4 and 11 were most consistent during the rapid expansion of the database. The CGMH isolates were distributed unevenly, especially in clades 3, 4 and 11

TABLE 2. MIC distribution of 73 *Candida albicans* bloodstream isolates from paediatric patients

	No. of isolates with MIC (mg/L) of:										MIC (mg/L)		
	≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	Range	MIC_{50}	MIC_{90}
Candida albicans (total *n* = 73 tested)													
5-Flucytosine					55	7	3	4	4		0.06–1	0.06	0.5
Amphotericin B							7	66			0.25–0.5	0.5	0.5
Fluconazole						5	29	38	1		0.12–1	0.5	0.5
Itraconazole			15	38	20						0.015–0.06	0.03	0.06
Posaconazole		14	51	8							0.008–0.03	0.15	0.03
Voriconazole		72	1								0.008–0.015	0.008	0.008
Micafungin		72	1								0.008–0.015	0.008	0.008
Caspofungin		1	3	13	54	3					0.008–0.12	0.06	0.06
Anidulafungin			14	13	40	6					0.015–0.12	0.06	0.06

MIC_{50} and MIC_{90}, MIC required to inhibit 50% and 90% of the isolates, respectively.

TABLE 3. Multilocus sequence typing of 72 *Candida albicans* isolates collected from paediatric patients stratified by wards

Clade	No. (%) of isolates	PICU (total *n* = 39)			NICU (total *n* = 19)			Ward (total *n* = 14)						Major DST	DST (*n*)[a]
		2L1	2L2	Surgical	3L1	3L2	5L	6L	7L	8L	9L	10L	11L		
1	16 (22.2)	4	3	1	3	1	2	0	0	2	0	0	0	1849	1082, 1097, 1849 (5), 2427*, 2513*, 2516*, 2519*, 2521*, 2522*, 2524*, 2527*, 2528*
3	7 (9.7)	2	1	0	1	0	1	1	0	0	1	0	0	693	693 (7)
4	10 (13.9)	3	0	2	4	0	0	0	0	0	1	0	0	659	659 (3), 1068, 1069, 1612, 1613, 1977, 2520*, 2524*
5	3 (4.2)	0	2	0	0	1	0	0	0	0	0	0	0	—	2511*, 2514*, 2534*
6	3 (4.2)	1	0	1	1	0	0	0	0	0	0	0	0	—	1889, 2515*,2525*
7	1 (1.4)	0	0	1	0	0	0	0	0	0	0	0	0	—	811
8	5 (6.9)	3	1	0	0	0	0	0	0	0	0	1	0	365	365 (3), 1895, 2533*
9	4 (5.6)	1	2	0	0	0	1	0	0	0	0	0	0	—	1371, 2518*, 2526*, 2530*
10	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0	0	—	609
11	3 (4.2)	2	0	0	0	0	1	0	0	0	0	0	0	—	461, 569, 1751
12	3 (4.2)	1	0	0	1	0	0	1	0	0	0	0	0	—	601, 719, 1752
14	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0	0	—	2517*
15	1 (1.4)	0	0	0	0	1	0	0	0	0	0	0	0	—	2532*
16	5 (6.9)	1	1	0	0	0	0	1	0	0	1	1	0	669, 1757	669 (2), 1723, 1757 (2)
17	4 (5.6)	1	0	1	0	0	0	1	1	0	0	0	0	443	443 (3), 2531*
18	4 (5.6)	1	1	0	0	0	0	0	0	0	1	0	1	732	732 (2), 2512*, 2523*
Singlet	1 (1.4)	0	0	0	1	0	0	0	0	0	0	0	0	—	2529*
Total	72 (100)	22	11	6	11	3	5	4	1	2	4	2	1		

DST, diploid sequence type; PICU, paediatric intensive care unit; NICU, neonatal intensive care unit.
[a]The DST numbers marked by an asterisk were newly assigned from this study.

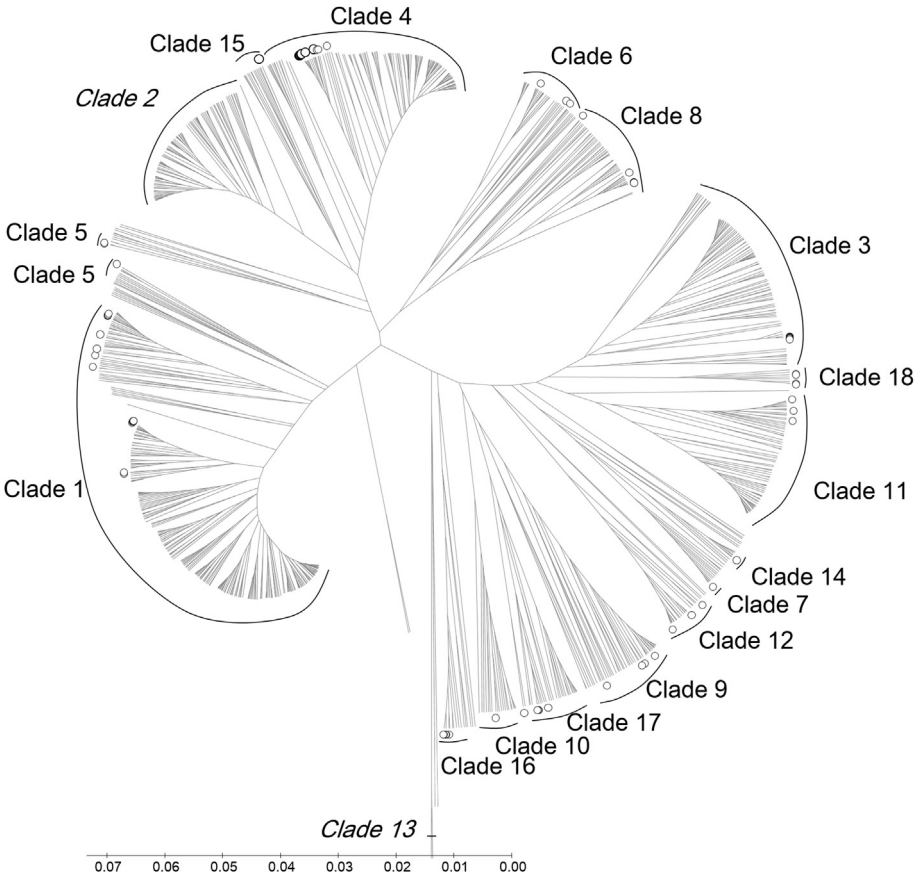

FIG. 1. The radial distribution of *Candida albicans* isolates from paediatric wards in the Chang Gung Memorial Hospital (CGMH) and reference strains. The allelic concatenated nucleic acid sequences of 72 CGMH isolates and 996 reference strains retrieved from a multilocus sequence typing database were phylogenetically analysed by UPGMA. The noted clade numbers are assigned according to reference [25]. Two clades (2 and 13) in italic letters contain no CGMH isolates. All open circles represent CGMH isolates. The scale bar indicates the *p*-distance.

(Fig. 1), which suggested close genetic relatedness of CGMH isolates within the same clades.

Eight (10.1%) isolates were categorized into four clusters (Table 4) by our definition. The majority of clustered isolates (seven isolates, 87.5%) were identified from the patients in PICUs or NICUs. In addition, one patient had two episodes of candidaemia caused by an indistinguishable strain during two hospitalizations. Neither the specific DST nor cluster-associated candidaemia was correlated with treatment outcomes or patient origin. The candidaemia-attributable mortality rate and case-fatality rates for cluster-associated cases did not differ statistically significantly from those of sporadic nosocomial infections.

Discussion

In addition to the clinical characteristics of *C. albicans* BSIs in children, this study aimed to contribute to a long-term genetic database of *C. albicans* BSI isolates of Taiwanese children for global epidemiology [32,33] and investigate whether different characteristics can be attributed to certain specific molecular types in our hospital. In the present study, we did not identify any specific molecular type that could be strongly correlated with certain patient characteristics, drug resistance, or treatment outcomes. However, results from the present study showed that *C. albicans* potentially accounted for small outbreaks inside the hospital and these escaped identification by the hospital infection control team.

In contrast to adult patients who have cancer or an immunocompromised status that places them at high risk for developing candidaemia [34,35], the majority of underlying chronic conditions in paediatric patients in this study were neurological and gastrointestinal co-morbidities. It is noticeable that the children with *C. albicans* BSIs in the present study almost all had previous antibiotic exposure, long-term hospitalization, or a catheter or artificial device in place. These findings are consistent with those previously reported, in which retained intravascular catheters, intestinal failure, presence of gastrostomy tube, and/or receipt of total parenteral nutrition were identified as independent risk factors of candidaemia [5,8,11,12,36].

TABLE 4. Clustered episodes of genetically related *Candida albicans* candidaemia

Clade	DST	Ward/location	Isolation date (month/day/year)
1	1849	2LPICU	1/25/2006
		3LNICU 1	2/15/2006
3	693	2LPICU	10/11/2009
		2LPICU→9L[a]	12/11/2009
3	693	3LNICU 1	12/4/2010
		5LNICU 1	12/31/2010
8	365	2LPICU	7/24/2006
		2LPICU	7/31/2006

PICU, paediatric intensive care unit; NICU, neonatal intensive care unit.
[a]Although the isolate was obtained when the patient was in the general ward, he had been in the PICU for a long time.

Clinical manifestations of candidaemia in paediatric patients are non-specific, like clinical sepsis, so empiric antifungal agents were administered to only one-third of the patients within 24 h of the onset of candidaemia in this cohort. Although all *C. albicans* isolates were susceptible to all antifungal agents tested in this study, only 54.4% of the patients had a good response within 3 days after initiating antifungal therapy, whereas nearly one-third had persistent candidaemia and the attributable mortality rate was up to 22.8%. The relatively high case-fatality rate may be partly explained by the high percentage of underlying chronic conditions in our cohort, which was consistent with previous studies [1,2,7,37]. All of these findings highlight the necessity for infection control measures to avoid the occurrence of candidaemia and a better treatment strategy to reduce mortality and morbidity in these patients.

There are several molecular typing methods to investigate *C. albicans* clones [38]. In addition to MLST, the methods include PCR fingerprinting and random amplification of polymorphic DNA [39], restriction fragment length polymorphism analysis [40], pulsed-field gel electrophoresis [19], and Southern blot hybridization with discriminating probes [38]. The strengths of MLST are not only that it is a highly discriminative tool for analysing genetic relatedness among sequential isolates from the same or different study sites, but also that it allows inter-laboratory comparisons worldwide. However, MLST may have some limitations in typing isolates from different patients, because some studies have shown different pulsed-field gel electrophoresis or fingerprinting patterns in shared DST isolates [19,20,41]. Although MLST has been found to be superior to pulsed-field gel electrophoresis and at least comparable to Southern hybridization [42,43], a strict definition of a nosocomial cluster as those occurring within a period of 90 days [18] would make the cluster identification more effective and reliable.

Compared with recent studies [19,20,41,44,45], the two most predominant clades (clade 1 and 4) for the isolates in this cohort were identified mainly in Asian countries. Interestingly, all seven CGMH isolates that clustered in clade 3 belonged to DST 693, which was first identified from the sputum of a patient with AIDS in 1996 in Taiwan [41] and which belongs to the global clonal complex 20 by eBURST analysis. These suggest that DST 693 might have some advantage to fit the niche in the hospital of northern Taiwan. In addition, although some studies indicated that clonal strains accounted for the majority of mortality cases [42], our data, consistent with most studies [19,20,43,45,46], showed no significant associations between clusters and clinical characteristics.

Although most studies collected *C. albicans* BSI isolates from several hospitals and suggested that interhospital strain

transmission was unlikely, even though some of their BSI isolates had identical DSTs [19,42], our study focused on strains within the same hospital and found nosocomial clusters accounted for 10.1% of total. Therefore, horizontal transmission of *C. albicans* between different wards, as well as within the unit, was possible [20,46]. A recent national multicentre study from Iceland showed as many as 23% of all cases of *C. albicans* BSIs were attributable to nosocomial clusters [18], but it was limited by the lower discriminatory method of randomly amplified polymorphic DNA analysis. There have been several studies that demonstrated single strains responsible for some outbreaks of candidaemia in the same hospital, and some of them lasted for a long period of time, sometimes for years [47,48].

There were some limitations in this study. This study was from a single centre and the results may be less generalizable than those from multicentre studies. Besides, not all isolates from the reported episodes were available for genotyping. Owing to the relatively small numbers in this study cohort, there would be an inadequate statistical power to either compare the differences between neonatal and paediatric *C. albicans* BSIs or allow for any determination of associations between molecular types and clinical data. Furthermore, although MLST is well known for its high discriminatory power and reproducibility, lack of the second method complementary to MLST, when an identical DST was shared by more than one isolate, may result in limitations in reflecting the real situation.

In conclusion, *C. albicans* constitutes an important cause of nosocomial BSI in children and causes a significant amount of mortality and morbidity, especially in those with underlying chronic co-morbidity. MLST is highly discriminating among *C. albicans* isolates. Although the possibly horizontal transmission and clustered nosocomial infections accounted for only a small percentage of candiaemia, further active surveillance network is warranted for better understanding of the molecular epidemiology of invasive candidiasis in children.

Transparency declaration

All authors declare no conflicts of interest in this article.

Authorship contribution

M-HT conceptualized and designed the study, drafted the initial manuscript and approved the final manuscript as submitted. S-HW performed the molecular typing of this study. J-FH and S-MC took care of these patients, collected and verified the data. L-CL performed experiments except molecular typing of this study. H-RH took care of these patients, and carried out the initial analyses. M-CC and R-HF took care of these patients, and helped data verification. J-JL designed the data collection instruments, and coordinated and supervised data collection and the whole study, critically reviewed the manuscript, and final approval of this manuscript. Y-CH critically reviewed the manuscript, revised the manuscript and approved the final manuscript as submitted.

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