Candidemia due to uncommon Candida species in children: new threat and impacts on outcomes

Ming-Horng Tsai1,5, Jen-Fu Hsu2,4, Lan-Yan Yang6, Yu-Bin Pan6, Mei-Yin Lai2,4, Shih-Ming Chu2,4, Hsuan-Rong Huang2,4, Ming-Chou Chiang2,4, Ren-Huei Fu2,4 & Jang-Jih Lu3,5

Many uncommon Candida spp. (species other than C. albicans, C. parapsilosis, C. glabrata, C. tropicalis, and C. krusei) have been shown to emerge in tertiary care facilities. We aimed to investigate these uncommon candidemia in children. Forty-six cases of candidemia caused by uncommon Candida spp. were identified during 2003–2015 from a medical center in Taiwan. The most common specie was C. guilliermondii (31.2%), followed by C. lusitaniae (18.8%) and C. metapsilosis (18.8%). These cases were analyzed and compared with 148 episodes of C. albicans candidemia. The incidence density of uncommon Candida spp. candidemia and the proportion to all candidemia episodes increased substantially during the study period. Prior exposure to azoles was uncommon in the 30 days prior to infection, but fluconazole resistant strains were significantly more common (n=19, 41.3%). The increased incidence density of uncommon Candida spp. candidemia was associated with increasing use of antifungal agents. No differences in demographics, underlying comorbidities, risk factors, clinical features, dissemination, and 30-day mortality were found between uncommon Candida spp. and C. albicans candidemia. Patients with uncommon Candida spp. candidemia were more likely to require modifications in antifungal treatment and receive echinocandin drugs (43.5% vs 21.6%, p = 0.007). Candidemia caused by uncommon Candida spp. had poorer response to antifungal treatment, led to longer duration of candidemia (median 4.0 versus 2.5 days, p = 0.008), and had a higher treatment failure rate (56.5% vs 38.5%, p = 0.040).

Candidemia is a major cause of morbidity and mortality in the health care setting, especially among critically ill or immunocompromised patients or those with complicated medical conditions. Among all Candida-associated invasive fungal diseases, C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, and C. krusei account for nearly 90% of isolates from blood or sterile site cultures. Candidemia caused by other uncommon species, including C. haemulonii, C. guilliermondii, and C. lusitaniae, is less well known and data have been reported only in small case series. However, these uncommon fungal species have emerged as a new health threat to hospitalized patients and are endemic in some areas. The widespread use of immunosuppressive therapies, broad-spectrum antibiotics, and antifungal prophylaxis may further increase the role of Candidal species as the causative pathogens among high-risk patients.

Recently, clinical isolates of C. haemulonii and C. guilliermondii have been reported to exhibit decreased in vitro susceptibility to antifungal agents, which highlights the importance of early identification and more updated treatment strategies. C. lusitaniae and C. famata have also presented as breakthrough candidemia in immunocompromised patients and lead to unfavorable outcomes. Clinical data of invasive candidiasis caused by these uncommon yeasts have mostly come from adult patients, whereas relevant studies reported in children are rare. Because most institutions have had limited experience with candidemia caused by these uncommon Candida spp. in children, we conducted an observational study of all candidemia cases caused by these pathogens that occurred at our institution during a 13-year period.

1Division of Neonatology and Pediatric Hematology/Oncology, Department of Pediatrics, Chang Gung Memorial Hospital, Yunlin, Taiwan. 2Division of Pediatric Neonatology, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan. 3Department of Laboratory Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan. 4College of Medicine, Chang Gung University, Taoyuan, Taiwan. 5Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan. 6Biostatistics Unit of Clinical Trial Center, Chang Gung Memorial Hospital, Linkou, Taiwan. Correspondence and requests for materials should be addressed to J.-J.L. (email: janglu45@gmail.com)
Materials and Methods

Study design, collection of isolates and antifungal susceptibility. This study was part of a collaborative, combined retrospective and prospective collected database, laboratory-based, single-center study of invasive yeast infection as previously described. We identified patients younger than 18 years of age with Candida bloodstream infection (BSI) caused by uncommon Candida spp. between January 2003 and December 2015. All Candida isolates were phenotypically identified by using the API 32C AUX yeast identification kit (bioMérieux SA, Marcy l’Étoile, France) and chromogenic culture media (CHROMagar, Becton Dickinson and Company, Franklin Lakes, NJ, USA). Beginning in December 2013, we applied Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF, Bruker Biotype, software version 3.0, USA), TSI1-5.8S-ITS2 rDNA gene sequencing and large-subunit (18S) ribosomal RNA gene D1/D2 domain sequencing to re-confirm all these species. The study was approved by the Institutional Review Board and Human Research Ethics Committee of Chang Gung Memorial Hospital (CGMH), and a waiver of informed consent for anonymous data collection was also approved. All methods in this current study were performed in accordance with the relevant guidelines mentioned in this manuscript.

We also enrolled all cases of candidemia in children caused by C. albicans during the study period for comparisons. We excluded cases of unidentified Candida spp. and selected only the first isolate recovered from the blood if a patient had several cultures positive for the same Candida spp. Antifungal susceptibility of all these Candida isolates to nine antifungal agents was determined by broth microdilution method using a Sensititre YeastOne system (Trek Diagnostic Systems Ltd., East Grinstead, UK) according to the manufacturer’s instructions. Minimum inhibitory concentration (MIC) was recorded as the highest concentration of antifungal agent resulting in the development of a blue color. The criteria for susceptibility of these Candida isolates to nine antifungal agents were based on MIC breakpoints of Candida spp. recommended by the Clinical & Laboratory Standards Institute (CLSI) guidelines. For uncommon Candida spp., other than C. guilliermondii, clinical breakpoints are undefined; therefore, isolates that showed MICs higher than the epidemiologic cutoff value were considered potentially resistant.

Data collection and definitions. An incident episode of candidemia was defined as the first positive blood culture drawn from a peripheral vein yielding a Candida species, with clinical symptoms and signs compatible with Candida BSI. Episodes were considered to be separate if they occurred at least 1 month apart and when at least one negative blood culture was noted between them. The clinical information was accumulated from a review of medical charts and included demographic characteristics, predisposing risk factors within the preceding 30 days from the onset of Candida BSI (defined as the day of first positive blood culture for Candida spp.), underlying diseases, and the presence of an intravenous catheter or any other artificial device at the time candidemia appeared. The clinical manifestations at the time of blood culture collection, ICU admission, and the antimicrobial regimens used were also collected.

An episode of candidemia was considered catheter-related only if the same Candida species was cultured from the catheter tip during the episode; the definition was suggested by the guidelines of the Infectious Diseases Society of America. Persistent candidemia was defined as repeated positive blood cultures for Candida spp. more than 3 days after antifungal agents were administered. Candidemia-attributable mortality was defined as patients died within 7 days after onset of candidemia or in the presence of persistent clinical sepsis or persistent candidemia, or those died of candidemia associated complications. Breakthrough candidemia was defined as new occurrence of candidemia while the patient was on antifungal agents for more than three days. The primary study outcome was clinical treatment failure, which was defined according to the Mycoses Study Group and European Organization for Research and Treatment of Cancer consensus criteria as the following: (1) all-cause mortality between days 3 and 30 after the initial positive blood culture, or (2) persistent fungal BSI for ≥72 hours after the initiation of antifungal therapy. The secondary outcome was all cause in-hospital mortality. Patients’ response to antifungal therapy following candidemia was defined according to the consensus criteria of the Mycoses Study Group and European Organization for Research and Treatment of Cancer.

Statistical analysis. The demographic, clinical, outcome variables and the in vitro susceptibility data were summarized using the descriptive statistics. All statistical analyses were performed using IBM SPSS software (version 22.0; IBM SPSS Inc., New York, USA). Categorical variables were compared using the χ2 or Fisher’s exact test, and continuous variables by the Mann-Whitney U test. A P value of 0.05 was considered significant. Poisson regression and the Cochran-Armitage test were used for trend analysis of the annual BSI incidence densities and the proportions of candidemia caused by uncommon Candida spp., respectively. We also compared BSI incidence densities for three time periods; 2003–2006, 2007–2011, and 2012–2015, using Poisson distribution and test-based methods. The correlation between the annual use of antifungals and time was evaluated by using the Spearman correlation. The associations between the incidence densities of uncommon Candida spp. BSI and the annual use of antifungals (defined as daily doses per 1,000 patient-days) were evaluated by using Poisson regression.

We used Cox regression analysis to identify factors that were significantly associated with death. Clinically relevant parameters in the univariate analysis (P < 0.1) were included at multivariate regression analysis. The full model was reduced to a final model by using a stepwise elimination procedure. The proportional hazards assumption was tested graphically and by building time-dependent variables.

Results

We identified 323 episodes of candidemia that occurred in hospitalized children over the 13-year study period. A total of 25 cultures that previously grew unspecified Candida spp. were rechecked by our ITS1-5.8S-ITS2 rDNA gene sequencing and MALDI-TOF and large-subunit (18S) ribosomal RNA gene D1/D2 domain sequencing. Twenty-one of the cultures were documented, and four unidentified Candida spp. were excluded. A total of 46
episodes of candidemia in 45 patients were caused by 10 uncommon *Candida* spp (Table 1). These data were compared with those reported for 148 episodes of candidemia caused by *C. albicans* in 136 patients.

The overall incidence of uncommon *Candida* spp. candidemia and their proportion relative to all episodes of candidemia increased significantly during 2003–2015 (incidence density $p < 0.001$; proportion $p < 0.001$) (Fig. 1). The overall incidence density of uncommon *Candida* spp. BSI was 3.61 episodes per 100,000 inpatient days, which increased from 1.48 (2003–2006) to 2.47 (2007–2011) and then to 7.29 (2012–2015; $p < 0.001$). Twenty-nine (63.4%) of the 46 episodes of uncommon *Candida* spp. candidemia occurred after January 2012. The overall proportion of uncommon *Candida* spp. candidemia relative to all episodes of candidemia in children was 14.4% and increased from 6.2% (2003–2006) to 9.3% (2007–2011) and then to 29.1% (2012–2015; $p < 0.001$). During 2012–2015, *C. guilliermondii* and *C. metapsilosis* had the highest incidence density (both 1.76 episodes/100,000 inpatient days) and had increased significantly compared with cases during 2003–2006 and 2007–2011. The incidence density rate of other uncommon *Candida* spp. did not increase.

Echinocandins have been available in our institute since 2004. Although the annual use of echinocandins,azole antifungals, and amphotericin B increased gradually during 2003–2015, there was no statistically significant increase in their use. The overall use of antifungal agents did increase significantly during this study period (Spearman $r = 0.68$; $p = 0.040$) (Fig. 1). The increase in incidence density of uncommon *Candida* spp. candidemia was correlated with the trend of increased voriconazole (VFEND®, Pfizer, New York, NY, USA) use ($p = 0.098$) and was significantly associated with the continuous increase in overall antifungal agent use ($p = 0.033$).

Most chronic comorbid conditions and associated risk factors were comparable between cases of candidemia caused by uncommon *Candida* spp. and *C. albicans* groups (Table 2). Although uncommon *Candida* spp. had a significantly higher MIC to fluconazole and relatively more commonly presented as breakthrough candidemia (17.4% vs 8.1%, $P = 0.094$), previous exposure to azoles was comparable between these two groups. The clinical characteristics, therapeutic regimens and treatment responses of uncommon *Candida* spp. candidemia and *C. albicans* candidemia are compared in Table 3. The severity of illness, judged by rates of severe sepsis and septic shock, were comparable between cases of candidemia caused by uncommon *Candida* spp. and those due to *C. albicans*. However, candidemia caused by uncommon *Candida* spp. led to significantly higher rates of persistent candidemia compared to *C. albicans* candidemia (76.1% vs 56.8%, $p = 0.024$).

Overall, 186 episodes (95.9%) of candidemia were treated with specific antifungal agents. The mean duration between onset of candidemia (time of the first positive blood culture for *Candida* spp.) and initiation of antifungal agents was 2.3 days (range 0–8 days). In 83 episodes (44.6%) of the 186 episodes, antifungal regimens were modified during the treatment course. Patients with uncommon *Candida* spp. candidemia had a significantly higher rate of antifungal regimen modification than did those with *C. albicans* candidemia, and were more often treated with echinocandins (43.5% vs 21.6%, $p = 0.007$). After modification of antifungal regimens, uncommon *Candida* spp. led to a significantly longer duration of candidemia (median 3.0 versus 1.0 days, $p < 0.001$), were slower to respond to antifungal agents, and had significantly higher rates of clinical treatment failure (56.5% versus 38.5%, $p = 0.040$), although the deaths due to fungemia and final in-hospital mortality were comparable between these two groups.

MICs for uncommon *Candida* spp. of eight antifungal agents are shown in Table 4. Overall, 19 (47.5%) of 40 available uncommon *Candida* spp. isolates were intermediate or resistant (minimum inhibitory concentration [MIC] $\geq 4$ mg/L). With the exception of three *C. haemulonii* isolates that had high MICs of amphotericin B (2.0 mg/L), all other strains were susceptible to amphotericin B (MIC $\leq 1.0$ mg/L). Seven *C. guilliermondii* isolates, three *C. haemulonii* isolates, and one isolate each of *C. lusitaniae*, *C. metapsilosis*, *C. orthopsilosis*, *C. pelliculosa* and *C. duobushaemulonii* were resistant (MIC $\geq 1$ mg/L) or susceptible-dose-dependent (MIC 0.25–0.5 mg/L) to itraconazole. Except for 2 *C. haemulonii* strains, all isolates were susceptible to voriconazole (MIC $\leq 1$ mg/L).

### Table 1. The uncommon *Candida* species causing 46 episodes of candidemia in children. *Newborn*: from neonatal intensive care unit, age <3 months old; children: ward or pediatric intensive care unit, age 3 months-18 years old. *Defined as repeated positive blood cultures for *Candida* spp. for more than 3 days after antifungal agents.

| Pathogens          | Total episode number, n (%) | Age category* | Years of occurrence | Treatment outcomes |
|---------------------|-----------------------------|---------------|---------------------|--------------------|
|                     |                             | Newborn | Children | 2003–2006 | 2007–2011 | 2012–2015 | Persistent candidemia* | Attributable mortality |
| *C. guilliermondii*  | 15 (31.2)                   | 7       | 8        | 3         | 4         | 8         | 9 (60.0) | 3 (20.0) |
| *C. lusitaniae*     | 7 (18.8)                    | 2       | 5        | 2         | 3         | 2         | 4 (57.1) | 1 (14.3) |
| *C. metapsilosis*   | 9 (18.8)                    | 3       | 6        | 0         | 2         | 7         | 6 (66.7) | 3 (33.3) |
| *C. orthopsilosis*  | 3 (6.3)                     | 1       | 2        | 0         | 0         | 3         | 3 (100)  | 1 (33.3) |
| *C. haemulonii*     | 4 (8.3)                     | 1       | 3        | 1         | 1         | 2         | 2 (50.0) | 1 (25.0) |
| *C. lipolytica*     | 2 (4.2)                     | 1       | 1        | 0         | 2         | 0         | 1 (50.0) | 0 (0)    |
| *C. dublensis*      | 2 (4.2)                     | 0       | 2        | 1         | 0         | 1         | 1 (50.0) | 0 (0)    |
| *C. pelliculosa*    | 2 (4.2)                     | 1       | 1        | 0         | 0         | 2         | 1 (50.0) | 1 (50.0) |
| *C. duobushaemulonii* | 1 (2.1)                   | 0       | 1        | 0         | 0         | 1         | 1 (100)  | 1 (100)  |
| *C. famata*         | 1 (2.1)                     | 0       | 1        | 0         | 0         | 1         | 0 (0)    | 0 (0)    |
| Total               | 46 (100)                    | 16      | 34.8     | 30 (65.2) | 7 (15.2)  | 13 (28.3) | 26 (56.5) | 28 (60.9) | 11 (23.9) |
| Controls: *C. albicans* | 148 (100)                | 50      | 33.8     | 98 (66.2) | 58 (39.2) | 53 (35.8) | 37 (25.0) | 58 (39.2) | 35 (23.6) |
and demonstrated posaconazole MICs of \( \leq 0.5 \) mg/L. Caspofungin and micafungin demonstrated good activity against all species and isolates; an exception to this was one episode of *C. guilliermondii* with MICs of 8 mg/L and 2.0 mg/L, respectively.

**Discussion**

Population-based surveillance studies have documented the shift of candidemia from *C. albicans* to non-*albicans* species over the past two decades\(^{2,4,5}\). Uncommon *Candida* species, which generally account for less than 10% of all cases of candidemia\(^{17,18}\), are emerging among critically ill patients. The reported prevalence of uncommon *Candida* spp. in children varies widely between 3.2–22%, depending on definitions, geographic region, and patient characteristics\(^{34-37}\). We found that uncommon *Candida* spp. accounted for 14.4% of all cases of candidemia in children, and our result is in agreement with a recent study that documented the incidence and proportion of uncommon *Candida* spp. BSIs has risen during the past decade. We found uncommon *Candida* spp. candidemia were more frequently associated with treatment failure than candidemia caused by *C. albicans*, as these isolates were more commonly resistant to azoles, which led to poorer response and longer duration of candidemia.

In contrast to non-*albicans* candidemia in adults, in which prior fluconazole exposure was often concluded as an independent risk factor\(^{18,38,39}\), studies in the pediatric populations found no difference between *C. albicans* and non-*albicans* candidemia in terms of demographics, underlying disease, risk factors, clinical features and outcomes\(^{32,40}\). However, previous studies attempting to investigate the risk factors of non-*albicans* candidemia in children have focused on the more common *Candida* spp., such as *C. parapsilosis* and *C. glabrata*\(^{32,41,42}\). To our knowledge, this is the first study to investigate uncommon *Candida* spp. candidemia in children. We found similar patients characteristics, risk factors and comparable outcomes between uncommon *Candida* spp. and *C. albicans*. These results are in agreement with other reports concluding host characteristics and underlying medical illness are the most powerful predictors of final outcomes\(^{14,42-44}\).

The overall incidence of candidemia during the study period remained stable and was not affected by the changes in antifungal treatment policies\(^{45,46}\). It is tempting to speculate that an increase in strains resistant to fluconazole and higher MICs values were associated with the changes of in antifungal treatment policies. Published guidelines encourage empiric use of echinocandins in patients with severe illness, history of azole exposure, or neutropenia\(^{47}\). This may have influenced the anti-fungal prescribing practices. It is possible that a significant
Within one month prior onset of candidemia, prior azoles exposure indicated patients received azoles drug in addition to the antifungal agents at time of candidemia. *Absolute neutrophil count ≤ 500 cells/μL.

Table 2. Demographic and clinical characteristics of 46 episodes of candidemia caused by uncommon Candida spp. versus 148 episodes of C. albicans candidemia. All data were expressed as number (percentage %), unless indicated otherwise; IQR: interquartile range. *Indicated the presence of underlying condition or risk factor at onset of candidemia, and most patients with candidemia had >1 underlying condition and/or risk factor. Within one month prior onset of candidemia, prior azoles exposure indicated patients received azoles drug in addition to the antifungal agents at time of candidemia. *Absolute neutrophil count ≤ 500 cells/μL.

| Characteristic | Uncommon Candida spp. candidemia (total n = 46) | C. albicans candidemia (total n = 148) | P value |
|---------------|-----------------------------------------------|--------------------------------------|---------|
| Neonatal episodes, n (%) | 16 (34.8) | 50 (33.8) | 0.809 |
| Patient age (days) of neonatal episodes, median (IQR) | 28.5 (18.5–65.8) | 25.5 (11.8–58.5) | 0.213 |
| Non-neonatal episodes, n (%) | 30 (65.2) | 98 (66.2) | 0.809 |
| Patient age (years) of non-neonatal episodes, years (IQR) | 3.9 (1.1–11.1) | 4.2 (1.0–8.2) | 0.711 |
| Sex, male subjects/female subjects | 21 (45.7)/25 (54.3) | 71 (48.0)/77 (52.0) | 0.866 |

Underlying conditions *

| Indicated the presence of underlying condition or risk factor | 5 (10.9) | 15 (10.1) | 0.886 |
| Neurological sequelae | 18 (39.1) | 48 (32.4) | 0.476 |
| Cardiovascular disease | 5 (10.9) | 17 (11.5) | 0.908 |
| Chronic lung disease and/or pulmonary hypertension | 16 (34.8) | 48 (32.4) | 0.858 |
| Gastrointestinal sequelae | 11 (23.9) | 44 (29.7) | 0.575 |
| Renal sufficiency with/without dialysis | 8 (17.4) | 19 (12.8) | 0.467 |
| Hematological/Oncology cancer | 9 (19.6) | 23 (15.5) | 0.503 |
| Immunodeficiency | 2 (4.3) | 2 (1.4) | 0.212 |
| Autoimmune disease | 1 (2.2) | 5 (3.4) | 0.680 |
| Hepatic failure or cholestasis | 0 (0) | 6 (4.1) | 0.165 |
| Days of hospitalization before candidemia onset, median (IQR) | 29.0 (14.8–50.0) | 29.0 (14.3–55.5) | 0.787 |

Sequences of episodes

| Characteristic | 0.608 |
| First episode | 39 (84.8) | 131 (88.5) |
| Recurrent episode | 7 (15.2) | 17 (11.5) |

Associated risk factors

| Receipt of systemic antibiotics * | 44 (95.7) | 138 (93.2) | 0.735 |
| Prior bacteremia * | 27 (58.7) | 67 (45.3) | 0.130 |
| Prior azoles exposure * | 6 (13.0) | 10 (6.8) | 0.176 |
| Presence of central venous catheter | 45 (97.8) | 141 (95.3) | 0.683 |
| Stay in an intensive care unit | 31 (67.4) | 110 (74.3) | 0.549 |
| Receipt of parenteral nutrition | 30 (65.2) | 94 (63.5) | 0.863 |
| Receipt of immunosuppressive drugs | 14 (30.4) | 29 (19.6) | 0.154 |
| Presence of artificial device other than central venous catheter | 27 (58.7) | 68 (46.0) | 0.176 |
| Prior surgery * | 16 (34.8) | 46 (31.1) | 0.719 |
| Neutropenia * | 14 (30.4) | 31 (20.9) | 0.230 |

Increase in consumption of antifungal agents during the first half of the study period accounted for the emergence of uncommon Candida species, which required longer periods of antifungal treatment and led to a vicious cycle of more uncommon Candida species. Therefore, the incidence density of uncommon Candida spp. BSIs was noted to be significantly higher after 2012.

During the 13-year study period, the approach and antifungal treatment policies at our institute changed in two aspects. In the neonatal intensive care unit, antifungal prophylaxis with fluconazole for extremely low birth weight infants was launched in 2011, and echinocandins became available since 2004. Caspofungin (Cancidas®, Merck, Sharp & Dohme, Kenilworth, NJ, USA) has been widely used since 2005 and micafungin (Micamine®, Astellas Pharma, Inc., Tokyo, Japan) became more common beginning in 2009. These changes in antifungal regimens may account for the changing epidemiologic characteristics of candidemia in children since 2011. Several studies have concluded that the increase of certain non-albicans or uncommon Candida species, such as C. glabrata and C. kefyr, are associated with the increasing use of echinocandin drugs. Another study found significant positive correlation between use of itraconazole and the increased incidence of C. parapsilosis and C. guilliermondii candidemia. However, no antifungal agent can account for the emergence of uncommon Candida spp. candidemia found in this study. In addition, our cases of uncommon Candida spp. candidemia were less commonly breakthrough candidemia or due to previous treatment with specific antifungal agents.

In several studies, C. guilliermondii has been the most commonly isolated uncommon Candida spp. among pediatric patients and C. lusitaniae was common in another international study in children. In other recent reports, more than half of all patients with candidemia caused by uncommon Candida spp. had breakthrough infections or underlying hematological malignancies. In our cohort, almost all cases of pediatric candidemia had specific chronic comorbidities and the majority of breakthrough candidemia cases were due to C.
parapsilosis and C. glabrata. These differences are a further reflection of the changing epidemiologic characteristics of pediatric candidemia and unique features of uncommon Candida spp. in children. Therefore, we concluded that uncommon Candida spp. distributions and clinical characteristics vary by patient population, geographic region, and antifungal practices.

Our study had some limitations. First, it was a retrospective study from a single institution with a small number of episodes caused by individual uncommon Candida spp.; therefore, further multicenter, prospective studies, or systemic review with meta-analysis are required to update information applicable to different geographic areas or specific groups at risk for candidemia caused by uncommon Candida species. Second, although we used the MALDI-TOF and DNA sequencing to re-identify all Candida isolates in the past 13 years, some Candida strains of pediatric candidemia more than five years ago were not available and were identified phenotypically at that time. Therefore, it is possible that during 2003–2011, some C. dublinensis and other Candida isolates were mis-identified as C. albicans, and the frequency of uncommon Candida spp was thus underestimated. Although MALDI-TOF has strengths of rapid, sensitive, and economical in terms of both costs and labor involved, it is also limited that the spectral database of MALDI-TOF must contain peptide mass fingerprints of the specific species before it can correctly identify new species. Finally, the uncommon Candida species are a heterogeneous "mixture" of many different organisms. Therefore, they may not share common clinical characteristics and treatment strategies should depend on individual cases.

| Clinical features                                      | Uncommon Candida spp. candidemia (total n = 46) | C. albicans candidemia (total n = 148) | P value |
|--------------------------------------------------------|-----------------------------------------------|---------------------------------------|---------|
| Severe sepsis                                          | 18 (39.1)                                     | 55 (37.2)                             | 0.862   |
| Septic shock                                           | 15 (32.6)                                     | 44 (29.7)                             | 0.717   |
| Progressive and deteriorated candidiasis*              | 6 (13.0)                                      | 33 (22.3)                             | 0.209   |
| Disseminated candidiasia†                               | 0 (0)                                         | 7 (4.7)                               | 0.133   |
| Breakthrough candidemia                                | 8 (17.4)                                      | 12 (8.1)                              | 0.094   |
| Duration of candidemia (days), median (interquartile range) | 4.0 (1.8–8.3)                       | 2.5 (1.0–5.0)                         | 0.008   |
| ≤2 days                                                | 16 (34.8)                                     | 74 (50.0)                             |         |
| 3–7 days                                               | 17 (37.0)                                     | 52 (35.1)                             |         |
| ≥8 days                                                | 13 (28.3)                                     | 22 (14.9)                             |         |
| Ultimate antifungal regimen for treatment              |                                               |                                       | 0.054   |
| Fluconazole/Voriconazole                               | 16 (34.8)                                     | 63 (42.6)                             |         |
| Amphotericin B                                         | 9 (19.6)                                      | 44 (29.7)                             |         |
| Echinocandins                                          | 20 (43.5)                                     | 32 (21.6)                             | 0.007   |
| Combination antifungal treatment                       | 0 (0)                                         | 2 (1.4)                               |         |
| None                                                   | 1 (2.2)                                       | 7 (4.7)                               |         |
| Effective antifungal agents given within 48 hours after onset of candidemia (based on antifungal susceptibility testing) | 16/46 (34.8)                   | 58/144 (40.3)                         | 0.601   |
| Total treatment duration (days), mean (interquartile range) | 20.0 (14.0–27.5)                   | 16.0 (14.0–22.0)                      | 0.116   |
| Catheter removal                                       | 23/45 (51.1)                                  | 91/141 (64.5)                         | 0.107   |
| Removal of central venous catheter within 3 days of onset | 16/45 (35.6)                      | 60/141 (42.6)                         | 0.406   |
| Table 3. Clinical features, treatment and outcomes of candidemia caused by uncommon Candida spp. versus C. albicans candidemia. All data were expressed as number (percentage %), unless indicated otherwise. *Defined as candidemia episodes with more disseminated candidiasis and/or progressive multi-organ failure even after effective antifungal agents. †Indicated positive Candida isolates recovered from more than two sterile sites, in addition to primary bloodstream infection. *Responsiveness to antifungal agents was defined according to the consensus criteria of the Mycoses Study Group and European Organization for Research and Treatment of Cancer.33.
Table 4. Available susceptibility data for uncommon Candida isolates associated with candidemia in children. AMB: amphotericin B; CAS: caspofungin; FLU: fluconazole; 5-FC: 5-flucytosine; ITC: itraconazole; MIC: micafungin; POS: posaconazole; VOR: voriconazole.

In conclusion, uncommon Candida spp. causing candidemia are emerging among hospitalized children. We did not find clinical variables that enable us early recognition or prediction of candidemia caused by these pathogens. Although clinical outcomes at day 30 are similar to those caused by C. albicans, uncommon Candida spp. more frequently result in prolonged fungemia and treatment failure. Because uncommon Candida species frequently show fluconazole MICs above the epidemiologic cutoff values, identification of all Candida organisms at the species level by advanced molecular methods is of value in guiding treatment directions.

Conclusion

Uncommon Candida species have now emerged among hospitalized children. These pathogens frequently are not susceptible to fluconazole and had higher rate of treatment failure; echinocandins are the treatment choice.

References

1. Russo, A. et al. Risk factors and clinical outcomes of candidaemia in patients treated for Clostridium difficile infection. Clin Microbiol Infect 21, 493.e1–493.e4 (2015).
2. Puig-Asensio, M. et al. Epidemiology and outcome of candidaemia in patients with oncological and haematological malignancies: results from a population-based surveillance in Spain. Clin Microbiol Infect 21, 491.e1–491.e10 (2015).
3. Oeser, C. et al. Neonatal invasive fungal infection in England 2004–2010. J Clin Microbiol 49, 547–551 (2011).
4. Hesstedal, L. et al. Twenty-two years of candidaemia surveillance: results from a Norwegian national study. Clin Microbiol Infect 21, 938–945 (2015).
5. Tan, B. H. et al. Incidence and species distribution of candidaemia in Asia: a laboratory-based surveillance study. Clin Microbiol Infect 21, 946–953 (2015).
6. Klingpor, L. et al. Invasive Candida infections in surgical patients in intensive care units: a prospective, multicentre survey initiated by the European Confederation of Medical Mycology (ECMM) (2006–2008). Clin Microbiol Infect 21, 87.e1–87.e10 (2015).
7. Lockhart, S. R., Messer, S. A., Pfaller, M. A. & Diekema, D. J. Loddromyces elongisporus Masquerading as Candida parapsilosis as a cause of bloodstream infections. J Clin Microbiol 46, 374–376 (2008).
8. Alcoba-Flórez, J. et al. Phenotypic and molecular characterization of Candida nivariensis sp. nov., a possible new opportunistic fungus. J Clin Microbiol 43, 4107–4111 (2005).
9. Khan, Z. U. et al. Outbreak of fungemia among neonates caused by Candida haemulonii resistant to amphotericin B, itraconazole, and fluconazole. J Clin Microbiol 45, 2025–2027 (2007).
10. Kim, M. N. et al. Candida haemulonii and closely related species at 5 university hospitals in Korea: identification, antifungal susceptibility, and clinical features. Clin Infect Dis 48, e57–e61 (2009).
11. Minari, A., Hachem, R. & Raad, I. Candida lusitaniae: a cause of breakthrough fungemia in cancer patients. Clin Infect Dis 32, 186–190 (2001).
12. Ramos, L. S. et al. Candida haemulonii complex: species identification and antifungal susceptibility profiles of clinical isolates from Brazil. J Antimicrob Chemother 70, 111–115 (2015).

13. Cuervo, G. et al. Breakthrough candidemia in the era of broad-spectrum antifungal therapies. Clin Microbiol Infect 22, 181–188 (2015).

14. Puig-Asensio, M. et al. Epidemiology and predictive factors for early and late mortality in Candida bloodstream infections: a population-based surveillance in Spain. Clin Microbiol Infect 20, 0245–0254 (2014).

15. Calmant, M. N. et al. A prospective, cohort, multicentre study of candidemia in hospitalized adult patients with hematological malignancies. Clin Microbiol Infect 20, 050–057 (2014).

16. González, G. M. et al. Species distribution and antifungal susceptibility of bloodstream fungal isolates in paediatric patients in Mexico: a nationwide surveillance study. J Antimicrob Chemother 68, 2847–2851 (2013).

17. Jung, D. S., Farmaikotis, D., Jiang, Y., Tarrand, J. J. & Kontoyiannis, D. P. Uncommon Candida species fungemia among cancer patients, Houston, Texas, USA. Emerg Infect Dis 21, 1942–1950 (2015).

18. Chen, S. C. et al. Candidaemia with uncommon Candida species: predisposing factors, outcome, antifungal susceptibility, and implications for management. Clin Microbiol Infect 15, 662–669 (2009).

19. Tsai, M. H. et al. Clinical and molecular characteristics of bloodstream infections caused by Candida albicans in children from 2003 to 2011. Clin Microbiol Infect 21, 1018.e1–1018.e8 (2015).

20. Alexander, B. D. et al. Comparative evaluation of Etest and Sensititre YeastOne panels against the Clinical and Laboratory Standards Institute M27-A2 reference broth microdilution method for testing Candida susceptibility to seven antifungal agents. J Clin Microbiol 45, 698–706 (2007).

21. Orasch, C. et al. Candida species distribution and antifungal susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing and new vs. old Clinical and Laboratory Standards Institute clinical breakpoints: a 6-year prospective candidaemia survey from the fungal infection network of Switzerland. Clin Microbiol Infect 20, 698–705 (2014).

22. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts: 4th informational supplement. Document M27-S4. Wayne, PA: CLSI (2012).

23. Pfaffer, M. A. & Diekema, D. J. Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. J Clin Microbiol 50, 2846–2856 (2012).

24. Santolaya, M. E. et al. Active surveillance of candidemia in children from Latin America: a key requirement for improving disease outcome. Pediatr Infect Dis J 33, e40–e44 (2014).

25. Nguyen, M. H. et al. Performance of Candida real-time polymerase chain reaction, β-D-Glucan Assay, and blood cultures in the diagnosis of invasive candidiasis. Clin Infect Dis 54, 1240–1248 (2012).

26. Mermel, L. A. et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 49, 1–45 (2009).

27. Muñoz, P. et al. Risk factors for late recurrent candidemia. A retrospective matched case-control study. Clin Microbiol Infect 22, 277, e11–e27.e20 (2016).

28. Tsai, M. H. et al. Clinical risk factors and outcomes for multidrug-resistant gram-negative bacteremia in the NICU. Pediatrics 133, e322–e329 (2014).

29. Tsai, M. H. et al. Clinical and microbiological characteristics, and impact of therapeutic strategies on the outcomes of children with candidemia. Sci Rep 7, 1083 (2017).

30. Lerolle, N. et al. Breakthrough invasive fungal disease in patients receiving posaconazole primary prophylaxis: a 4-year study. Clin Microbiol Infect 20, O952–O959 (2014).

31. Brosh-Nissimov, T. & Ben-Ami, R. Differential association of fluconazole doses and does/MIC ratio with mortality in patients with Candida albicans and non-albicans bloodstream infection. Clin Microbiol Infect 21, 1011–1017 (2015).

32. Dotis, J., Prasad, P. A., Zaoutis, T. & Rolides, E. Epidemiology, risk factors and outcome of Candida parapsilosis bloodstream infection in children. Pediatr Infect Dis J 31, 557–560 (2012).

33. Segal, B. H. et al. Defining responses to therapy and study outcomes in clinical trials of invasive fungal diseases: Mycoses Study Group and European Organization for Research and Treatment of Cancer consensus criteria. Clin Infect Dis 47, 674–683 (2008).

34. Steinbach, W. J. et al. Results from a prospective, international, epidemiologic study of invasive candidiasis in children and neonates. Pediatr Infect Dis J 31, 1252–1257 (2012).

35. Rodriguez-Nunez, A. Incidence and mortality of proven invasive Candida infections in pediatric intensive care patients. Infect Control Hosp Epidemiol 22, 477–478 (2001).

36. Vogtjatz, L. et al. Invasive candidiasis in pediatric intensive care in Greece: a nationwide study. Intensive Care Med 39, 2188–2195 (2013).

37. Jordan, L. et al. Per-species risk factors and predictors of invasive Candida infections in patients admitted to pediatric intensive care units: development of ERICAP scoring systems. Pediatr Infect Dis J 2014, e187–e193 (2014).

38. Chow, J. K. et al. Factors associated with candidemia caused by non-albicans Candida species versus Candida albicans in the intensive care unit. Clin Infect Dis 46, 1206–1213 (2008).

39. Playford, E. G. et al. Candidemia in nonneutropenic critically ill patients: risk factors for non-albicans Candida spp. Crit Care Med 36, 2034–2039 (2008).

40. Dutta, A. & Palazzi, D. L. Candida non-albicans versus Candida albicans fungemia in the non-neonatal pediatric population. Pediatr Infect Dis J 30, 664–668 (2011).

41. Pemán, J. et al. Epidemiology and antifungal susceptibility of bloodstream fungal isolates in pediatric patients: a Spanish multicenter prospective study. J Clin Microbiol 49, 4158–4163 (2011).

42. Puig-Asensio, M. et al. Impact of therapeutic strategies on the prognosis of candidemia in the ICU. Crit Care Med 42, 1423–1432 (2014).

43. Fernández-Ruiz, M. et al. Initial use of echinocandins does not negatively influence outcome in Candida parapsilosis bloodstream infection: a propensity score analysis. Clin Infect Dis 58, 1413–1421 (2014).

44. Bassetti, M. et al. A multicenter multinational study of abdominal candidiasis: epidemiology, outcomes and predictors of mortality. Intensive Care Med 41, 1601–1610 (2015).

45. Lai, C. C. et al. Association between incidence of candidemia and consumption of antifungal agents at a medical center in Taiwan. Int J Antimicrob Agents 40, 349–353 (2012).

46. Bassetti, M. et al. Incidence of candidemia and relationship with fluconazole use in an intensive care unit. J Antimicrob Chemother 64, 625–629 (2009).

47. Pappas, P. G. et al. Clinical practice guideline for the management of candidiasis: 2016 updated by the Infectious Disease Society of America. Clin Infect Dis 62, e1–e50 (2016).

48. Dufresne, S. F. et al. Epidemiology of Candida kefyr in patients with hematologic malignancies. J Clin Microbiol 52, 1830–1837 (2014).

49. Charisiotis, A. et al. Candidemia in children caused by uncommon species of Candida. Arch Pediatr Infect Dis 6(2), e11895 (2018).

50. Singhal, N., Kumar, M., Kanauja, P. K. & Virdi, J. S. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol 6, 791 (2015).
Acknowledgements
This work was supported by a grant from Chang Gung Memorial Hospital (CMRPG3B1302, CMRPG3D1241, and CMRPG3E1491). The authors acknowledge the statistical assistance provided by the Clinical Trial Center, Chang Gung Memorial Hospital, Linkou, Taiwan, which was founded by the Ministry of Health and Welfare of Taiwan; MOHW106-TDU-B-212-113005. Chang Gung Medical Research Program Foundation (grants CMRPG3E1491).

Author Contributions
Ming-Horng Tsai: Dr. Tsai conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted. Jen-Fu Hsu: Dr. Hsu designed the data collection instruments, and coordinated and supervised data collection and the whole study. Lan-Yan Yang: Dr. Yang performed the statistical analysis of this study. Yu-Bin Pan: Dr. Pan helped to perform the statistical analysis of this study and complete the figure 1. Mei-Ying Lai: Dr. Lai helped to collect and verify the data. Shih-Ming Chu: Dr. Chu performed the microbiological characteristics of this study. Hsuan-Rong Huang: Dr. Huang took care of these patients, and carried out the initial analyses. Ming-Chou Chiang: Dr. Chiang took care of these patients, and helped data verification. Ren-Huei Fu: Dr. Fu took care of these patients, and helped data verification. Jang-Jih Lu: Dr. Lu critically reviewed the manuscript, revised the manuscript, and approved the final manuscript as submitted.

Additional Information
Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018