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://