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ORIGINAL ARTICLE

Distribution and drug susceptibilities of *Candida* species causing candidemia from a medical center in central Taiwan

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Abstract Invasive fungal infections have increased significantly in the past few decades because of the increase in high-risk populations. To investigate the distribution and drug susceptibilities of such infections, we analyzed all 152 *Candida* isolates causing candidemia from 2004 to 2006 at the China Medical University Hospital, a medical center in central Taiwan. *Candida albicans* was the most common species, accounting for 52.6 % of the isolates, followed by *C. tropicalis* (19.7 %), *C. parapsilosis* (14.5 %), *C. glabrata* (8.6 %), *C. guilliermondii* (3.9 %), and *C. pelliculosa* (0.7 %). All isolates were susceptible to amphotericin B, anidulafungin, micafungin, and voriconazole according to minimum inhibitory concentrations (MICs) after a 24-h incubation; 0.7 %, 6.6 %, and 7.9 % of isolates were resistant to amphotericin B, fluconazole, and voriconazole, respectively, after 48-h incubation. Both *C. albicans* and *C. parapsilosis* had high degrees of agreement for azoles between 24- and 48-h incubation periods, whereas *C. glabrata* (38.5–46.2 %) and *C. tropicalis* (56.7–63.3 %) did not. The majority of the isolates with high azole MICs displayed a trailing growth phenotype. Hence, the MICs of different drugs after 24-h incubation may be considered for prognosis of candidemia.

Keywords Amphotericin B · Azoles · *Candida* species · Candidemia · Drug susceptibility · Echinocandins

Introduction

The epidemiology of invasive fungal infections has become important in the past few decades because of the increased number of immunocompromised patients, the augmentation of invasive medical devices, and the extensive use of broad-spectrum antibiotics [1–3]. *Candida* species are the most frequently isolated fungal pathogens,

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responsible for significant morbidity and mortality [4–9], and the leading causes of bloodstream infections globally [2, 8–14]. According to the results of hospital-based surveillance, European countries reported 0.5–0.7 cases per 10,000 patient-days in association with invasive candidiasis [15, 16], and in the United States, it was 1.5 cases per 10,000 patient-days. Globally, Brazil has the highest reported incidence of 3.7 cases per 10,000 patient-days [11, 17]. On average, it is about 1.5 cases per 10,000 patient-days in Taiwan [18–20].

Hundreds of species of *Candida* have been described, but only 30–40 species among these have been reported to cause diseases in humans [21, 22]. *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, and *Candida krusei* are the five most common species causing candidemia, in combination accounting for more than 95 % of the cases [2, 9, 11, 15].

Azole (fluconazole and voriconazole), echinocandin (anidulafungin, caspofungin, and micafungin), and polyene (amphotericin B) are the three classes of drugs most commonly prescribed for treating systemic fungal infections. The emergence of fungal pathogens resistant to these drugs is a growing concern [3, 23, 24]. Hence, we have conducted studies on 152 *Candida* isolates causing candidemia in central Taiwan, collected from 2004 to 2006, to determine the species distribution and their susceptibility profiles to five antifungal agents (amphotericin B, anidulafungin, fluconazole, micafungin, and voriconazole). We also compared the categorical agreement of minimum inhibitory concentrations (MICs) of amphotericin B, fluconazole, and voriconazole after 24- and 48-h incubation.

Materials and methods

Clinical isolates and patient age groups

All isolates causing candidemia were collected from October 2004 to December 2006 in central Taiwan at the China Medical University Hospital (CMUH), a medical center with approximately 2,000 beds. A total of 152 *Candida* isolates were collected and characterized in the present study. In addition to the sources of isolates, the patient age data were also recorded, and the patients were divided into three groups by age: <18 years, 19–65 years, and >65 years [25, 26].

Identification of isolates

All *Candida* isolates were first identified by the CMUH and reassessed in the laboratory at National Health Research Institutes (NHRI). The identification procedure at NHRI for the candidemia isolates was based on our previous reports [27–29]. All isolates were tested with the VITEK Yeast Biochemical Card (YBC) (bioMérieux, Marcy l'Etoile, France) for species identification. When the YBC identification probability was less than 90 % or when the identification of an organism was inconsistent between the hospital and the NHRI laboratories, sequences of the internal transcribed spacer (ITS) region and/or the D1/D2 region of ribosomal DNA were used for species identification. The ITS region was amplified with the primers ITS1, 5′-TCCGTAGGTGAACCTGCGG-3, and ITS4 5′-TCCTCCGCTTATTGATATGC-3′, and the D1/D2 region was amplified with the primers NL1 5′-GCATATCAAT AAGCGGAGGAAAAG-3′ and NL4 5′-GGTCCGTG TTTCAAGACGG-3′ [30, 31].

Antifungal susceptibility testing

The MICs of the five agents were determined by the same in vitro antifungal susceptibility testing established in our laboratory [32], according to the guidelines of M27-A3 recommended by the Clinical and Laboratory Standards Institute (CLSI) [33]. RPMI medium 1640 (31800-022; Gibco BRL, Gaithersburg, MD, USA) was used for growth and dilution of the yeasts. Strains from the American Type Culture Collection (ATCC), including *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019), were used as the standard controls. Growth of each isolate was measured by the Biotrak II plate spectrophotometric reader (Amersham Biosciences, Biochrom, Cambridge, England) after incubation at 35 °C for 24 h for all five drugs and 48 h for amphotericin B, fluconazole, and voriconazole.

Standard powders of amphotericin B kindly provided by Bristol Myers Squibb, anidulafungin, fluconazole, and voriconazole by Pfizer, and micafungin by Astellas Pharms were dissolved in dimethyl sulfoxide (DMSO). The final concentrations of anidulafungin, micafungin, and voriconazole were 0.0156–8 mg/l, amphotericin B, 0.0313–16 mg/l, and fluconazole, 0.125–64 mg/l.

The newly defined species-specific breakpoints after 24-h incubation for the five common *Candida* species *C. albicans*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* were applied in the present study [34]. For the species for which clinical breakpoints have not been established, we applied epidemiological cutoff values instead [34].

The MICs were defined as the concentration of drugs capable of reducing the turbidity of cells to greater than 50 % for anidulafungin, fluconazole, micafungin, and voriconazole and completely inhibiting cell growth for amphotericin B. The MICs of 50 % and 90 % of the total population were defined as MIC_{50} and MIC_{90}, respectively.

The epidemiological cutoff value of amphotericin B was 2 mg/l. For fluconazole, with *C. albicans*, *C. tropicalis*, and