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Species distribution and drug susceptibilities of *Candida* isolates in TSARY 2010 ☆

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ABSTRACT

Susceptibilities to antifungal drugs of 1083 *Candida* isolates collected in Taiwan Surveillance of Antimicrobial Resistance of Yeasts in 2010 were determined. There were 422 (39%) *C. albicans*, 270 (24.9%) *C. tropicalis*, 258 (23.8%) *C. glabrata*, 87 (8%) *C. parapsilosis*, 18 (1.7%) *C. krusei*, and 28 (2.6%) of 13 other species. In the present study, we have applied species-specific clinical breakpoints for common species and epidemiological cutoff values for rare species. We found that majority of isolates were susceptible to tested drugs. A total of 15, 3, 2, and 0 isolates were not susceptible to fluconazole, voriconazole, amphotericin B, and anidulafungin, respectively. We found that three of the four fluconazole non-susceptible *C. albicans* isolates were resistant to voriconazole. Hence, there is an issue of cross-resistance among azole-type drugs.

1. Introduction

The increased prevalence of fungal infections is due to the expanding number of immunocompromised patients, increased use of invasive medical devices, and extensive use of broad spectrum antibiotics (Pappas et al., 2009; Pfaller and Diekema, 2010; Yang and Lo, 2001). Hence, the epidemiology of fungal infections has become important in the last few decades. *Candida* species are the most frequently isolated fungal pathogens responsible for significant morbidity and mortality (Chen et al., 2006; Chen et al., 2011; Cheng et al., 2004; Pfaller and Diekema, 2007; Warnock, 2007; Yang et al., 2010) and they are leading causes of fungal infections in the world (Chuang et al., 2010; Colombo et al., 2006; Edwards, 1995; Pfaller and Diekema, 2010; Ruan and Hsueh, 2009; Warnock, 2007; Wisplinghoff et al., 2004; Yang et al., 2010). Among the 30 to 40 *Candida* species causing diseases in human (Johnson, 2009; Richardson, 1991), *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* are the five most common species causing candidiasis (Almirante et al., 2005; Colombo et al., 2006; Pfaller and Diekema, 2010; Yang et al., 2010).

Azoles (fluconazole and voriconazole), echinocandins (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 drug-resistant fungal pathogens is a growing concern in medical settings (Sanglard and Odds, 2002; White et al., 1998; Yang and Lo, 2001).

In 1999 and again in 2002 and 2006, three national surveys, Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY), have been conducted. The drug susceptibilities of the 632, 909, and 964 Candida isolates collected in 1999, 2002, and 2006 respectively, have been determined (Yang et al., 2004; Yang et al., 2005a; Yang et al., 2008). Among them, 0.5% (1999), 2.5% (2002), and 1.8% (2006) were with amphotericin B MICs ≧2 mg/L after 48-hour (h) incubation. There were 8.4%, 1.9%, and 17.1% of the isolates in 1999, 2002, and 2006, respectively, with fluconazole MICs ≧64 mg/L after 48 h (Yang et al., 2004; Yang et al., 2005a; Yang et al., 2008).

Among the phenomena associated with resistance, "trailing" describes the reduced but persistent growth that some isolates exhibit at drug concentrations above the MIC in broth dilution tests with azoles (Arthington-Skaggs et al., 2002; Lee et al., 2004). In TSARY 2006 (Yang et al., 2008), we have found, among the isolates with fluconazole MICs ≧64 mg/L after incubation for 48 h, 72.7% (96/132) *C. tropicalis*, 70% (7/10) *C. albicans*, and 44.4% (4/9) *C. glabrata* isolates exhibited the trailing phenomenon, whereas none of the *C. krusei* isolates did. Epidemiological cutoff values for *Candida* species and species-specific clinical breakpoints for fluconazole (Pfaller et al., 2010), voriconazole (Pfaller et al., 2011a), and the echinocandins (Pfaller et al., 2011b) for common species have been established to minimize the trailing and to support a shorter time for reporting MICs. In the present study, we have conducted a follow-up TSARY in 2010. We have applied newly defined species-specific breakpoints for fluconazole, voriconazole, and echinocandins for common species and epidemiological cutoff values for the rare species (Pfaller and Diekema, 2012) to investigate drug susceptibilities of 1083 Candida isolates.

☆ Declaration of interest: Authrors declare that they have no conflict of interest.

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2. Materials and methods

2.1. Organisms and media

Yeast isolates were collected from the 23 hospitals participating in TSARY from July 1 to September 30 in 2010 (Yang et al., 2013). Each hospital was asked to submit all yeast pathogens from sterile sites and the first ten *C. albicans* and 40 non-*C. albicans* yeast isolates from non-sterile sites. In principle, only one isolate was accepted from each specimen. Nevertheless, when there were multiple species isolated from one specimen, one isolate from each species was analyzed. All the collected isolates were stored frozen at −70 °C in vials containing 50% glycerol. After their arrival at the laboratory at National Health Research Institutes (NHRI), these isolates were first sub-cultured on sabouraud dextrose agar (SDA, BBL, Becton Dickinson Cockeysville, MD, USA) to assess the purity and identification. When there were more than one species from one frozen vial, the cells from the vial will be streaked onto CHROMagar *Candida* medium (BBL, Becton Dickinson Cockeysville, MD, USA) and pure single colony of each morphotype was labeled and stored in vials containing 50% glycerol at −70 °C awaiting further analyses.

2.1.1. Identification

The identifications of the isolates were first performed by the contributing TSARY hospitals and then reassessed in the laboratory at NHRI. The procedure for the identification was established previously (Lo et al., 2001; Yang et al., 2013). In brief, all isolates identified as *C. albicans* by the TSARY hospitals were subjected to germ tube assay in RPMI medium 1640 (Gibco BRL, 31800–022) containing 10% fetal bovine serum (GibcoBRL, US-628531). The germ tube-positive isolates failing to grow at 42 °C were identified by sequencing their ribosomal DNA (rDNA) (Leaw et al., 2007). All isolates identified as non-*C. albicans* by the TSARY hospitals were tested with VITEK 2 (bioMérieux, Marcy l'Etoile, France). The sequences of the internal transcribed spacer (ITS) and/or the D1/D2 regions of rDNA were used for species identification when one of the followings occurred: the identification probability of the VITEK 2 was less than 85%, the identification of an organism was inconsistent between the hospital and the NHRI laboratory, and when uncommon species were reported. The ITS regions were amplified by the primers ITS1, 5′-TCCGTAGGTGAACCTGCGG-3, and ITS4 5′-TCCTCCGCTTATTGATATGC-3′, and the D1/D2 regions were amplified by the primers NL1 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and NL4 5′-GGTCCGTGTTTCAAGACGG-3′ (Leaw et al., 2007). In addition, to determine whether *Candida nivariensis* isolates were recovered in the present study, we have streaked out all isolates identified as *C. glabrata* onto CHROMagar *Candida* medium (Alcoba-Florez et al., 2005b). The rDNA sequences of the cells showing white color on the CHROMagar *Candida* medium were then determined (Alcoba-Florez et al., 2005a).

2.1.2. Antifungal susceptibility testing

The MICs of the four antifungal agents were determined by the *in vitro* antifungal susceptibility testing established in our laboratory (Yang et al., 2004), according to the guidelines of M27-A3 recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2008). RPMI medium 1640 (31800–022, Gibco BRL) was used for growth and dilution of the yeast. Strains from 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. The 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.

Standard powders of amphotericin B, kindly provided by Bristol Myers Squibb, and anidulafungin, fluconazole, and voriconazole, by Pfizer, were dissolved in dimethal sulfoxide (DMSO). The final concentrations of the anidulafungin and voriconazole were from 0.0156 mg/L to 8 mg/L, amphotericin B, 0.0313–16 mg/L, and fluconazole, 0.125–64 mg/L.

The MICs were defined as the concentration of drugs capable of reducing the turbidity of cells to greater than 50% for anidulafungin, fluconazole, and voriconazole and completely inhibiting cell growth for amphotericin B. The epidemiological cutoff values for amphotericin B after 24-h incubation was 2 mg/L for all species (Pfaller et al., 2012). The newly defined species-specific breakpoints for the five common *Candida* species, *C. albicans*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* were applied in the present study (Pfaller and Diekema, 2012). For fluconazole, the clinical breakpoints of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* are as following: MICs ≤2 mg/L were considered to be susceptible, ≥8 mg/L resistant, and 4 mg/L susceptible-dose dependent (SDD); for *C. glabrata*, MICs ≤32 mg/L were SDD, ≥64 mg/L resistant. For voriconazole, the clinical breakpoints of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were MICs ≤0.125 mg/L susceptible, ≥1 mg/L resistant, and 0.25–0.5 mg/L intermediate. For *C. krusei*, MICs ≤0.5 mg/L were susceptible, ≥2 mg/L resistant, and 1 mg/L intermediate. For anidulafungin, the clinical breakpoints of *C. albicans*, *C. krusei*, and *C. tropicalis* were MICs ≤0.25 mg/L susceptible, ≥1 mg/L resistant, and 0.5 mg/L intermediate. For *C. parapsilosis* and *C. guilliermondii*, the breakpoints were changed to MICs ≤2 mg/L susceptible, ≥8 mg/L, resistant, and 4 mg/L, intermediate. For *C. glabrata*, they were ≤0.125 mg/L susceptible, ≥0.5 mg/L resistant, and 0.25 mg/L intermediate. For the species of which clinical breakpoints have not been established, we applied epidemiological cutoff values instead (Pfaller and Diekema, 2012). The MICs of 50% and 90% of the total population were defined as MIC_{50} and $MIC_{90,}$ respectively.

2.1.3. Database and analysis

The database for this study contained the characteristic information of each submitted isolate: hospital origin, location and type of the hospital, identification and source of the isolate. The statistical significance of the differences in frequencies and proportions was determined by the chi-square test with Mantel-Haenszel correction or fisher exact with 2-tailed correction.

3. Results

3.1. Distribution of Candida species

In the present study, we collected the first ten *C. albicans* and 40 non-*C. albicans* yeast isolates from non-sterile sites. Therefore, the prevalence of *C. albicans* was underestimated. Even so, it was still the most frequently isolated species in the present study, accounting for 39% of the total isolates. *Candida tropicalis* (270, 24.9%) and *C. glabrata* (258, 23.8%) were the two most frequently isolated non-*C. albicans Candida* species followed by *C. parapsilosis* (87, 8%), *C. krusei* (18,1.7%), *C. guilliermondii* (7, 0.6%), and others (21, 1.9%) (Table 1). When classified according to the sources, there were 489 (45.2%) isolates from urine, 213 (19.7%) from blood, 141 (13%) from sputum, 53 (4.9%) from catheter tip, 40 (3.7%) from wound, 33 (3%) from ascites, 28 (2.6%) from pus, 22 (2%) from bronchial washing, and 64 (5.9%) from other 23 different sites. *Candida albicans* was still the most common cause for candidemia (103, 48.4%), followed by *C. tropicalis* (41, 19.2%), *C. parapsilosis* (35, 16.4%), *C. glabrata* (22, 10.3%), *C. krusei* (2, 0.9%), and others (10, 4.7%).

3.2. Susceptibilities to amphotericin B and anidulafungin of Candida species

The range of amphotericin B MICs of the 1083 isolates was from 0.0131 to 2 mg/L (Table 2). The MIC_{50} and MIC_{90} were 0.25 and 0.5 mg/L, respectively. Only two *C. albicans* isolates were with amphotericin B MIC ≧2 mg/L. The range of anidulafungin MICs was from 0.0156