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ORIGINAL ARTICLE

An emerging issue of mixed yeast cultures

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Identification

Background: Different yeast species have different susceptibilities to commonly prescribed antifungal drugs. Thus, it is important to accurately determine the species of pathogenic yeasts, especially when more than one species are in a specimen.
Methods: Clinically significant yeast isolates were collected via the Taiwan Surveillance of Antimicrobial Resistance of Yeasts from July to September in 2010. The identifications of isolates were assessed in the core laboratory at the National Health Research Institutes.
Results: Of the 1127 isolates recovered, 1088 were of *Candida* genus, accounting for 96.53% of the total isolates, followed by *Cryptococcus* (15, 1.33%), *Trichosporon* (12, 1.06%), *Kodamaea* (4, 0.35%), *Pichia* (4, 0.35%), and three others. In all, 38 out of 1116 (3.4%) specimens had mixed yeast cultures. One ascites specimen had three species, *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*. In the remaining 37 specimens, 16 had a combination of *C. albicans* and *C. glabrata*, eight *C. albicans* and *C. tropicalis*, five *C. glabrata* and *C. tropicalis*, three *Candida krusei* and *C. tropicalis*, and five with different combinations.
Conclusion: The high prevalence of cultures with mixed yeasts may be an emerging issue. Thus, to determine mixed yeast cultures in the same specimen, we highly recommend CHROMagar Candida medium to culture yeast isolates directly from the specimen.

Introduction

Due to the increased size of the populations at risk, the prevalence of yeast nosocomial infections has increased in the past decades. In the United States, yeast infections

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rank as the fourth most common cause of nosocomial bloodstream infection.[1,2] In Taiwan, the prevalence of nosocomial candidemia increased 27-fold from 1981 through 1993.[3,4] *Candida* species are the most frequently isolated fungal pathogens causing morbidity and mortality in seriously immunocompromised hosts. Although *Candida albicans* is still the most prominent cause of candidemia, the prevalence of non-*C. albicans* yeast species has increased.[5–8]

Candida krusei, *Candida glabrata*, and *Candida tropicalis* are less susceptible to fluconazole than other *Candida* species.[8–14] *Candida lusitaniae* is relatively resistant to amphotericin B.[15] Accurate identification to the species level is therefore crucial for clinical management, since different species have various degrees of susceptibility to common antifungal drugs.

Recently, we reported that among healthy volunteers, 5% had yeast colonization in oral cavities and 6.1% of those incidences were by multiple species.[16] Furthermore, we have also found that there has been an increase in the number of HIV-infected outpatients colonized by more than one species (from 7.9% before 2002 to 19.9% in 2005 in a Medical center in northern Taiwan and 18.7% and 20.4% in 2009 in a Medical center and a regional hospital, respectively, in southern Taiwan).[17–19] In the present study, we characterized isolates collected in the Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 2010. The data indicated that mixed yeast colonization is an emerging issue.

Materials and methods

Organisms and media

From the 23 hospitals participating in TSARY, yeast isolates were collected according to procedures reported in previous studies[14,20] from July 1 to September 30, 2010. Each hospital was asked to submit all yeast pathogens from sterile sites and the first 10 *C. albicans* and 40 non-*C. albicans* yeast isolates from non-sterile sites to the core laboratory at National Health Research Institutes (NHRI). Due to the collection criteria, the percentage of *C. albicans* from different sources was not calculated. 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 at −70°C in vials containing 50% glycerol.

Identification

Primary identification of the isolates was performed by the contributing TSARY hospitals. Then, the results were reassessed in the laboratory at the NHRI. All isolates identified as *C. albicans* by the TSARY hospitals were subjected to germ tube assay in media containing 10% fetal bovine serum (GibcoBRL, US-628531, Gel Company, CA, USA) at 37°C for 3—4 hours. The germ tube-positive isolates that failed to grow at 42°C were further analyzed by sequencing ribosomal DNA (rDNA).[21] All isolates identified as non-*C. albicans* yeasts by the TSARY hospitals were subjected to VITEK 2 (bioMérieux, Marcy l'Etoile, France). Cells in the vials were streaked onto CHROMagar Candida medium (CHROMagar, Paris, France) and the sequences of the rDNA were used for identification when one of the followings occurred: the identification probability of the VITEK 2 (bioMérieux, Marcy l'Etoile, France) was less than 85%; the identification of an organism was inconsistent between the hospital and the NHRI laboratories; uncommon species were reported; and potential mixed yeast cultures were observed during the germ tube assay. The internal transcribed spacer (ITS) region was amplified by the primers ITS1, 5′-TCCGTAGGTGAACCTGCGG-3′, and ITS4 5′-TCCTCCGCTTATTGATATGC-3′ and/or the D1/D2 region of rDNA was amplified by the primers NL1 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and NL4 5′-GGTCCGTGTTTCAAGACGG-3′.[21]

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 procedure for yeast identification used by each hospital was also collected. The statistical significance of the differences in frequencies and proportions was determined using the chi-square test with Mantel-Haenszel correction.

Results

Distribution of body sites

The mean number of isolates collected from the hospitals participating in the TSARY was 49 (ranging from 8 to 100) per hospital. The distribution of the 1127 isolates in different body sites is shown in Table 1. Among 34 different body sites, urine (44.3%) was the most common source of yeast clinical isolates, followed by blood (19.8%), sputum (13.1%), catheter tip (4.8%), wound (3.7%), ascites (2.9%), pus (2.7%), bronchial washing (2%), and 23 other different body sites (6.7%).

Distribution of species

Candida was the most common genus found, accounting for 96.53% (1088/1127) of the total isolates, followed by *Cryptococcus* (15, 1.33%), *Trichosporon* (12, 1.06%), *Kodamaea* (4, 0.35%), *Pichia* (4, 0.35%), and one each of *Rhodosporidium*, *Rhodotorula*, and *Sterigmatomyces*. Among the 223 isolates recovered from blood, *C. albicans* (103, 46.2%) was the most common species, followed by *C. tropicalis* (41, 18.4%), *C. parapsilosis* (35, 15.7%), *C. glabrata* (22, 9.8%), *Cryptococcus neoformans* (8, 3.6%), *C. krusei* (2, 0.9%) and 10 other species (12, 5.4%), see Table 1. Although the prevalence of *C. albicans* was underestimated due to the collection criteria, it was still the most common species among the 1127 isolates collected in the present study (423 isolates, 37.6%). *C. tropicalis* (270, 24%) and *C. glabrata* (262, 23.2%) were the two major non-*C. albicans* yeast species, followed by *C. parapsilosis* (87, 7.7%), *C. krusei*