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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.

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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 lusitanae* 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 *Rhodospiridium*, *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*

Table 1 The distribution of body sites and yeast species

Source	<i>C. albicans</i> *		<i>C. tropicalis</i>		<i>C. glabrata</i>		<i>C. parapsilosis</i>		<i>C. krusei</i>		<i>C. neoformans</i>		Others		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Urine	133	50.7	137	50.7	201	76.7	14	16.1	3	16.7	0	0.0	11	21.2	499	44.3
Blood	103	15.2	41	15.2	22	8.4	35	40.2	2	11.1	8	53.3	12	23.1	223	19.8
Sputum	69	17.8	48	17.8	14	5.3	1	1.1	7	38.9	0	0	9	17.3	148	13.1
Catheter tip	24	5.2	14	5.2	5	1.9	7	8.0	1	5.6	1	6.7	2	3.8	54	4.8
Wound	17	2.6	7	2.6	4	1.5	9	10.3	0	0	0	0	5	9.6	42	3.7
Ascites	23	1.1	3	1.1	6	2.3	0	0	0	0	0	0	1	1.9	33	2.9
Pus	17	1.9	5	1.9	1	0.4	4	4.6	1	5.6	0	0	2	3.8	30	2.7
Bronch washing	11	2.6	7	2.6	2	0.8	0	0	1	5.6	0	0	1	1.9	22	2.0
Others	26	3.0	8	3.0	7	2.7	17	19.5	3	16.7	6	40.0	9	17.3	76	6.7
Total	423	100	270	100	262	100	87	100	18	100	15	100	52	100	1127	100

* Due to the collection criteria, the percentage of *C. albicans* from different sources was not calculated.

(18, 1.6%), *C. neoformans* (15, 1.3%), and other species (52, 4.6%), see Table 1.

Mixed yeast cultures

Since we asked the hospitals to collect the first 10 *C. albicans* and 40 non-*C. albicans* yeast isolates from non-sterile sites, there is a possibility that the specimens in the vials just represented part of those cultured in the hospital laboratories. Thus, the rate (38/1116, 3.4%) of mixed yeast cultures in the present study is most likely underestimated. Among the 38 specimens containing mixed yeast cultures, 16 were from urine, 13 from sputum, two from ascites, two from blood, one each from bronchial washing, catheter tip, nail, skin, and pus (Table 2). *C. albicans* appeared to be the most common species co-recovered with others, accounting for 73.7% of the cases, followed by *C. glabrata* (57.9%) and *C. tropicalis* (44.7%). These three species were co-recovered from one ascites specimen. Furthermore, 16 out of the remaining 37 specimens had a combination of *C. albicans* and *C. glabrata*.

Methods of identification

Different participating hospitals applied different combinations of methods for yeast identification in the present study. CHROMagar Candida medium (52.2%) was the most frequently used method by the 23 participating hospitals, followed by germ tube assay (47.8%), cornmeal agar (43.5%), ID20C (bioMérieux, Marcy l'Etoile, France) (30.4%), ID32C (bioMérieux, Marcy l'Etoile, France) (21.7%), VITEK 2 (17.4%), VITEK Yeast Biochemical Card (bioMérieux, Marcy l'Etoile, France) (8.7%), the assimilation method (8.7%), eosin-methylene blue (4.3%), Gram staining (4.3%), microscan (Siemens Healthcare Diagnostics, West Sacramento, CA, USA) (4.3%), and rapid yeast (Innovative Diagnostic Systems, Norcross, GA, USA) (4.3%).

Even though we requested the participating hospitals keep only one species in an individual vial, 18 of the 38 mixed yeast cultures were not identified by the hospitals. Four hospitals contributed one vial containing mixed yeast cultures, two contributed two such vials, one hospital contributed four and another hospital six such vials. The majority of the vials (12/18) were contributed by hospitals using methods other than the CHROMagar Candida medium one.

In general, on CHROMagar Candida medium, *C. albicans* colonies were green, *C. glabrata* were pink to dark purple, *C. krusei* fuzzy rose with white edges, *C. parapsilosis* white, and *C. tropicalis* steel blue accompanied by purple pigment diffusion into the surrounding agar (Fig. 1A). When the cells from the 18 vials containing mixed yeast culture were streaked onto CHROMagar Candida medium in the NHRI laboratory, 16 had colonies in two different colors. The combination of *C. albicans* and *C. glabrata* (Fig. 1A a–j), *C. krusei* and *C. tropicalis* (Fig. 1A o–p), *C. glabrata* and *C. tropicalis* (Fig. 1A q), as well as *C. albicans* and *C. parapsilosis* (Fig. 1A r), could be distinguished when the cells were streaked onto CHROMagar Candida medium. However, under this condition, *C. albicans* from two vials containing the combination of *C. albicans* and *C. tropicalis*

Table 2 The distribution of yeast species in specimens having mixed yeast cultures

Source	<i>C. albicans</i>				<i>C. tropicalis</i>				<i>Kodamaea ohmeri</i> .	
	<i>C. glabrata</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>	<i>C. catenulata</i>	<i>C. nivariensis</i>	<i>C. parapsilosis</i>	<i>C. glabrata</i>	<i>C. krusei</i>	<i>C. parapsilosis</i>	<i>C. guilliermondii</i>
Urine (16)*	0	8 (5) [†]	4 (3)	0	0	0	4 (1)	0	0	0
Sputum (13)	0	6 (5)	3 (1)	0	1	0	1	1 (1)	1	0
Ascites (2)	1	1	0	0	0	0	0	0	0	0
Blood (2)	0	1	0	1	0	0	0	0	0	0
Bronchial washing (1)	0	0	0	0	0	0	0	1	0	0
Catheter tip (1)	0	0	1	0	0	0	0	0	0	0
Nail (1)	0	0	0	0	0	0	0	0	0	1
Skin (1)	0	0	0	0	0	1 (1)	0	0	0	0
Pus (1)	0	0	0	0	0	0	0	1 (1)	0	0
Total (38)	1	16 (10)	8 (4)	1	1	1 (1)	5 (1)	3 (2)	1	1

* Number of specimens.

[†] Total number of specimens having mixed yeast cultures (number of specimens having mixed yeast cultures identified by the NHRI laboratory).

failed to show up (Fig. 1A k and l). When streaking more cells (one vial per agar plate) from the oYM100104 and oYM100109 vials onto CHROMagar Candida medium, we were able to find several green colonies (arrows) of *C. albicans* from the oYM100104 vial (Fig. 2A) but not from the oYM100109 (Fig. 2B). When the mixed yeast cells were patched onto CHROMagar Candida medium (Fig. 1B), only the color of the dominant species would show up.

Discussion

The distribution of species in the present study was similar to those in previous TSARYs.^{12,14,20} With the exception of blood, the distributions of species found at various body sites in the present study were not significantly different from those in previous TSARY surveys.^{14,20,22} The reason that the proportion of isolates from blood in the present study (223/1127) was higher than those in the 2002 (130/945 $p = 0.0003$) and 2006 TSARY (160/1015 $p = 0.02$) needs further investigation.

Among the 38 specimens containing mixed yeast cultures, 17 had a combination of *C. albicans* and *C. glabrata*. Since the clinical treatments for infections caused by these two species may be different,^{8,11,23} it is important to accurately differentiate these two species, especially when they are present in the same specimen.

There are some limitations of CHROMagar Candida medium. For example, when the ratio of two species was significantly different, the medium failed to detect the minority (Figs. 1A k and l, and 2B). The medium is not capable of detecting mixed yeast cultures in the same specimen if the cells were patched onto it (Fig. 1B). The fact that in the present study, the hospital using only CHROMagar Candida medium for yeast identification misidentified *C. parapsilosis* as *C. glabrata* demonstrates that CHROMagar Candida medium provides presumptive but not definite identification of yeast species. Despite the limitations, CHROMagar Candida is still a helpful tool in facilitating the recognition of mixed yeast cultures.²⁴ Application of CHROMagar Candida medium to culture yeast isolates directly from the specimen is therefore highly recommended, particularly the specimens from patients who did not respond well to the treatment or the specimens from the sterile sites. The most critical point is that hospital technologists should be aware of the increasing possibility that one patient may be infected by more than one fungal species.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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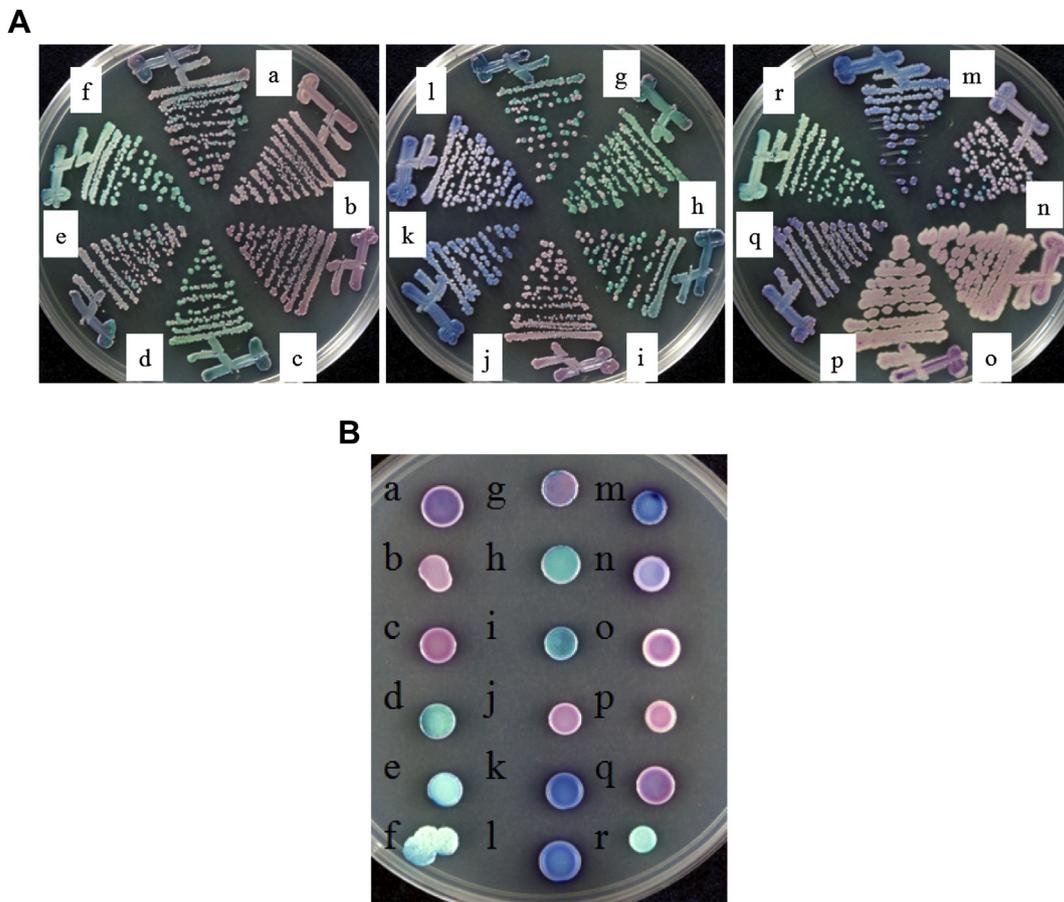


Figure 1. Mixed yeast cultures on CHROMagar Candida medium. Cells from 18 vials containing mixed yeast cells were (A) streaked and (B) patched onto CHROMagar Candida medium and then incubated at 35°C for 48 hours. (a) oYM100085, (b) oYM100108, (c) oYM100482, (d) oYM100835, (e) oYM100839, (f) oYM100858, (g) oYM100863, (h) oYM100866, (i) oYM100870, and (j) oYM101014, with *C. albicans* and *C. glabrata*; (k) oYM100104, (l) oYM100109, (m) oYM100865, and (n) oYM1001079 with *C. albicans* and *C. tropicalis*; (o) oYM100125 and (p) oYM100096 with *C. krusei* and *C. tropicalis*; (q) oYM100513 with *C. glabrata* and *C. tropicalis*, and (r) oYM100961, with *C. albicans* and *C. parapsilosis*.

Christian Hospital, Chung Shan Medical Dental College Hospital, Hua-Lien Hospital, DOH, the Executive Yuan, Kaohsiung Military Hospital, Kuan-Tien General Hospital,

Lo-Hsu Foundation Inc. Lo-Tung Poh Ai Hospital, Miin Sheng General Hospital, National Cheng Kung University Hospital, Shalu Tung's Memorial Hospital, Show Chwan Memorial

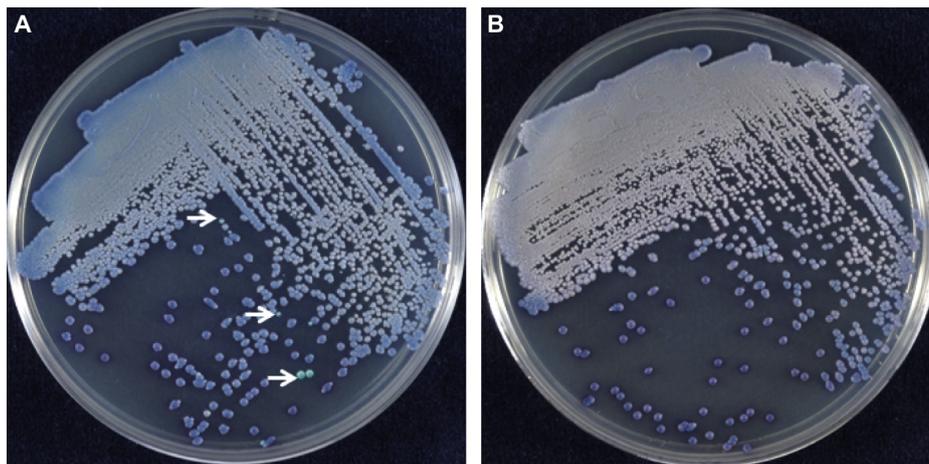


Figure 2. Cells from (A) oYM100104 and (B) oYM100109 vials containing mixed *C. albicans* and *C. glabrata* were streaked onto CHROMagar Candida medium and then incubated at 35°C for 48 hours.

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