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Original Article

Surveillance of pathogenic yeasts in hospital environments in Taiwan in 2020

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Abstract *Background:* A predominate azole-resistant *Candida tropicalis* clade 4 genotype causing candidemia has been detected in not only Taiwan but also China, Singapore, and Australia. It can also be detected on fruit surfaces. In addition to determining distribution and drug susceptibilities of pathogenic yeasts in environments of intensive care units of 25 hospitals in Taiwan, we would also like to investigate whether the azole-resistant *C. tropicalis* exists in Taiwan's hospital environment.
Methods: The swabs of hospital environments were collected from August to November in 2020 and were cultured for yeasts. The yeasts were identified by rDNA sequence and the antifungal susceptibilities of those isolates were determined by the broth microdilution method.
Results: The average yeast-culture rate of hospitals was 9.4% (217/2299). Sinks had the highest yeast-positive culture rate (32.7%), followed by bedside tables (28.9%), floors (26.0%), water-dispenser buttons (23.8%), and TV controller/touch panels (19.0%). Of 262 identified isolates, *Candida parapsilosis* was the most common species, accounting for 22.1%, followed by *Filobasidium uniguttulatum* (18.3%), *Candida albicans* (9.5%), *C. tropicalis* (8.0%), *Candida glabrata* (*Nakaseomyces glabratus*) (6.9%), and 30 other species (35.1%). Of the 21 *C. tropicalis* isolates from 11 units in 9 hospitals, 15 diploid sequence types (DSTs) were identified. The two DST506 fluconazole-resistant ones belonged to clade 4.
Conclusion: We detected not only various pathogenic yeast species but also the predominant clade 4 genotype of azole-resistant *C. tropicalis*. Our findings highlight and re-emphasize the importance of regular cleaning and disinfection practices.

Introduction

Fungal infections range from superficial to life-threatening and are associated with approximately 1.5 million deaths annually,[1] of which half are due to invasive candidiasis.[2] Infection-causing *Candida* species can be acquired either endogenously[3] or exogenously, including from the environment.[4] Recently, several reports demonstrated the possibility of transmission of the emerging multidrug-resistant *Candida auris* in healthcare settings,[5] making a better understanding of the sources of acquisition of *Candida* species urgently needed.

Even though *Candida* is not airborne, like multidrug-resistant *C. auris* causing outbreaks in the healthcare settings,[6] *C. parapsilosis* can be transmitted by various human sources.[7,8] During COVID-19 pandemic, there were several outbreaks caused by drug-resistant *C. parapsilosis*.[9—13] Furthermore, hospital sinks have been reported to be frequently contaminated with *Candida* species. Splattering of water from the sink drain results in dispersal *Candida* species to the sink bowl and countertop.[14] Hence, contaminated sinks and other areas may be a potential source for dissemination of *Candida* species.

To monitor trends of species distribution and drug susceptibility of clinical yeast pathogens, we initiated a national survey, the Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY), in 1999. To date, six TSARYs have been conducted.[15—17] We found that *Candida tropicalis* is the most common non-albicans *Candida* species causing diseases in Taiwan.[18] Moreover, we found that *C. tropicalis* has a higher fluconazole-resistant rate than other *Candida* species.[19] Recently, we reported that a predominate azole-resistant *C. tropicalis* genotype circulated in Taiwan widely from 2014 to 2018.[20,21] In addition, the clade 4 azole-resistant *C. tropicalis* was detected on the surface of fruits in a supermarket.[17]

C. tropicalis is often found in intensive care unit (ICU) patients, accounting for approximately 5%—10% of ICU yeast infections.[22] Cross-contamination is a major risk factor for outbreaks caused by *C. tropicalis* in ICUs. To investigate the distribution of pathogenic yeasts, especially azole-resistant *C. tropicalis* in ICUs, we conducted a surveillance of pathogenic yeasts in ICUs of 25 hospitals in Taiwan.

Materials and methods

Sampling

The samples were collected in 25 hospital ICU environments from August to November 2020 and sent to the National Health Research Institutes for testing. Medical centres were defined according to government guidelines (https://www.mohw.gov.tw/np-129-2.html). Every site was sampled by a sponge swab (EZ Culturette; Becton Dickinson, Sparks, MD) pre-moistened with sterile saline before daily environmental cleaning. One public-area site, the water-dispenser button, was sampled. At nurse stations, eight sites were sampled: chart, computer mouse, desk/table, dressing cart, ipad, nurse-station call button, nursing cart, and telephone. Around beds, 22 sites were sampled: bed control, bed table, bedrail, bedrail switch, bedside table, curtain, doorknob, electrocardiogram wire, feeding pump, feeding syringe, floor, intravenous pole, IV pump, light switch, oxygen flow meter, physical monitor panel, sink, sphygmomanometer cuff, suction-flow meter, TV controller/touch panel, ventilator panel, and wall. In general, samples from nurse stations in both medicine and surgery departments were collected. But four hospitals had only one ICU nurse station. Thus, only 8 swabs around a nurse station were sent. Each hospital sampled environments from four beds. Thus, each hospital would send a total of 88 swabs of environment around beds. Nevertheless, there are less swabs collected from hospitals when the patients did not use the devices.

Microbiologic processing

All swabs were maintained at room temperature and transported to the laboratory within 24 h. Yeasts were cultured by streaking the swab on Yeast Extract Peptone Dextrose (YPD) agar medium (BBL) containing 0.5 mg/ml chloramphenicol. Plates were incubated at 25 °C for 3 days. Swabs were kept at 4 °C for further procedure. To determine whether yeasts on the YPD medium had the same morphotype, we transferred all colonies onto Chromagar Candida (BBL) medium when the colony number on the YPD medium was less than 10. When the colony number was greater than 10, we streaked the original swab kept at 4 °C on Chromagar Candida (BBL) medium. Three independent colonies (if available) of each morphotype were selected from each positive culture on Chromagar Candida. The sequences of the internal transcribed spacer (ITS) region and/or the D1/D2 region of ribosomal DNA were used for species identification. Table S1 lists the primers.[23] Trichosporon was further identified according to the sequence of intergenic spacer regions.[24]

Antifungal susceptibility testing

Drug susceptibilities of *Candida* species were determined by *in vitro* antifungal susceptibility testing established in the core laboratory,[16] according to the Clinical and Laboratory Standards Institute's M27-A3 guideline.[25] 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), *Candida krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019), were used as the standard controls. The growth of each isolate was measured by Multiskan FC Microplate Photometer (Thermo Fisher Scientific, USA) after incubation at 35°C for 24 and 48 h.

Standard powders of fluconazole, voriconazole, and anidulafungin (Pfizer) and amphotericin B (A10073, Adooq Bioscience) were dissolved in dimethyl sulfoxide (DMSO). Final concentrations were 0.125—64 mg/L (fluconazole), 0.0156—8 mg/L (anidulafungin and voriconazole), and 0.0313—16 mg/L (amphotericin B).

MICs were defined as the concentration of drugs capable of reducing the turbidity of cells to greater than 50% for fluconazole, voriconazole, and anidulafungin. Amphotericin B completely inhibited cell growth. The epidemiological cut-off value for amphotericin B after 24 h incubation was 2 mg/L for all species.[26] Newly defined species-specific breakpoints for common *Candida* species (*C. albicans*, *C. glabrata* (*N. glabratus*), *C. tropicalis*, and *C. parapsilosis*) were applied.[27] For fluconazole, the clinical breakpoints of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were as follows: MICs $\leq$2 mg/L were considered susceptible, $\geq$8 mg/L resistant, and 4 mg/L susceptible-dose dependent (SDD). For *C. glabrata*, MICs $\leq$32 mg/L were SDD, $\geq$64 mg/L resistant. For voriconazole, the clinical breakpoints of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were MICs $\leq$0.125 mg/L susceptible, $\geq$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 SDD. Breakpoints for *C. glabrata* remain undetermined. For anidulafungin, the clinical breakpoints of *C. albicans* and *C. tropicalis* were MICs ≤0.25 mg/L susceptible, ≥1 mg/L resistant, and 0.5 mg/L intermediate. For *C. parapsilosis* and *Candida guilliermondii*, the breakpoints were ≤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. The MICs of 50% and 90% of the total population were defined as MIC_{50} and MIC_{90}, respectively.

When the MIC of an isolate measured after 48 h incubation was approximately 4-fold higher than at the 24 h point,[28] the isolate was defined as having trailing growth phenotype.

Multilocus sequence typing

Multilocus sequence typing (MLST) for *C. tropicalis* was conducted as in previous reports[21,29,30] Table S1 lists the primers. Phylogenetic analysis was performed using MEGA X software's unweighted pair group method with arithmetic average (UPGMA).[31] A cut-off *P* distance of 0.01 was chosen because it separated clades that contained known examples of isolates. The system could not provide an allele number of *SAPT 4* gene from both HE200006 and HE200017 isolated from the same bed site since a position of the DNA fragment had three signals. We constructed a DST-based phylogenetic tree global *C. tropicalis* comprised of 1368 DSTs according to the *C. tropicalis* MLST database, and clades containing more than 10 genetically closely related DSTs were labelled.

Statistical methods

SPSS software for Windows (version 12.0) was used to analyse the data. Since yeast-culture positive rate of the present study was low (9.4%, 217/2299), we analysed 262 identified isolates from 217 samples to determine the prevalence of different species at different sampling sites, location or type of hospitals. No report shows that there is an association of occurrence among pathogenic yeast species in hospital environment. Hence, the 262 isolates were assumed to be independent from each another even though more than one isolate were detected in one specimen.

The chi-squared or Fisher's exact test with 1-tailed correction was applied for categorical variables to determine whether specific sampling sites distributed differently in type and location of hospitals, ward, and different yeast species. We also determined whether occurrence of different yeast species was associated with type and location of hospitals. To avoid the problem of small sample sizes, we re-grouped regions into south and non-south in the following analysis. We conducted Logistic regression to assess the independent effects of factors, including type (medical center vs. non-medical center) and location (south vs. non-south) of hospitals, type (general, medical, or surgical) of wards and yeast species (one species vs. remaining species) controlling for confounders. To further determine the association between occurrence of yeast species with factors, we conducted a full logistic regression model including types (medical center vs. non-medical center) and locations (south vs. non-south) of hospitals. A p value less than 0.05 was considered significant.

Results

Sample collections

A total of 2299 swabs (79—106 per hospital) were collected from 25 hospitals. The average yeast-culture rate of hospital was 9.4% (217/2299), ranging from 0 to 26.3%. The yeast-culture positive rate varied at different sampling sites and types and locations of hospitals. Of the 33 yeast-positive culture sites, yeasts were detected and recovered from sinks (32.7%, 32/98), bedside tables (28.9%, 28/97), floors (26.0%, 26/100), water-dispenser buttons (23.8%, 10/42), TV controller/touch panels (19.0%, 4/21) from, 18.9% (7/37) from feeding pump, 16.2% (6/37) nurse-station call button, 15.3% (9/59) from bed table, 10.6% (10/94) from sphygmomanometer cuff, 10.0% (10/100) from suction-flow meter, 9.1% (3/33) from ipads, and ventilator panels and others (8.3%, 7/84).

Some samples had more than one species detected (Table 1). Samples from different departments of hospitals had similar yeast-culture positive rates. According to the results of logistic regression analysis, water-dispenser buttons in medical centre have higher yeast-culture positive rate than those in non-medical centre (OR, 4.602; 95% CI 1.353—15.644; p = 0.014). Water-dispenser buttons (OR, 5.598; 95% CI 1.655—18.93; p = 0.006) from hospitals in central Taiwan have higher yeast-culture positive rate than those in other regions. In addition, floor from hospitals in southern Taiwan have higher yeast-culture positive rate than those in other regions (OR, 3.565; 95% CI 1.561—8.141; p = 0.003).

Distribution of species

A total of 262 isolates were identified (Table 1), covering 16 genera and 35 species, of which 24 have been reported as able to cause diseases in humans. Of those 249 potential human pathogens, 157 (63.1%) belonged to *Candida* species. *C. parapsilosis* was the most common species identified, accounting for 22.1%, followed by *F. uniguttulatum* (18.3%), *C. albicans* (9.5%), *C. tropicalis* (8.0%), *C. glabrata* (6.9%), *Candida haemulonis* (5.3%), *F. magnum* (3.8%), and 28 other species (26.0%).

The distribution of species varied at different sampling sites according to the results of logistic regression analysis. Less *C. parapsilosis* was detected in the floor samples than other sites (OR, 0.071; 95% CI 0.009—0.554; p = 0.012). More *C. tropicalis* were detected on suction-flow meters (OR, 16.539; 95% CI 4.243—64.473; p < 0.001) and bed table (OR, 8.116; 95% CI 1.689—39.008; p = 0.009) than other sites. There were more *C. haemulonis* detected in sink (OR, 8.879; 95% CI 2.764—28.523; p < 0.001), *F. uniguttulatum* in feeding pump (OR, 9.127; 95% CI 2.041—40.82; p = 0.004), *C. glabrata* in electrocardiogram wire (OR, 20.495; 95% CI 2.205—190.496; p = 0.008), *Cystobasidium slooffiae* in light switch (OR, 46.863; 95% CI 1.328—1654.01;

Table 1 Distribution of 262 yeast isolates according to sampling sites.

	Sink	Bedside table	Floor	Water dispenser button	Sphygmo-manometer cuff	Suction flow meter	Bed table	Wall	Bedrail switch	Feeding pump	Ventilator panel	Light switch	Nurse station call button	Bedrail	Curtain	Electro-cardiogram wire	Feeding syringe	IV pole	TV controller/ touch panel	Other 14 sites[c]	Total
Total	43	34	33	15	12	12	10	10	8	8	7	6	6	5	5	5	5	5	4	29	262
%	16.4	13.0	12.6	5.7	4.6	4.6	3.8	3.8	3.1	3.1	2.7	2.3	2.3	1.9	1.9	1.9	1.9	1.9	1.5	11.1	100
Type of hospital																					
MC (12)	21	10	16	10*	5	4	2	2	1	3	0	0*	2	1	2	2	5*	1	0	11	98
non-MC (13)	22	24	17	5	7	8	8	8	7	5	7	6	4	4	3	3	0	4	4	18	164
Location of hospital																					
Central (8)	16	16	9	11*	2	7	3	2	1	2	2	1	2	1	1	0	0	1	0	9	86
North (7)	15	7	8	3	6	2	3	6	4	1	3	1	3	1	3	1	5*	1	1	4	78
South (7)	8	6	13*	0*	1	2	2	1	2	2	1	0	1	1	0	3	0	1	3*	8	55
East (3)	4	5	3	1	3	1	2	1	1	3	1	4*	0	2	1	1	0	2	0	8	43
Ward																					
Surgical	19	14	14	9	4	7	6	0	2	5	1	3	3	2	3	2	3	3	1	21	122
Medical	20	14	16	6	6	4	4	4	1	3	4	2	2	2	2	3	2	1	3	7	106
General	4	6	3	0	2	1	0	6	5	0	2	1	1	1	0	0	0	1	0	1	34
Species																					
Candida parapsilosis[a]	7	6	1*	3	5	1	4	1	2	1	2	2	1	3	1	2	0	2	3*	11	58
Filobasidium uniguttulatum[a]	8	7	9	3	1	0	0	4	1	5*	1	1	0	0	1	0	0	2	0	5	48
Candida albicans[a]	2	6	4	0	3	1	3	0	0	0	0	1	0	0	0	1	3	0	0	1	25
Candida tropicalis[a]	5	1	2	0	1	6*	3*	0	0	1	0	0	0	0	0	0	1	0	0	1	21
Candida glabrata[a]	2	2	4	0	1	2	0	0	0	0	0	0	0	1	0	2*	1	0	0	3	18
Candida haemulonis[a]	8*	0	2	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	1	14
Filobasidium magnum[a]	3	0	0	1	0	1	0	1	0	1	1	0	0	0	1	0	0	0	0	1	10
Candida guilliermondii[a]	1	2	2	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	9
Cryptococcus liquefaciens[a]	0	3	0	3*	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	8
Candida pararugosa[a]	2	0	1	2*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Cryptococcus curvatus[a]	0	2	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	5
Cryptococcus flavescens[a]	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	4
Trichosporon asahii[a]	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	4
Candida lusitaniae[a]	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	3
Cryptococcus diffluens[a]	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	3
Candida duobushaemulonis[a]	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
Cystobasidium slooffiae	0	0	1	0	0	0	0	0	0	0	0	1*	0	0	0	0	0	0	0	0	2
Moesziomyces antarcticus[a]	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
Rhodosporidium paludigenum	0	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	0	0	0	1	2
Rhodotorula dairenensis[a]	0	0	2*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Trichosporon dermatis[a]	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2
Other 14 spp.[b]	1	2	3	3	0	1	0	0	0	0	0	0	2	0	0	0	0	1	1	0	14

[a] Species causing diseases in human.*$P < 0.05$.

[b] One each of Arthrographis kalraea, *Cystobasidium calyptogenae*, and Pseudozyma siamensis from floor; Candida oleophila, Pichia caribbica, and *Pichia kluyveri* from water dispenser button; Cyberlindnera fabianiia and *Rhodotorula diobovata* from nurse station call button; *Cystobasidium oligophagum* and *Sterigmatomyces elviae* from bedside table; Pseudozyma aphidisa from IV pole; Candida metapsilosisa from TV controller/touch panel; Wickerhamomyces anomalusa from sink; and Zalaria obscura from suction flow meter.

[c] 3 each of bedside call button, desk/table, ipad, IV pump, and oxygen flow meter; 2 each of bed control, chart, doorknob, nursing cart, and physical monitor panel; one of each computer mouse, dressing cart, telephone, and vibratory vest.

MC, medical center.

p = 0.034), and *Rhodosporidium paludigenum* in nurse-station call button (OR, 60.456; 95% CI 2.644–1382.48; p = 0.01), respectively, than other sites.

The distribution of species varied at different locations and types of hospitals (Table 2). More *F. magnum*, *C. haemulonis*, and *Cryptococcus flavescens* were detected in central (OR, 8.839; 95% CI 1.83–42.917; p = 0.007), northern (OR, 3.39; 95% CI 1.093–10.516; p = 0.035), and southern Taiwan (OR, 16.069; 95% CI 1.465–176.264; p = 0.023), respectively, than other regions. In medical centres, more *F. uniguttulatum* (OR, 2.489; 95% CI 1.24–4.996; p = 0.01) and *C. glabrata* (OR, 3.69; 95% CI 1.115–12.21; p = 0.032) were found but fewer *C. parapsilosis* (OR, 0.378; 95% CI 0.193–0.739; p = 0.004). The present survey did not detect *C. auris* or *C. krusei* (*Pichia kudriavzevii*).

Susceptibilities to antifungal drugs

Of the 158 *Candida* species, 21 isolates (14 *C. haemulonis*, 6 *Candida pararugosa*, and 1 *Candida oleophila*) failed to grow in RPMI medium. Susceptibilities to clinical antifungal drugs of the remaining 137 isolates were determined (Table 3). The MIC_{50} and MIC_{90} of amphotericin B were 0.5 mg/L and 1 mg/L, respectively. All but four isolates (one each of *Candida duobushaemulonis*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*) were with MIC ≥2. The MIC_{50} and MIC_{90} of anidulafungin were 0.0625 mg/L and 1 mg/L, respectively. As expected, *C. parapsilosis* and *C. guillermondii* were with higher anidulafungin MICs than other species. The MIC_{50} and MIC_{90} of fluconazole were 0.25 mg/L and 1 mg/L, respectively. Five isolates (2 *C. tropicalis*, one each of *C. duobushaemulonis*, *C. glabrata*, and *C. guillermondii*) had fluconazole MICs ≥8 mg/L. The MIC_{50} and MIC_{90} of voriconazole were 0.0156 and 0.0313 mg/L, respectively. The two fluconazole-resistant *C. tropicalis* isolates, from bed table and feeding pump from one patient having surgery in a hospital located in central region, were cross-resistant to voriconazole.

Genotypes of *C. tropicalis*

Of the 21 *C. tropicalis* isolates from 11 units of 9 hospitals, 15 DSTs were identified (Table 4 and S2). The two azole-

Table 2 Distribution of 262 yeast isolates according to location and type of hospitals.

Species	Location				Type		Total
	Central (8)	North (7)	South (7)	East (3)	MC (12)	Non-MC (13)	
Candida parapsilosis[a]	14	14	18[c]*	12	15[c]*	43	58
Filobasidium uniguttulatum[a]	20	9	8	11	25[b]*	23	48
Candida albicans[a]	7	11	1[c]*	6	8	17	25
Candida tropicalis[a]	9	5	5	2	12	9	21
Candida glabrata[a]	5	5	2	6	10	8	18
Candida haemulonis[a]	4	8[b]*	0[c]*	2	5	9	14
Filobasidium magnum[a]	8[b]*	1	1	0	2	8	10
Candida guilliermondii[a]	1	4	4	0	2	7	9
Cryptococcus liquefaciens[a]	5	2	0	1	2	6	8
Candida pararugosa[a]	1	3	2	0	3	3	6
Cryptococcus curvatus[a]	2	3	0	0	3	2	5
Cryptococcus flavescens[a]	0	0	3[b]*	1	2	2	4
Trichosporon asahii[a]	1	1	2	0	2	2	4
Candida lusitaniae[a]	0	3[b]*	0	0	0	3	3
Cryptococcus diffluens[a]	1	1	1	0	1	2	3
Candida duobushaemulonis[a]	0	0	1	1	0	2	2
Cystobasidium slooffiae	0	0	1	1	1	1	2
Moesziomyces antarcticus[a]	1	1	0	0	0	2	2
Rhodosporidium paludigenum	1	1	0	0	1	1	2
Rhodotorula dairenensis[a]	0	0	2[b]*	0	0	2	2
Trichosporon dermatis[a]	1	1	0	0	0	2	2
Other 14 spp.	5[d]	5[e]	4[f]	0	4[g]	10[h]	14
Total	86	78	55	43	98	164	262

[a] Species causing diseases in human. MC: medical center.
[b] Refers to higher frequency.
[c] Represents lower frequency among species.*P < 0.05.
[d] Arthrographis kalraea, Candida oleophila, *Pichia kluyveri*, *Sterigmatomyces elviae*, and Zalaria obscura from Center.
[e] Candida metapsilosisa, Pichia caribbica, Pseudozyma aphidisa, *Rhodotorula diobovata*, and Wickerhamomyces anomalusa from North.
[f] Cyberlindnera fabianiia, *Cystobasidium calyptogenae*, *Cystobasidium oligophagum*, and Pseudozyma siamensis from South.
[g] Arthrographis kalraea, Candida oleophila, *Cystobasidium oligophagum*, and *Pichia kluyveri* from medical centers.
[h] Candida metapsilosisa, Cyberlindnera fabianiia, *Cystobasidium calyptogenae*, Pichia caribbica, Pseudozyma aphidisa, Pseudozyma siamensis, *Rhodotorula diobovata*, *Sterigmatomyces elviae*, Wickerhamomyces anomalusa, and Zalaria obscura from non-medical centers.

Table 3 Antifungal susceptibilities of 137 Candida species.

mg/L	Total	*C. parapsilosis*	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. guilliermondii*	other 3[d]
Amphotericin B							
MIC_{50}	0.5	0.5	0.25	0.5	0.5	0.25	0.5
MIC_{90}	1	1	0.5	1	1	0.5	2
≦0.5	105	39	25	15	13	9	4
1	28	18	0	5	4	0	1
2	3	1	0	1	0	0	1[c]
4	1	0	0	0	1	0	0
Anidulafungin							
MIC_{50}	0.0625	1	0.0156	0.0313	0.0313	0.25	0.0313
MIC_{90}	1	2	0.0156	0.0625	0.0313	1	0.25
≦0.125	71	0	25	21	18	2	5
0.25	11	5	0	0	0	5	1
0.5	14	13	0	0	0	1	0
1	31	30	0	0	0	1	0
2	10	10	0	0	0	0	0
Fluconazole							
MIC_{50}	0.25	0.25	0.125	0.25	0.5	1	0.5
MIC_{90}	1	2	0.125	0.5	1	4	8
≦2	132	58	25	19	17[a]	8	5
4	0	0	0	0	0	0	0
8	2	0	0	0	1[a]	0	1[c]
16	1	0	0	0	0	1	0
32	1	0	0	1[b]	0	0	0
64	1	0	0	1[b]	0	0	0
Voriconazole							
MIC_{50}	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156
MIC_{90}	0.0313	0.0313	0.0156	0.0625	0.0156	0.125	0.0625
≦0.25	134	58	25	19	18	8	6
0.5	1	0	0	0	0	1	0
1	1	0	0	1[b]	0	0	0
2	1	0	0	1[b]	0	0	0
Total	137	58	25	21	18	9	6

[a] Susceptible-dose dependent.
[b] Resistant.
[c] *C. duobushaemulonis*.
[d] 3 *C. lusitaniae*, 2 *C. duobushaemulonis*, and 1 *C. metapsilosis*.

resistant ones belonged to DST506, classed to the predominant genotype, clade 4 detected previously. A total of 8 isolates (3 DST140, 2 DST525, and one of each DST987, DST1188, and DST1189) exhibited fluconazole trailing growth phenotype (Table 4).

Discussion

We found that sinks had a high yeast-culture positive rate, consistent with a previous report that hospital sinks are a potential nosocomial source of Candida infections.[14] The

Table 4 Association between genotype and fluconazole susceptibilities of the 21 *Candida tropicalis* isolates.

Clade	1						3		4	5		6		7	N1187	ND	Total
DST	1192	987	1191	1193	1188	1189	506	1190	140	525	960	1304	184	1187			
	MICs at 48h (mg/L)																
	≦0.5	>64	0.5	0.5	>64	>64	>64	4	>64	≧64	1	0.5	0.5	0.25	0.5		
MICs at 24h (mg/L)																	
≦0.25	2	1[a]	1	1	0	0	0	1	0	2[a]	1	1	1	1	2	14	
0.5	0	0	0	0	1[a]	1[a]	0	0	3[a]	0	0	0	0	0	0	5	
≧32	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	
Total	2	1	1	1	1	1	2	1	3	2	1	1	1	1	2	21	

[a] Fluconazole trailing growth phenotype.

observation that different sites in different hospital types or location had different yeast-culture positive rate suggests that each hospital should conduct its own survey to monitor the level of microbial contamination in environment.

C. parapsilosis was the most common species detected in the present study, consistent with several previous reports.[32–34] Unlike previous reports in other areas, all 58 *C. parapsilosis* isolates were susceptible to all antifungal drugs tested. The fact that no *C. auris* was detected in the present study supports the findings that there were only few cases of infections caused by drug-susceptible *C. auris* in Taiwan.[35–37]

In addition to detect azole-resistant clade 4 *C. tropicalis* in hospital environment, several isolates with azole trailing phenotype were detected. The phenomena associated with susceptibility, 'trailing', describes the reduced but persistent growth that some isolates exhibit at drug concentrations above the MIC in broth-dilution tests with azoles after 48 h incubation point.[28,38,39] Hence, those isolates may be able to survive in the presence of low levels of drug and are prone to be resistant. According to the results of TSARYs, DST140 was the predominate azole-resistant genotype until 2006[19] and is still one of common genotypes causing infections in patients.[21] Furthermore, five out of eight isolates exhibiting trailing growth in the present study belonged to clade 5 (Table S2). It has been reported that hand carriage by the healthcare personnel may be an important source of *C. tropicalis* causing outbreaks.[40] In order to prevent the transmission of drug-resistant *C. tropicalis*, we need to elucidate the determining factor(s) contributing to the drug-resistance and persistence of *C. tropicalis* in Taiwan.

There are several limitations of the present study. We did not conduct yeast colonization of healthcare professionals and patients when we collected environmental samples. We did not set a criterion how many days have patients been admitted when sampling is conducted. The clinical significance of the detection of one species to other species among different sampling sties remains further investigations. Thus, another survey needs to be conduct to distinguish whether a strain detected in the environment also existed in healthcare professionals or patients. Furthermore, a long duration survey on the same sites may help to distinguish whether patient, healthcare professional or environment is the original source of pathogenic yeasts.

The observation that each hospital has different distribution of pathogenic yeasts emphasizes the importance of a regular survey of each hospital. A large multiple-center survey of both environments, healthcare professionals, and patients at the same time is needed to address the association between the strains in the environment and humans. Importantly, our findings demonstrate that the azole-resistant clade 4 *C. tropicalis* exists in the ICU environment and also re-emphasize the importance of regular cleaning and disinfection practices.

CRediT authorship contribution statement

De-Jiun Tsai: Writing — original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Li-Yun Hsieh:** Writing — original draft, Formal analysis, Data curation. **Pei-Jung Chung:** Methodology, Formal analysis, Data curation. **Yin-Zhi Chen:** Methodology, Conceptualization. **Yi-Jyun Jhou:** Methodology. **Kuo-Yun Tseng:** Methodology, Data curation. **Chia-Jui Yang:** Resources. **Yen-Cheng Yeh:** Resources. **Seng-Yi Lin:** Resources. **Susan Shin-Jung Lee:** Resources. **Ting-I Wu:** Resources. **Tsung-Ta Chiang:** Resources. **Chien-Hsuan Chou:** Resources. **Wei-Chieh Miu:** Resources. **Po-Yu Liu:** Resources. **Chin-Te Lu:** Resources. **Yuan-Ti Lee:** Resources. **Yu-Ling Syu:** Resources. **Yee-Chun Chen:** Resources. **Nan-Yao Lee:** Resources. **Chang-Hua Chen:** Resources. **Ching-Cheng Yang:** Resources. **Lih-Shinn Wang:** Resources. **Jien-Wei Liu:** Resources. **Chin-Chuan Kao:** Resources. **Ya-Ting Chang:** Resources. **Keh-Sen Liu:** Resources. **Bor-Shen Hu:** Resources. **Che-Han Hsu:** Resources. **Yi-Ching Huang:** Resources. **Hsiu-Jung Lo:** Writing — review & editing, Writing — original draft, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.08.011.