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Prevalent Drug Resistance Among Oral Yeasts from Asymptomatic Patients in Hainan, China

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Abstract The oral cavity is a significant niche of the human microbiome and a gateway for the microbiota in many other human body sites. As a result, understanding the oral microbiota has broad implications for the prevention and management of human infectious diseases. Opportunistic yeast infections are among the most prevalent fungal infections of humans, and most opportunistic yeast pathogens are common residents of the oral mucosa. However, relatively little is known about the drug susceptibility profiles of oral yeasts. Here, we report the species distribution and patterns of antifungal susceptibility profiles among 313 yeasts isolated from the oral cavities of 301 asymptomatic hospitalized patients in Hainan Province in southern China. These yeasts were tested for their susceptibilities to the following five drugs: amphotericin B, fluconazole, itraconazole, ketoconazole, and fluorocytosine. Since none of the sampled hosts had taken any antifungal drugs at least 3 months before samples were taken, we hypothesized that little or no drug resistance should be observed. Contrary to our expectations, our analyses identified that 29 % (91/313) of the isolates were resistant to at least one drug and 14.3 % (45/313) were resistant to two or more of the five common drugs. The potential sources of the observed resistance were discussed.

Keywords Antifungal resistance · Oral yeasts · Candida · Geographic differences · Origin of drug resistance

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Introduction

Yeast infections are among the most common diseases caused by opportunistic pathogens in both immunocompromised and immunocompetent hosts [1–4]. Pathogenic yeasts can infect almost all human tissues and organs, from nails to the skin and mucosal surfaces, and from gastrointestinal to respiratory, reproductive, cardiovascular, and central nervous systems [1–4]. With the increasing number of immunocompromised hosts, opportunistic yeast infections have become more common. The broad application of antifungal agents to treat yeast infections over the last two decades has led to significant changes in yeast species distributions in favor of intrinsically drug-tolerant/resistant species [1–3, 5–7]. Interestingly, with few exceptions [8], the rates of drug-resistant strains within individual yeast species have remained relatively low and largely consistent across broad geographic regions [5–7, 9–13]. However, most of the

analyzed strains were associated with systemic infections in patients from developed countries, and relatively little is known about drug susceptibility profiles from commensal yeasts from developing countries. In addition, current evidence suggests that endogenous commensal yeasts of the *Candida* genus are responsible for a significant proportion of both superficial and systemic yeast infections [3, 4]. Therefore, understanding the patterns of antifungal susceptibilities of commensal yeasts has significant implications in the management of yeast infections.

The oral cavity is the major entry of the microbiome in the human gastrointestinal (GI) tract. Thus, the oral microbiome impacts not only the health of the oral cavity, but also that of the entire GI tract and by association, other organ systems such as the cardiovascular and lymphatic systems. In a typical individual, the oral cavity contains thousands of microbial species [14]. While the majority of the microbial species are prokaryotic [14], culture-based studies have revealed that oral yeasts can be commonly found in 20–70 % of the surveyed populations [15, 16, and references therein]. However, culture-independent and DNA sequence-based studies have shown a greater variation in yeast carriage rate [17, 18], with one study found no *Candida* or other fungi in the oral cavities of 25 volunteers [17] while another found all 20 volunteers had fungi in their oral cavities and 15 of the 20 hosts contained *Candida* [18].

Yeasts can cause a diversity of diseases in the oral cavity such as pseudomembranous candidiasis, erythematous candidiasis, angular cheilitis, and median rhomboid glossitis [19]. Because the oral cavity is only a transient entry point for food, drinks, and for patients' medicine, it has not been considered an important site in which selection for antibiotic resistance occurs. As a result, there is a little investigation on the antibiotic susceptibility profiles of oral yeasts (and other oral microbes in general). The objective of this study was to investigate antifungal susceptibility profiles of yeasts from the oral cavities of humans on Hainan Island in southern China.

Materials and Methods

Oral swabs were taken from 451 hospitalized patients at three hospitals on Hainan Island in southern China. The Island is geographically isolated from Mainland China and is surrounded by the South China Sea, with a tropical climate. Among the 451 patients, 192 were from Hainan General Hospital located in the provincial capital city Haikou at the north of the island; 145 were from Wenchang General Hospital in Wenchang city on the east coast; and 114 were from Sanya General Hospital in Sanya city in the southernmost part of the island. The sampled hosts ranged from 5 to 97 years old and no two people belonged to the same family or were closely related to each other. All oral swabs were collected between 2010 and 2012. At the time of sampling, none of the 451 hosts had the symptoms of an oral yeast infection. The demographic features of the hosts are summarized in Table 1. These patients had disease symptoms at a variety of body sites, including pulmonary, cardiovascular, gastrointestinal, and lymphatic systems. However, none of the hosts were diagnosed of having systemic yeast infection, and none had directly and knowingly taken any antifungal drugs at least 3 months before oral swab samples were collected. Most of them had never taken antifungal drugs through their entire life. Sample collections and yeast strain isolations followed protocols described previously [15, 16]. All obtained yeast isolates were stored at −80 °C freezer until use.

Yeast Species Identification

For species identifications, the stored isolates were first plated on the yeast extract–peptone–dextrose agar (YEPD: 1 % yeast extract, 2 % peptone, 2 % dextrose, and 2 % agar) and incubated for 48–72 h at 33 °C. Their genomic DNA were extracted followed the protocol described in Xu et al. [20]. To identify these isolates to the species level, the inter-transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene cluster were amplified through PCR using the universal fungal primers ITS1 (5′-TCCGTAGGTGA ACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTG A-3′). Each PCR had 20 μL and contained 0.15 μmol of the primers, ~5–10 ng/mL template DNA, and 1X GoTaq (Promega, Madison, Wisconsin). The PCR protocol was run as follows: denaturation at 95 °C for 4 min; then, 40 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 60 s, followed by one cycle of 72 °C at 7 min. The PCR products were then analyzed with gel electrophoresis on a 0.8 % agarose gel using 1X Tris borate EDTA (TBE) buffer at 100 V for 45 min and stained with ethidium bromide for confirmation of successful PCR amplification.

Table 1 Oral yeast carriage and species distributions among asymptomatic patients on Hainan Island, China

Host features	Host characteristics	Number of sampled hosts	No. of hosts with yeasts (% positive)	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	Number of strains belonging to other yeast species[a]
Gender	Female	177	118 (66.7)	70	27	11	14
	Male	274	183 (66.8)	100	41	28	22
Age group	≤59 years old	133	74 (56.4)	45	14	9	9
	≥60 years old	318	227 (71.7)*	125	54	30	27
Geographic region	Haikou	192	131 (68.2)	72	23	28	14
	Wenchang	114	81 (71)	31	38	7	11
	Sanya	145	89 (61.4)	67	7	4	11
Underlying disease condition	Pulmonary disease	86	60 (69.8)	33	4	11	12
	Cardiovascular and blood problems	197	124 (62.9)	69	36	16	14
	Gastrointestinal disorder	60	44 (73.3)	25	9	4	6
	Cancer	20	18 (90.0)	7	9	1	2
	Endocrine (including diabetic) disorder	28	15 (53.6)	11	2	0	2
	Wounds and other physical injuries	52	35 (67.3)	20	8	7	0
	Others	8	5 (62.5)	5	0	0	0
Total		451	301 (66.7)	170	68	39	36

[a] The other yeast species were *Candida krusei (*syn. *Issatchenkia orientalis*, 10 isolates), *Kodamaea ohmeri* (9 isolates), *Candida dubliniensis* (4 isolates), *Trichosporon asahii* (3 isolates), *Pichia anomala* (3 isolates), *Candida parapsilosis* (3 isolates), *Candida intermedia* (2 isolates), and one strain each of *Rhodotorula mucilaginosa* and *Candida metapsilosis*

The sign "*" indicates that people of 60 years and older have a significantly higher yeast carriage rate than those of 59 years and younger

Sequencing of the ITS PCR products was done at BGI-Shenzhen, China. The ITS regions have been recommended as the fungal barcode [21], and there is a large number of yeast ITS sequences in GenBank (and other databases) for comparisons and species determination. The obtained sequences were compared to those in the GenBank through the BLASTn search option. For species identifications, the primary criterion we used was the best match based on the full-length sequence to the type strain of a known taxon in the GenBank database. In addition, we used an E-score of $<10^{-10}$ and at least 97 % nucleotide sequence identity through the full length of ITS (covering both regions 1 and 2 of the ITS) as cutoffs [15, 16].

Antifungal Susceptibility Testing

For each of the obtained yeast strains, we tested its susceptibilities to the following five antifungal drugs: fluconazole, itraconazole, ketoconazole, fluorocytosine, and amphotericin B. These drugs represent three distinct drug targets within the cell, and they are commonly used in China to treat fungal infections [22, 23]. The agar disk diffusion method was used in our tests, following the protocol described in CLSI M44-A2 [24]. Disks containing individual drugs were purchased from ROSCO [25] through Guangzhou Dijing Biotechnology Company (Guangzhou, China). Reference strains and interpretive criteria for susceptible (S), intermediate (I), and resistance (R) for each of the drugs followed those described in CLSI M44-A2 [24] and the manufacturer's manual [25]. Briefly, for each of the five drugs, the diameters for the zones of inhibition (in mm) representing *S*, *I* (also called dose dependent), and *R* are as follows: fluconazole (25 μg/disk; $S \geq 19$; *I* 18–15; and $R \leq 14$); amphotericin B (10 μg/disk; $S \geq 15$; *I* 14–10; and $R < 10$); itraconazole (10 μg/disk; $S \geq 23$; *I* 22–14; and $R \leq 13$); ketoconazole (15 μg/disk; $S \geq 28$; *I* 27–21; $R \leq 20$); and fluorocytosine (1 μg/disk; $S \geq 20$; *I* 19–12; and $R \leq 11$).

Data Analyses

To compare the rates of yeast carriage between and among different demographics groups, we used the chi-square contingency table test [26] embedded in the Microsoft Excel program. Comparisons were made for each of the four examined host features (Table 1): age (59 years and younger; 60 years and older), sex (male or female), underlying disease conditions, and geography (Sanya, Wenchang, and Haikou). The potential differences in yeast species compositions among samples from different groups of hosts were also compared using the chi-square contingency table test [26], similar to those for oral yeast carriage rate comparisons described above. Due to the small number of strains for many of the species in our samples (see below), for statistical robustness, only the three most common yeast species (*Candida albicans, Candida glabrata,* and *Candida tropicalis*, see below) were included in the comparisons. In addition, to minimize false positive results, when the expected numbers were smaller than five for certain species in a host group, the smallest categories were combined in the test as one group [26].

Results and Discussion

Yeast Isolation Rate

Among the 451 oral swabs, 301 were found to contain yeasts, representing an oral yeast isolation rate of 66.7 %, significantly higher than those in the general healthy population in Hainan (23.1 %) but similar to patient oral samples from other Hainan hospitals (68.2 %, ref. 16). Table 1 presents the summary information of oral yeast carriage rates for the groups of hosts from different hospitals, of different genders, and with different underlying disease conditions. Briefly, no difference in yeast isolation rates was found between oral samples from the two sexes ($P = 0.221$) or among samples from the three geographic regions ($P = 0.127$). However, oral swabs from patients aged 60 years and older had a significantly higher yeast carriage rate (71.7 %) than that from those aged 59 years and younger (56.4 %) ($P = 0.0012$). This pattern is similar to that reported previously for both the healthy and patient samples from Hainan [16]. Significant differences were also found among host groups with different underlying conditions ($P = 0.0003$); those undergoing cancer treatments had the highest carriage rate (90 %), while patients with endocrine disorders had the lowest yeast carriage rate (53.6 %).

Yeast Species Distribution

Our ITS sequence comparisons identified that of the 301 hosts with oral yeast, two had three yeast species each, eight had two yeast species each, and the remaining 291 patients had one yeast species each. The 313 yeast isolates were classified into the following 12 species in order of their prevalence (number of isolates, percentage of the species in the total yeast population): *Candida albicans* (170 isolates, 54.3 %), *Candida glabrata* (68, 21.7 %), *Candida tropicalis* (39, 12.5 %), *Candida krusei* (syn. *Issatchenkia orientalis*, 10 strains, 3.2 %), *Kodamaea ohmeri* (9, 2.9 %), *Candida dubliniensis* (4, 1.3 %), *Trichosporon asahii* (3, 0.96 %), *Pichia anomala* (3, 0.96 %), *Candida parapsilosis* (3, 0.96 %), *Candida intermedia* (2, 0.64 %), and one strain each of *Rhodotorula mucilaginosa* and *Candida metapsilosis* (0.32 %). Of the 10 hosts with more than one colonizing yeast species each, two had three species each (both hosts contained the same three species *C. albicans, C. glabrata*, and *C. tropicalis*); and three had both *C. albicans* and *C. tropicalis*; other three had both *C. albicans* and *C. glabrata*; one had both *C. albicans* and *C. krusei*, and one had both *C. glabrata* and *C. krusei*.

No difference in yeast species distribution was found between samples from the two sexes ($P = 0.322$) or the two age groups ($P = 0.619$) (Table 1). However, the three geographic populations differed significantly in their yeast species distributions ($P = 3.52 \times 10^{-11}$), with the Sanya, Wenchang, and Haikou populations having significantly greater than expected numbers of *C. albicans*, *C. glabrata*, and *C. tropicalis,* respectively (Table 1). This result is consistent with previous observations that showed geography playing a significant role in oral yeast species distributions [15, 16 and references therein]. Importantly, compared to previous samples obtained between 2006 and 2008 from Hainan [16], the samples analyzed here (obtained from 2010 to 2012) had higher percentages of *C. albicans* (54.3 vs. 42.2 %), *C. glabrata* (21.7 vs. 5.5 %), and *Candida krusei* (3.2 vs. 1.9 %), while the frequencies of *C.*

tropicalis (12.5 vs. 20 %) and *C. parapsilosis* (0.96 vs. 4.1 %) were lower in the current sample than the previous sample ($P = 2.75 \times 10^{-10}$). The Wenchang yeast sample and the patient group with cardiovascular and blood problems in general had especially high ratios of *C. glabrata*, at 43.7 % (38/87) and 26.7 % (36/135), respectively (Table 1). Several hypotheses have been proposed to explain the differential yeast species distributions among geographic populations [15, 16]. These hypotheses apply equally to the observations here, and they are not described further.

Antifungal Susceptibility Profile

The summary results of antifungal susceptibility testing are presented in Table 2. Similar to previous observations [5–12], the frequencies of strains with resistant (R) and/or intermediate (I) susceptible phenotypes varied significantly among the species and the drugs (Table 2). A high percentage of the isolated species (10/12, 83.3 %) had at least one isolate with the R and/or I phenotypes to at least one of the five drugs. Of the 12 yeast species, only two (*C. dubliniensis* and *C. metapsilosis*, representing five isolates total) did not show either the R or I phenotype. Interestingly, among the four non-*Candida* species isolated here (*K. ohmeri, T. asahii, P. anomala,* and R. *mucilaginosa*), each had at least one strain showing either the R and/or I phenotype to at least one of the five tested antifungal drugs (Table 2). In comparison, a recent study from Taiwan found non-*Candida* oral yeasts were susceptible to antifungal drugs [27]. However, due to the relatively limited sample sizes for most of the non-*Candida* oral yeast species analyzed so far, it is difficult to determine the statistical significance in the differences between our samples and those published previously.

Similar to those found in other studies [5–12], *C. glabrata* and *C. krusei* were found to have the highest frequency of drug resistance among the isolated yeast species in our sample. Furthermore, the frequencies of strains in these two species (41/68 = 60.3 % for *C. glabrata* and 10/10 = 100 % for *C. krusei*) resistant to at least one of the five antifungal drugs were significantly higher than those found in several recent large-scale studies of bloodstream isolates [5–7, 9–12]. For example, in the study by Pfaller et al. [11] that examined 571 isolates of *C. glabrata* from across the globe, about 20 % were resistant to one or more of the six tested antifungal drugs. Similarly, Lockhart et al. [6] examined 670 bloodstream isolates of *C. glabrata* from the USA and found a lower percentage of isolates (11.9 %) than ours (17.6 %) that were resistant to fluconazole.

Overall, 0.96 % of all strains (3/313) were resistant to amphotericin B, 8 % (25/313) to fluconazole, 16.3 % to itraconazole, 16.6 % to ketoconazole, and 10.5 % to fluorocytosine. Our overall rates of resistance to amphotericin B, fluconazole, itraconazole, and ketoconazole were within the range or higher than most bloodstream yeast populations from the Americas, Europe, and Asia [e.g., 5–12, 28, 29], most of which were from patients who had been exposed to antifungal drug treatments. However, one recent study of 131 bloodstream yeast isolates from Nanjing, China, showed greater rates of triazole resistance than ours [8]. In this study by Ma et al. [8], the four most common yeast species were *C. tropicalis* (28.6 %), *C. albicans* (23.3 %), *C. parapsilosis* (19.5 %), and *C. glabrata* (8.3 %). About half of the 131 candidemia patients were treated with fluconazole. Among the 131 isolates, 30.5 % were resistant to itraconazole, 53.9 % resistant to fluconazole, and 61.1 % resistant to ketoconazole [8], significantly higher than what we found in our study, or as far as we are aware of, any other study reported so far. Unfortunately, because Ma et al. [8] did not examine the rates of resistance to amphotericin B and fluorocytosine, we are unable to compare their susceptibility patterns with ours. However, the rates of resistance to fluorocytosine (at 7.6–30 % for the five most common yeast species, Table 2) in our samples were significantly higher than those reported previously, e.g., over 8 times of those reported by Lockhart et al. [6].

We found that the resistant strains were not clustered in a specific hospital and/or patient group. Instead, antifungal resistance was found in all three hospitals/geographic regions, from both gender groups and all age groups, and from patients with all types of underlying health conditions (data not shown). The only association between geography/hospital and high frequency of antifungal resistance was in Wenchang due to its high prevalence of *C. glabrata* (Tables 1, 2). However, within Wenchang General Hospital, there was no significant difference in the distribution of *C. glabrata* among the patient groups with different underlying condition (data not shown).

Table 2 In vitro susceptibilities of the most frequent five yeast species to five antifungal agents determined by CLSI M44-A2

Yeast species[a] (no. isolates)	Antifungal drug	S[b]	I[c]	R[d] (% total)	$I + R$ (% total)
Candida albicans (170)	Amphotericin B	168	2	0 (0)	2 (1.2)
	Fluconazole	157	11	2 (1.2)	13 (7.6)
	Itraconazole	129	34	7 (4.1)	41 (24.1)
	Ketoconazole	135	16	9 (5.3)	25 (14.7)
	Fluorocytosine	153	4	13 (7.6)	17 (10.0)
Candida glabrata (68)	Amphotericin B	66	2	0 (0)	2 (2.9)
	Fluconazole	37	19	12 (17.6)	31 (45.6)
	Itraconazole	16	26	26 (38.2)	52 (76.5)
	Ketoconazole	16	24	28 (41.2)	52 (76.5)
	Fluorocytosine	62	0	6 (8.8)	6 (8.8)
Candida tropicalis (39)	Amphotericin B	36	2	1 (2.6)	3 (7.7)
	Fluconazole	34	1	4 (10.3)	5 (12.8)
	Itraconazole	10	20	9 (23.1)	29 (74.4)
	Ketoconazole	19	15	5 (12.8)	20 (51.3)
	Fluorocytosine	35	0	4 (10.3)	4 (10.3)
Candida krusei (10)	Amphotericin B	8	1	1 (10)	2 (20)
	Fluconazole	0	4	6 (60)	10 (100)
	Itraconazole	0	4	6 (60)	10 (100)
	Ketoconazole	0	0	10 (100)	10 (100)
	Fluorocytosine	7	0	3 (30)	3 (30)
Kodamaea ohmeri (9)	Amphotericin B	8	0	1 (11.1)	1 (11.1)
	Fluconazole	8	1	0 (0)	1 (11.1)
	Itraconazole	7	1	1 (11.1)	2 (22.2)
	Ketoconazole	7	2	0 (0)	2 (22.2)
	Fluorocytosine	7	0	2 (22.2)	2 (22.2)

Susceptibilities for the remaining seven species are described in the footnote

[a] The *R. mucilaginosa* strain (1) obtained here was resistant to both itraconazole and fluorocytosine. One of the three strains of *Pichia anomala* showed "intermediate" susceptibility to both itraconazole and ketoconazole. Of the three strains of *T. asahii*, one was resistant to fluorocytosine, the second was intermediate to itraconazole, and the third was intermediate to Amphotericin B, itraconazole, and ketoconazole and resistant to fluorocytosine. Of the two strains of *C. intermedia*, one was resistant to fluconazole, itraconazole, and fluorocytosine ,while the second was intermediate to itraconazole. Of the three strains of *C. parapsilosis*, one was intermediate to Amphotericin B and resistant to fluorocytosine. All other strain–drug combinations were susceptible. All strains in *C. dubliniensis* (4) and *C. metapsilosis* (1) were susceptible to all five antifungal drugs. [b] *S* susceptible, [c] *I* intermediate susceptible, [d] *R* resistant

In addition to the high frequencies of resistance to individual drugs, multidrug resistance was also common among the Hainan strains. Specifically, of the 91 strains showing resistance phenotype to at least one of the five tested drugs, 45 were resistant to two or more drugs, with 23, 17, 4, and 1 strains displaying resistance to 2, 3, 4, and 5 drugs, respectively. Excluding strains of the two known intrinsically resistant species *C. glabrata* and *C. krusei* from our analyses still resulted in 17 % (40/235) of the strains showing resistance to at least one of the five drugs, with 6.4 % resistant to two or more drugs. In addition, including the strains with intermediate susceptibility in our analyses would yield a total of 170 R + I strains (=54.3 %), with 129 of which not completely susceptible to at least two of the five drugs. Furthermore, 100 of the 170 R + I strains belonged to yeast species other than *C. glabrata* or *C. krusei*, and 65 of these 100 were not completely susceptible to at least two of the drugs.

Among the 45 multidrug-resistant strains (the R category only), 38 were found resistant to two or all three azoles. Interestingly, 15 strains were also resistant

to both azole and fluorocytosine. Furthermore, three strains, one each of *C. krusei, C. tropicalis*, and *K. ohmeri*, were found resistant to all three types of drugs (i.e., amphotericin B, 1–3 of the azoles and fluorocytosine). At present, the molecular and cellular mechanisms responsible for the observed widespread resistance are unknown. However, previous studies have shown that intrinsic drug resistance and acquired multidrug resistance in fungi are often due to intrinsically efficient efflux pumps for the specific drugs or to mutations that increase the efficiency of such efflux pumps [30].

Potential Origin(s) of Resistant Yeasts

The high frequency of drug-resistant yeasts found here was unexpected. As mentioned above, none of the hosts had taken any antifungal drug at least 3 months before our sampling. In addition, the human oral cavity is generally considered only a transient entry point for food and drinks into the GI tract, and the oral mucosal surface has not been considered an effective site for selecting drug-resistant microorganisms. At present, the mechanism(s) for such a high rate of drug resistance in our sample is not known. However, there are three possibilities.

The first is related to selection pressure from agricultural fungicide application. Due to its tropical climate, Hainan has been one of the agriculturally most productive regions in China and a large number of fruits, vegetables, and cereals are produced year around. In recent years, large quantities of pesticides and fungicides are used in China to ensure agricultural productivity. Indeed, China is among the largest pesticide and fungicide-consuming countries in the world, about 700,000 tons of fungicides are consumed annually for agriculture and the use of agriculture triazoles increased by 48 % between 2000 and 2011 [31, news.agropages.com]. These agricultural fungicides could have selected for resistant yeasts in the environment and some of the yeasts were subsequently transmitted to humans via food and water. For example, the extensive use of triazole fungicides in agriculture has shown to be most likely responsible for the recent emergence and rapid spread of multiple triazole-resistant clinical strains of the filamentous human fungal pathogen *Aspergillus fumigatus* in many parts of the world, including China [32, 33].

The second possibility might be associated with rising antifungal drug use in hospitals. Similar to that in agriculture, clinical antifungal drug use has been increasing rapidly in China [8, 22, 23, 34]. At present, sales of clinical antifungal drugs have reached over USD $1 billion in China, with an annual growth rate of over 20 % since 2006 and azole drugs account for over 70 % of the current clinical antifungal drug use in China [33]. The mutated and selected drug-resistant yeasts in hospitals could be then passed on to patients. Several recent studies have identified genotype clusters of yeasts causing bloodstream infections for hospitalized patients in both Iceland and Canada, consistent with persistent nosocomial transmission of yeast pathogens [35, 36].

The third possibility is that drug-resistant yeasts in Hainan could have come from other surrounding regions such as Mainland China and Southeast Asia. Long-distance dispersal of human fungal pathogens, including those resistant to antifungal drugs, has been reported [especially, for those capable of producing airborne spores such as *A. fumigatus* and *Cryptococcus neoformans* [e.g., 33, 37]. Though airborne dispersal is unlikely, resistant yeasts associated with humans can disperse to wherever human hosts migrate to [e.g., 38]. In recent years, Hainan has become a popular destination for tourists from both Mainland China as well as outside of China. Indeed, a 2007 study identified that the ITS sequence diversity for strains of *C. albicans* from Hainan was comparable to the global phylogenetic diversity of *C. albicans,* consistent with recent migration of *C. albicans* from other regions to Hainan [39].

The above possibilities are not mutually exclusive, and all could have contributed to the prevalent drug resistance among oral yeasts from asymptomatic hosts in Hainan. However, to distinguish the above possibilities, genetic analyses of these strains as well as additional ones from a diversity of sources (e.g., from fruits and vegetables and other foods in Hainan, from hospital environments and agricultural fields, and from surrounding regions outside of Hainan) are needed. Such information will have significant public health implications. Indeed, the patients sampled here represented some of the most vulnerable to systemic yeast infections, and some of the common drugs would have failed to treat the infections by these yeasts.

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