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Abstract

Objective To describe a prospective laboratory-based surveillance of Candida species that were collected from different anatomical sites of patients admitted to the University of Malaya Medical Centre, Malaysia, from the year 2000 to 2013.

Methods Conventional (culture, microscopic examination and carbohydrate assimilation test) and molecular (PCR amplification and DNA sequencing) techniques were used to identify Candida species.

Results A total of 16 Candida species isolated from 34,392 clinical samples were from the oral cavity (oral swabs and throat swabs), blood, respiratory tract (sputum, tracheal secretions, nasopharyngeal aspirates, bronchoalveolar lavage), high vaginal swab, pus and urine. C. albicans (66.70%, 22,941/34,392), C. glabrata (11.71%, 4029/34,392), C. parapsilosis (10.74%, 3692/34,392), C. tropicalis (9.19%, 3162/34,392) and C. krusei (1.15%, 396/34,392) were the five predominant Candida species. C. albicans was the predominant species isolated from the oral cavity, respiratory tract and high vaginal swab; while the Candida species isolated from blood, urine and pus were predominant non-albicans Candida. Uncommon Candida species, such as C. lusitaniae, C. haemulonii, C. humicola, Pichia ohmeri and C. ciferrii, were also isolated in this study.

Conclusion Our study expands the current knowledge of the epidemiology of non-invasive and invasive candidiasis in Malaysia. The variability of the Candida species distribution from different anatomical sites highlights the significance of local epidemiology in disease management and selection of antifungal agents.

Keywords candidiasis, epidemiology, Candida albicans, bloodstream infection

Introduction

Candida is a large genus of Ascomycetous yeast, consisting of about 150 species, and more than 20 species are clinical importance. Candida yeasts are common inhabitants of the skin and mucous membranes of tracheal, gastrointestinal and genitourinary tracts [1]. Candida species are the leading cause of nosocomial bloodstream infections, candidaemia. Patients admitted to an intensive care unit (ICU) or patients with immune dysfunctions, bone marrow transplantation and underlying malignancy are at high risk of candidaemia [2]. Among the Candida species, Candida albicans is most commonly associated with candidaemia [3].

It is notable that although the distribution of Candida species varied across geographic regions, C. albicans remains the predominantly isolated species [4–9]. However, a shift towards non-albicans Candida species has been observed [9, 10], mainly due to severe immunosuppression, use of broad-spectrum antibiotics and empirical use of antifungal drugs. The non-albicans Candida species are usually indistinguishable based on symptoms alone due to their similarity in clinical presentations. Also, several of them are inherently resistant or acquire resistance to antifungal drugs [11].

The isolation of diverse Candida species from patients with different underlying medical conditions has been documented in various studies. As previously reported [12], C. dubliniensis is highly prevalent in the oral cavities of HIV-infected patients and patients with AIDS. C. parapsilosis has emerged as a significant nosocomial pathogen associated with invasive procedures [13]. This species is also predominantly related to ailments in patients receiving continuous ambulatory peritoneal dialysis (CAPD) [14] and causes invasive fungal infection in very low birthweight infants [15]. C. glabrata was reported commonly in patients with prolonged duration of hospitalisation, and it is innately resistant to azoles, especially fluconazole [16]. C. tropicalis is particularly virulent in patients with prolonged neutropenia that have
acute leukaemia or after undergone bone marrow transplantation [17]. On the other hand, C. krusei is an emerging nosocomial pathogen and associated with high mortality. C. krusei is found primarily in patients with haematological malignancies or bone marrow transplant patients who are receiving fluconazole prophylaxis [18]. It is inherently resistant to fluconazole and exhibits a reduced susceptibility to other antifungal drugs.

The aim of this present work was to document the temporal trends of Candida species isolated from different anatomical sites of patients in a tertiary hospital in Kuala Lumpur, Malaysia, over the study period of 14 years.

Methods
Study design
A prospective laboratory-based surveillance study was conducted at the University of Malaya Medical Centre (UMMC), Malaysia between 2000 and 2013. All yeasts isolated from the oral cavity (oral and throat swabs), blood, respiratory tract (sputum, tracheal secretions, nasopharyngeal aspirates, bronchoalveolar lavage), high vaginal swab, pus and urine were accrued for this study.

Laboratory identification of Candida species
Initially, gram stain and urease test were used to identify the Candida species. The yeasts were presumptively identified as Candida if the urease test was negative. The urease production medium was prepared by mixing urease base (1 g peptone, 5 g NaCl, 2 g KH2PO4, 1 g glucose, 20 g agar in 1000 mL distilled water) and 20% (w/v) urea stock solution (100 g urea in 500 mL distilled water). Klebsiella pneumoniae ATCC 700603 was used as a positive control and Escherichia coli ATCC 25922 as a negative control.

The germ tube production test was performed by inoculating the yeast into plasma and incubated at 35 °C for 2.5–3 h. The germ tube test-positive isolates were presumptively identified as C. albicans or C. dubliniensis. All germ tube test-negative isolates were grouped as non-albicans Candida species. The carbohydrate assimilation test was performed on all yeast isolates. All media and sugar discs (xylose, α-methyl-D-glucoside, glucose, sucrose, trehalose, cellobiose, arabinose, galactose and maltose) used in this study were prepared as previously described [19]. Cornmeal Tween 80 agar (BBL Microbiology Systems, Cockeysville, MD, USA) was used to study the morphological characteristics, pseudohyphal and chlamydoconidia production of the Candida species. The isolate was then cultured on Sabouraud dextrose agar (SDA) at 37 and 45 °C to differentiate C. albicans from C. dubliniensis, which fails to grow or grow poorly. The identity of yeasts that could not be confirmed by the auxanographic carbohydrate assimilation test was determined by API 20C AUX (bioMérieux, Marcy l’Etoile, France), complemented by observation of morphological characteristics on cornmeal Tween 80 agar.

Molecular identification by PCR amplification and sequencing of the ITS1-5.8S-ITS2 region [20, 21] was used to identify the yeast isolates that were unable to be determined by the auxanographic carbohydrate assimilation test and the API 20C AUX system. All sequenced data were subjected to a BLASTn search against the non-redundant (nr) NCBI-nucleotide database for yeast identification.

Results
Between 2000 and 2013, we obtained 34 392 Candida isolates from high vaginal swab (67.55%, 23 231/34 392), sputum (8.47%, 2913/34 392), nasopharyngeal aspirates (1.01%, 346/34 392), tracheal secretions (2.84%, 978/34 392), bronchoalveolar lavage (1.97%, 676/34 392), urine (11.37%, 3911/34 392), blood cultures (4.99%, 1716/34 392), oral swabs (0.63%, 216/34 392), 112 throat swabs (0.33%, 112/34 392) and 293 pus samples (0.85%, 293/34 392). A total of 16 Candida species were identified from these ten different clinical sample types. Overall, the most dominant Candida species isolated from the patients was C. albicans. Among the non-albicans Candida species, C. glabrata was the most frequently detected, followed by C. parapsilosis, C. tropicalis and C. krusei. In addition, numerous uncommon non-albicans Candida species were also detected from the patients’ samples, including C. rugose, C. dubliniensis, C. guilliermondii, C. kefyr, C. lusitaniae, C. utilis, C. baemuloni, C. pelliculosa, P. ohmeri, C. eiféri and C. bamicola (Table 1).

Over the 14-year period, C. albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. krusei constituted 99.50% (34 220 isolates) of the total isolates (Table 1). There was an increase in the number of C. albicans from 2000 to 2007, but the number decreased gradually from 2008 to 2013. As shown in Table 1, the trend of the distribution of C. parapsilosis from 2000 to 2013 was similar to that of C. albicans. Also, a slightly incremental pattern of C. glabrata, C. parapsilosis and C. tropicalis was observed (Table 1). However, our results demonstrate that there was no shift to non-albicans Candida species.
In this study, the Candida species isolated from the blood were predominant non-albicans Candida (Figure 1). A total of 73.14% of the Candida isolates were identified as non-albicans Candida species, including C. tropicalis (47.49%), C. parapsilosis (35.38%) and C. glabrata (11.08%). The other ten non-albicans Candida accounted for 4.43% (Figure 2).

C. albicans contributed 26.86% of the total Candida species isolated from blood culture.

The Candida species isolated from high vaginal swabs was predominantly C. albicans (72.19%). The remaining isolates consisted of 12 non-albicans Candida species (27.81%), of which C. glabrata (52.30%), C. parapsilosis (31.20%), C. tropicalis (11.17%) and C. krusei (4.75%) constituted 99.43% of the (Figures 1 and 2).

The Candida species isolated from urine was mainly non-albicans (52.54%): C. tropicalis (59.03%), C. parapsilosis (24.96%), C. glabrata (12.36%), C. krusei

| Table 1 Candida species isolated from clinical samples from year 2000 to 2013 |
|-----------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | C. albicans | C. glabrata | C. parapsilosis | C. tropicalis | C. krusei | C. rugosa | C. dubliniensis | C. guilliermondii | C. kefyr | C. lusitaniae | C. haemulonii | C. humicola | C. pelliculosa | C. ohmeri | P. ohmeri | C. utilis | C. ciferri | Total |
| 2000 | 890 | 107 | 124 | 119 | 29 | 16 | 5 | 2 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 1296 |
| 2001 | 1289 | 171 | 245 | 167 | 18 | 16 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1906 |
| 2002 | 1311 | 210 | 45 | 183 | 11 | 11 | 2 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1931 |
| 2003 | 1504 | 221 | 251 | 245 | 10 | 11 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2382 |
| 2004 | 1620 | 293 | 345 | 323 | 50 | 11 | 3 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2449 |
| 2005 | 1749 | 318 | 251 | 325 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2634 |
| 2006 | 1611 | 271 | 321 | 235 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2554 |
| 2007 | 2226 | 362 | 220 | 258 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3099 |
| 2008 | 2175 | 286 | 283 | 335 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3195 |
| 2009 | 1633 | 259 | 229 | 255 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2973 |
| 2010 | 1856 | 229 | 285 | 221 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2482 |
| 2011 | 1734 | 222 | 214 | 236 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2671 |
| 2012 | 1324 | 206 | 206 | 245 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2657 |
| 2013 | 22 941 | 237 | 106 | 3162 | 23 | 78 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 34 392 |
| Total (%) | 66.70 | 11.71 | 10.74 | 9.19 | 1.15 | 0.23 | 0.10 | 0.03 | 0.06 | 0.03 | 0.01 | 0.01 | 0.003 | 0.003 | 0.01 | 0.01 | 0.01 | 0.01 | 100

**Figure 1** Comparisons of C. albicans and non-albicans Candida isolated from the clinical samples.
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(1.36%) and C. rugosa (1.80%) constituted 99.51% of the total non-albicans Candida isolated from urine. C. albicans accounted for 47.46%.

Seven Candida species were isolated from oral cavities, of which 73.17% (240 isolates) were C. albicans. The six non-albicans were C. parapsilosis (45.45%), C. tropicalis (29.55%), C. glabrata (17.05%), C. krusei (4.55%), C. dubliniensis (2.27%) and C. rugosa (1.14%) (Figure 1).

C. albicans accounted for 70.65% of isolates recovered from respiratory tract samples, non-albicans Candida species for 29.35% (Figure 1). Of the latter C. parapsilosis accounted for 42.09%, followed by C. tropicalis (38.56%), C. glabrata (15.40%) and C. krusei (13.33%).

The Candida species isolated from pus were predominantly non-albicans (51.19%), including C. parapsilosis (48.00%), C. tropicalis (32.67%), C. glabrata (13.33%) and C. krusei (4.00%).

Discussion

The increased incidence and prevalence of Candida infections has attracted the attention of many researchers due to the increased population of immunocompromised and chronically ill patients in hospitals. In this study, we report the first large-scale surveillance study of the distribution of Candida species in Malaysia. Our results showed a high diversity of Candida species (16 species) isolated from all samples, in contrast to the previous epidemiological studies that identified only 5–8 common Candida species [4, 6, 7] which underscores the importance of other uncommon species. The distribution of common Candida species in this study was similar to the reports from other countries [4, 6–8, 22, 23]. In this study, we identified additional uncommon non-albicans Candida species, including C. dubliniensis, C. lusitaniae, C. kefyr, C. haemulonii, C. humicola, C. pelliculosa, P. ohmeri, C. utilis and C. ciferrii. Our data for these contemporary Candida species corroborate previous findings [4–9] which demonstrated that C. albicans was the predominant species. The non-albicans Candida accounted for 33.30% of the total Candida isolates, which is markedly less than C. albicans. Several studies have shown that non-albicans Candida species, especially C. glabrata has emerged as an important causative agent of candidiasis [22–24]. Also, previous epidemiological studies displayed a mycological shift from predominately C. albicans to C. glabrata [25–27]. Although C. glabrata was the second most common Candida species in our study, the result did not indicate a shift towards C. glabrata as the emerging causative agent of candidiasis in our hospital. Our data were in contrast to the previous surveillance studies that showed the shifting patterns towards the non-albicans Candida [16, 28–30].

Analysis of the Candida species isolated from different anatomical sites showed a remarkable diversity of Candida species distribution. We noted that C. albicans were isolated mainly from the oral cavity, respiratory tract and high vaginal swabs. However, non-albicans Candida species were the most frequent isolates recovered from blood, pus and urine. These findings confirm those of other Asian studies [4, 19, 31–33] showing the predominance of non-albicans Candida species isolated from the blood. However, this trend was in contrast to the series

**Figure 2** Distribution of non-albicans Candida isolated from the clinical samples.
we have isolated numerous uncommon decreased susceptibility to antifungal agents, including *Candida* species such as *C. dubliniensis*. Further studies are required to determine the antifungal susceptibility of superificial candida infections, which has been isolated from the erythematous skin papulae of a patient with acute myeloid leukaemia [45], skin and nails [46, 47]. Interestingly, three *C. ciferrii* isolates were isolated from the high vaginal swab in this study, which may represent the first isolation of this species from non-cutaneous site. However, at this stage of knowledge, the change of its anatomical niches and its adaptation in a plethora of niches remain unknown.

The changing epidemiology of uncommon *Candida* species infections has generated concern about the emergence of antifungal agents’ resistance and their clinical relevance. As previously noted, *C. guilliermondii*, *C. haemulonii*, *C. humicola*, *C. guilliermondii*, *C. dubliniensis*, *C. lusitaniae* and *C. ciferrii* exhibit resistant or decreased susceptibility to antifungal agents, including amphotericin B and azoles [9, 16, 48–50]. In this study, we have isolated numerous uncommon *Candida* species; however, *in vitro* susceptibility testing of *Candida* was not performed on isolates during the study period. Further studies are required to determine the antifungal susceptibility of *Candida* isolates in our hospital as this data will be crucial to guide the clinicians in the empirical treatment of candidiasis.

In conclusion, our working hypothesis that *C. albicans* was still the major isolate in this hospital and the shift towards isolation of non-*albicans Candida* was not confirmed. The isolation of uncommon non-*albicans Candida* species such as *C. lusitaniae*, *C. haemulonii*, *C. humicola*, *P. ohmeri* and *C. ciferrii* in our study indicated that these rare species should be added to the growing list of opportunistic fungal pathogens that require monitoring in the laboratory. Our findings also suggest that the relative pathogenicity of *Candida* species is related to the micro-environments of mucosal surfaces. Describing the distribution of *Candida* species in different anatomical sites is important for establishing efficient clinical management strategies.

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