Prevalence of *Candida dubliniensis* among Oral *Candida* Isolates in Patients Attending the Kuwait University Dental Clinic

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Key Words

Prevalence • *Candida* • Oral flora

Abstract

Objectives: The aim of this study was to determine the oral candidal carriage of patients seeking dental treatment at the Kuwait University Dental Clinic and to ascertain the *Candida* species composition among them. **Methods:** 370 oral rinse samples were collected from patients. The germ tube test, CHROMagar *Candida* medium and VITEK 2 yeast identification system were used for species identification. *C. dubliniensis* isolates were confirmed by the production of rough colonies with hyphal fringes and chlamydoconidia on simplified sunflower seed agar. **Results:** Of the 370 samples investigated, 160 (43.24%) showed *Candida* in culture. The isolation of *Candida* was significantly higher in individuals who were smokers or were under medication for either diabetes or asthma [99 (62%)] compared to healthy individuals [61 (38%)]. Of the 210 samples which did not yield *Candida*, 131 (62.38%) were healthy and 79 (37.62%) were associated with smoking or with usage of drugs for aforementioned conditions. Species isolated were *C. albicans* [102 (63.7%)], *C. dubliniensis* [23 (14.3%)], *C. krusei* [13 (8.1%)], *C. tropicalis* [12 (7.5%)] and *C. glabrata* [10 (6.2%)]. **Conclusions:** *Candida* species

were more prevalent in patients having predisposing factors implicated in oral candidosis, such as in smokers, diabetic patients and asthmatic patients using inhalation steroids. *C. albicans* was the most prevalent species isolated, followed by *C. dubliniensis*.

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Introduction

Due to the increasing number of immunocompromised hosts, infections caused by opportunistic pathogens have become a serious problem worldwide. Species of *Candida* are among the most important opportunistic pathogens, which constitute part of the normal oral microbiota. Since species of *Candida* differ in virulence properties and susceptibility to antifungal drugs, understanding the human commensal flora will have a significant impact on designing treatment and prevention strategies against yeast infection. Although opportunistic yeast infections are considered a global problem and there is global interest in these causal agents, we know relatively little of the global distribution of *Candida* pathogens. Therefore, it is worth investigating the distribution of these pathogenic yeasts in different geographical locations.

Most data on the distribution of pathogenic yeast species have come from patient populations in Europe and North America. These studies have reported yeast carriage rates of about 20–40% in typical population samples [1, 2]. In these studies, *C. albicans* has been found to be the dominant species, accounting for up to about 90% of all isolated yeasts. In samples obtained from patients in developing countries such as those in Africa, the yeast carriage rates were similar to or higher than those reported in developed countries [3]. Interestingly, the diversity of yeast species was typically higher in developing countries, and the frequency of *C. albicans* was usually much lower than frequencies found in developed countries [3]. Several other surveys showed that the distribution of opportunistic yeast pathogens from healthy hosts in North America was similar to that in patient populations in North America and most other Western countries regarding both the yeast carriage rate and the yeast species distribution, with *C. albicans* as the dominant species [2]. In addition, different groups of healthy hosts in Hong Kong have yeast carriage rates and yeast species compositions highly similar to those in North America and Europe [4]. In contrast, a comparable survey of healthy hosts in several regions of mainland China identified a significantly higher rate of yeast carriage (66.9%) than those reported in other regions [5]. Furthermore, *C. albicans* was ranked only fourth most prevalent, accounting for only 9.4% of all isolated yeast strains [5].

C. dubliniensis is an emerging pathogen capable of causing oropharyngeal, vaginal and systemic infections [6]. Though similar to *C. albicans* in several phenotypic characteristics, it differs from it with respect to epidemiology, certain virulent attributes and the ability to rapidly develop resistance to fluconazole [6]. There have been no previous studies on oral yeast carriage in the Middle Eastern population in general, and in the Kuwaiti population in particular. Furthermore, the prevalence of *C. dubliniensis* isolates among oral *Candida* isolates in this segment of the population is unknown. As such a study would add further knowledge to the understanding of the global distribution of this opportunistic species, the main aim of this study was to determine the oral candidal carriage of patients seeking dental treatment at the Kuwait University Dental Clinic (KUDC), and to ascertain the *Candida* species composition among the yeast carriers. The other aim was to determine the antifungal susceptibility of these isolates to antifungal drugs such as amphotericin B, fluconazole, ketoconazole and caspofungin.

Subjects and Methods

Study Group

A total of 370 patients attending the KUDC for dental treatment were enrolled in the study. The KUDC is affiliated to the Faculty of Dentistry and situated in the Jabriya Kuwait University Campus and functions as an outpatient clinic. The KUDC provides a full range of dental treatment for those who have dental treatment needs that correspond to the teaching needs of dental students. Hence, in general, the majority of the patients attending the clinic do not belong to the category of having an increased risk for higher carriage of oral *Candida*. The ethical clearance for this study was granted by the Health Sciences Center Ethics Clearance Committee, Faculty of Medicine, Kuwait University, and the study was undertaken with the informed consent of each patient. Of the total of 370 patients, 176 were males (16–67 years) and 194 were females (17–71 years). All patients came for a routine dental checkup at the time of attending the KUDC for dental treatment. There were 178 patients (107 males and 71 females) who were heavy smokers, while others were under treatment for diabetes mellitus and bronchial asthma. The diabetic patients were under oral hypoglycemic drugs and the asthmatic patients under steroid inhalation therapy, and they were otherwise healthy at the time of attending the KUDC. The patients who smoked more than 25 cigarettes per day were categorized as heavy smokers. For the purpose of description, this group is identified as 'compromised patients' in the text. The remaining 192 patients (69 males and 123 females) were not associated with either smoking or medication. None of the 370 patients had clinically proven oral candidosis.

Isolation and Identification

The standard oral rinse technique was used for the isolation of *Candida* species. All yeast isolates were tested for germ tube formation. Thereafter, a presumptive identification of the *Candida* isolates was performed on the basis of the characteristic colony color on CHROMagar *Candida* medium (Becton, Dickinson and Company, Sparks, Md., USA). All the isolates were further identified based on their carbohydrate assimilation pattern by the VITEK 2 yeast identification system (bioMérieux, Craponne, France). The identity of *C. dubliniensis* was confirmed by the production of rough colonies with hyphal fringes and chlamydospores on simplified sunflower seed agar [7], which has shown to accurately identify *C. dubliniensis* when compared with molecular identification methods using seminested PCR amplification of the internal transcribed spacer-2 region of rDNA followed by direct DNA sequencing of the internal transcribed spacer region of rDNA as described previously [8].

Antifungal Susceptibility

Antifungal susceptibility of the yeast isolates was determined against amphotericin B, fluconazole, ketoconazole and caspofungin by E test performed according to the manufacturer's recommendations (AB BIODISK, Solna, Sweden). Each test isolate was freshly subcultured. Five isolated colonies were uniformly suspended in sterile saline, and turbidity was adjusted to 0.5 McFarland standard. This inoculum was swabbed onto the agar plates (150 mm diameter) and allowed to dry for 10–15 min before the E test strips were applied. RPMI 1640 agar supplemented with 2% glucose and buffered with MOPS (0.165 M; pH 7.0) was used for susceptibility testing according to the method recommended by

Table 1. Variance in the distribution of patients (n = 160) who yielded *Candida* in culture (n)

	Gender distribution		Number of patients
	males (n = 67; 42%)	females (n = 93; 58%)	
Healthy	14	47	61
Smoking	34	0	34
Medication for diabetes	2	29	31
Medication for asthma	16	11	27
Other	1	6	7
Total	67	93	160 (100%)

the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards; M27-A2). The plates were incubated at 35°C, and minimum inhibitory concentrations (MIC) were read after 24–48 h of incubation. The point where inhibition ellipses intercepted the scale on the antifungal strip was taken as the MIC for each test isolate: complete inhibition (100%) of growth for amphotericin B and caspofungin, marked decrease in growth intensity (80%) for fluconazole and ketoconazole. Reference strains of *C. albicans*, ATCC 90028, and *C. parapsilosis*, ATCC 22019, were used for quality control of the susceptibility testing. Interpretive susceptibility breakpoints for fluconazole were those recommended by Clinical and Laboratory Standards Institute document M27-A2. Due to the lack of defined susceptibility breakpoints for amphotericin B and ketoconazole, an isolate was considered susceptible with an MIC breakpoint of ≤ 1.0 µg/ml for amphotericin B and 0.125 µg/ml for ketoconazole. For caspofungin, an isolates with MIC of ≤ 2 µg/ml were considered as susceptible.

Statistical Analysis

To compare the potential differences in yeast carriage rates among groups of hosts, the χ^2 contingency table test was used. The differences among the compromised and healthy groups were analyzed, and a $p < 0.05$ was considered significant.

Results

Prevalence and Distribution of Oral *Candida* Species

Of the 350 patients investigated, 160 (43.24%) yielded *Candida* in culture; 93 (58%) were females and 67 (42%) males. The isolation rate of *Candida* was higher in individuals who were compromised in addition to the dental complaint [99 (62%); 46 females and 53 males] compared to healthy individuals ($p < 0.01$). Among the individuals who had only the dental complaint, the isolation rate was only 38% (n = 61; 47 females and 14 males). The distribution of the patients who yielded *Candida* in culture is given

Table 2. Distribution and percentage of different oral *Candida* species in 160 patients that yielded *Candida* in culture

	Distribution A ¹	Distribution B ²	Total species
<i>C. albicans</i>	73	29	102 (63.7%)
<i>C. dubliniensis</i>	11	12	23 (14.3%)
<i>C. krusei</i>	7	6	13 (8.1%)
<i>C. tropicalis</i>	6	6	12 (7.5%)
<i>C. glabrata</i>	2	8	10 (6.2%)
Total	99	61	160 (100%)

¹ Distribution in patients with dental complaint associated with either heavy smoking or usage of medication.

² Distribution in healthy patients with dental complaint only.

in table 1. The distribution and percentage of different oral *Candida* species in these 160 patients that yielded *Candida* in culture are given in table 2. Accordingly, the species isolated were *C. albicans* [102 (63.7%)], *C. dubliniensis* [23 (14.3%)], *C. krusei* [13 (8.1%)], *C. tropicalis* [12 (7.5%)] and *C. glabrata* [10 (6.2%)]. The isolation of *C. albicans* in the compromised group was significantly higher (n = 73) than in the group which only had the dental complaint (n = 29). Of the 180 samples which did not yield any *Candida*, 131 (62.38%) were healthy apart from the dental complaint, and only 79 (37.62%) were compromised patients in addition to the dental complaint. This difference too was statistically significant ($p < 0.01$).

Antifungal Susceptibility

The data on antifungal susceptibility as determined by the E test are presented in table 3. All *Candida* isolates tested were susceptible to amphotericin B and caspofungin. All *C. albicans*, *C. dubliniensis* and *C. tropicalis* isolates were also susceptible to fluconazole and ketoconazole. *C. glabrata* isolates showed reduced susceptibility to fluconazole and some were determined as resistant to ketoconazole. However, all *C. krusei* were determined as resistant to fluconazole and ketoconazole.

Discussion

The present study to our knowledge is the first study to survey the prevalence of oral *Candida* species in a Middle Eastern country. Of the 370 patients investigated, 43.24% yielded *Candida* in culture. Previous studies in Europe and North America have reported yeast carriage rates of about 20–40% [1, 2]. A slightly higher oral candi-

Table 3. Antifungal susceptibility of different oral *Candida* species by E test (µg/ml)

	<i>C. albicans</i>	<i>C. dubliniensis</i>	<i>C. krusei</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>
Amphotericin B	0.004–0.19	0.002–0.125	0.008–0.25	0.004–0.38	0.032–0.25
Ketoconazole	0.004–0.032	0.002–0.012	0.19–0.75	0.012–0.19	0.008–0.38
Fluconazole	0.047–0.125	0.016–0.38	32–64	0.38–1.5	2–16
Caspofungin	0.004–0.125	0.003–0.19	0.004–0.5	0.004–0.38	0.032–0.19

Values denote MIC ranges.

dal carriage rate of 53% has been reported in a study conducted in South Africa [9]. The observed prevalence of candidal carriage of 43.24% falls within the range of 20–60% reported by other researchers [10]. The results of the present study also showed a higher prevalence of *Candida* in females than in males, similar to other studies [11]. The isolation rate of *Candida* was significantly higher in individuals who were associated with smoking or were receiving medication for diabetes and asthma in addition to the dental complaint (62%) compared to individuals who only had the dental complaint (38%). Hence, the current finding on the prevalence of *Candida* in the oral cavity further adds credence to the fact that *Candida* is an opportunistic pathogen.

On the distribution of different *Candida* species among the patients who yielded *Candida* in culture, our analysis identified that *C. albicans* was the dominant yeast species in our sample, accounting for 63.7% of all *Candida* isolates. Previous studies in North America and Europe have also reported *C. albicans* to be the dominant species isolated, accounting to up to 90% of all isolates tested [1, 2]. Others have revealed that though the most dominant, the frequency of *C. albicans* could be lower than frequencies found in North America and Europe [3]. Moreover, *C. albicans* was ranked fourth most prevalent, accounting for only 9.4% of all isolates tested, in a study conducted in mainland China [5]. Hence, our finding falls within the range of previous findings on the prevalence of *C. albicans* species [1–3, 5].

C. glabrata and *C. tropicalis* are considered as the commonest non-*albicans* oral *Candida* species isolated after *C. albicans* [12]. In contrast, in our sample, among the non-*albicans* *Candida* species isolated, *C. dubliniensis* was the most dominant species, accounting for 14.3% of all *Candida* species tested. Conversely, in studies on oral carriage of *Candida* species in patient populations involving both healthy and compromised, but not HIV-positive subjects, *C. dubliniensis* was not isolated at all among

all *Candida* species tested [13–17]. Furthermore, in another study on carriage of *Candida* species in the oral cavities of HIV-infected patients and healthy individuals in South India, *C. dubliniensis* was not isolated among all *Candida* species tested [18]. However, in a study on oral yeast carriage in HIV-infected populations in Argentina, the isolation rate of *C. dubliniensis* was 20.2% in HIV-positive patients, whereas it was 2.1% in HIV-negative individuals [19]. The isolation rate of this pathogen in HIV-positive patients in a study conducted in India was 16.3% of all *Candida* species isolated [20]. *C. dubliniensis* is now well recognized as an opportunistic pathogen associated with recurrent oral candidiasis in HIV infection, and this could be the reason for the findings of the later two studies. In a study with diabetic patients conducted in Ireland, the isolation rate of *C. dubliniensis* was also higher, with 18.2% of the *Candida* species isolated being *C. dubliniensis* [21]. In contrast, in another study with diabetic patients conducted in Turkey, none of the *Candida* isolates recovered were *C. dubliniensis* [22]. However, compared to all the aforementioned studies, in our study none of the patients were HIV-positive but were healthy, even though some patients were smokers and some were under medication for diabetes and asthma. Hence, taken together, our finding suggests a high prevalence of oral carriage of *C. dubliniensis* isolates compared to the majority of previous studies. Although the exact reasons for such differences in diversity remain unknown, several reasons could be proposed for this high prevalence rate as seen in our study. Firstly, there may be a racial preference of *C. dubliniensis* isolates for the tested population. In fact, a study by McCullough et al. [23] reported that genotypic differences among *C. dubliniensis* may be related to ethnic or racial differences between Jews, Arabs and Druse, despite the fact that they inhabit the same geographic area. A racial preference of *C. dubliniensis* has also been suggested in another study from South Africa, where 16% of oral cavities of healthy white individuals were colonized with

the species, whereas the isolation rate among healthy blacks was 0% [24]. Secondly, *C. dubliniensis* is closely related to *C. albicans* in evolutionary terms, sharing the latter's properties of commensalism and opportunistic infection with genomic similarities between these two species, with the vast majority of genes being approximately 90% homologous. Given this similarity, it is not surprising that previous studies may have misidentified *C. dubliniensis* as *C. albicans* due to the unavailability of reliable methods for accurately differentiating these two species [25]. However, analysis of data from a larger number of the population in Kuwait is important before arriving at these conclusions.

All *Candida* isolates tested were susceptible to amphotericin B and caspofungin. Furthermore, all *C. albicans*, *C. dubliniensis* and *C. tropicalis* isolates were also susceptible to fluconazole and ketoconazole. However, *C. glabrata* isolates showed reduced susceptibility to fluconazole and some of these isolates were also resistant to ketoconazole. All *C. krusei* isolates were resistant to both ketoconazole and fluconazole. *Candida* species differ in their susceptibility to antifungal agents. For instance, *C. krusei* is often innately azole resistant, *C. glabrata* has been reported to acquire resistance in vitro and in vivo, and *C. dubliniensis* isolates have been observed to rapidly develop resistance to fluconazole [26, 27]. In our study, too, all *C. krusei* isolates were resistant to both azoles (fluconazole and ketoconazole). *C. glabrata* showed reduced susceptibility to fluconazole and some of these isolates were also resistant to ketoconazole. Though *C. dubliniensis* isolates have been observed to rapidly develop resistance to fluconazole, all isolates in our study were suscep-

tible to this drug. Reduced susceptibility as well as frank resistance to drugs such as fluconazole and ketoconazole, as observed in our study, could be an issue of clinical importance in a scenario if these patients develop oral candidal lesions. Hence, antifungal susceptibility testing could be a valuable tool for predicting the efficacy of a given agent, and could help guide empiric therapy for high-risk patients with known predisposing factors for developing serious candidal infection (i.e. smokers and patients under medication as seen in our study). On the other hand, identification of the infecting species such as *C. krusei* or *C. glabrata* could also be highly predictive of the likely drug susceptibility to azoles and could be used as a guide to therapy.

Conclusion

C. albicans was the most prevalent species isolated from the oral cavity, followed by *C. dubliniensis*, in the population tested. Compared to other prevalence studies on oral candidal microbiota, a high prevalence of *C. dubliniensis* over other non-*albicans* species was seen in this study. Analysis of data from a larger number of the population in Kuwait is important before arriving at this conclusion.

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