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Rare Fungal Species Isolated from the Oral Mucosa of Libyan Diabetic Patients

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Abstract

Background and objectives: The emerging resistance of *Candida* species to antifungals routinely used to treat candidiasis in HIV patients and in patients with diabetes mellitus (DM) has resulted in the frequent isolation of non-albicans *Candida* species.
This study aimed to establish the prevalence and fluconazole resistance profiles of yeasts other than commonly identified *Candida* species which may be found colonizing the oral mucosa of Libyan patients with DM.
Methods: Fungal species were isolated from the oral cavity of DM-positive patients attending a diabetes clinic in Misrata Diabetes Centre in Libya. This study included patients aged between 35 and 95 years and excluded subjects who had been on antifungal therapy within two weeks prior to sample collection. The identification of the isolated species was done by growing the isolates on selective and chromogenic media and by API ID 32C biochemical testing. Antimicrobial susceptibility testing of the isolates to the antifungal fluconazole was performed using disk diffusion. The study complied with the Declaration of Helsinki (2013).
Results: Forty-four rare fungal isolates representing ten fungal species were identified from the oral mucosa of 194 patients, with 28.6% of rare *Candida* species demonstrating resistance to fluconazole. *Saprochaete capitata* and *Cryptococcus humicola* isolates demonstrated high levels of resistance to fluconazole, with other yeast species showing lower resistance levels.
Conclusion: The methodologies used in this study allowed for the accurate identification of rare fungal species. The API 32 ID system was found to be a better identification method when compared to chromogenic media, as some species could not be identified with the latter. This study emphasizes the importance of accurate species identification and antifungal surveillance in patients with underlying chronic diseases such as DM who have higher morbidity and mortality rates due to less known and resistant fungal infections.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from a deficiency in insulin secretion and/or action. With the number of diabetics worldwide having more than doubled over the past three decades, it is fast becoming one of the most significant chronic diseases globally.[1]

The increased levels of salivary glucose in DM patients are known to enhance yeast growth.[2] Yeast adhesion to epithelial cell surfaces is considered an initial and critical stage in the process of *Candida* colonization and further occurring infection.

The prevalence and distribution of *Candida* species varies according to geographical location and may be influenced by several factors such as immunosuppression, behavioural patterns, diet and socio-economic status. In addition, recent studies have demonstrated the increasing resistance of *Candida* infections to antifungals routinely used to treat oral candidiasis in HIV-positive patients[3] and in patients with DM,[4] including in particular, fluconazole. This has been attributed to both the emergence of resistant *C. albicans* strains as well as the more frequent isolation and detection of non-albicans *Candida* species with innate resistance.
Misrata is a fast growing city in the north of Libya with a population of just over 550,000 in 2006 and the third largest city in Libya after Tripoli and Benghazi. Due to the lack of information on the profile of DM in this city, the study investigated the prevalence of *Candida* species in diabetic patients visiting a specialized centre, in order to study the general profile of the disease including its risk factors and complications.

Methods

Sample collection was performed by swabbing the patient's oral mucosa using sterile cotton swabs. Isolation of cultures was done on Sabouraud dextrose agar (SDA) (Cat. no. BO0408T, Oxoid, UK), while presumptive identification of species was achieved by inoculation onto choromogenic agars (Cat. no. 94382, Sigma-Aldrich, USA and Cat. no. CM1002A, Oxoid, UK). Biochemical testing was done using API 32 ID (bioMérieux, Marcy l'Etoile, France).

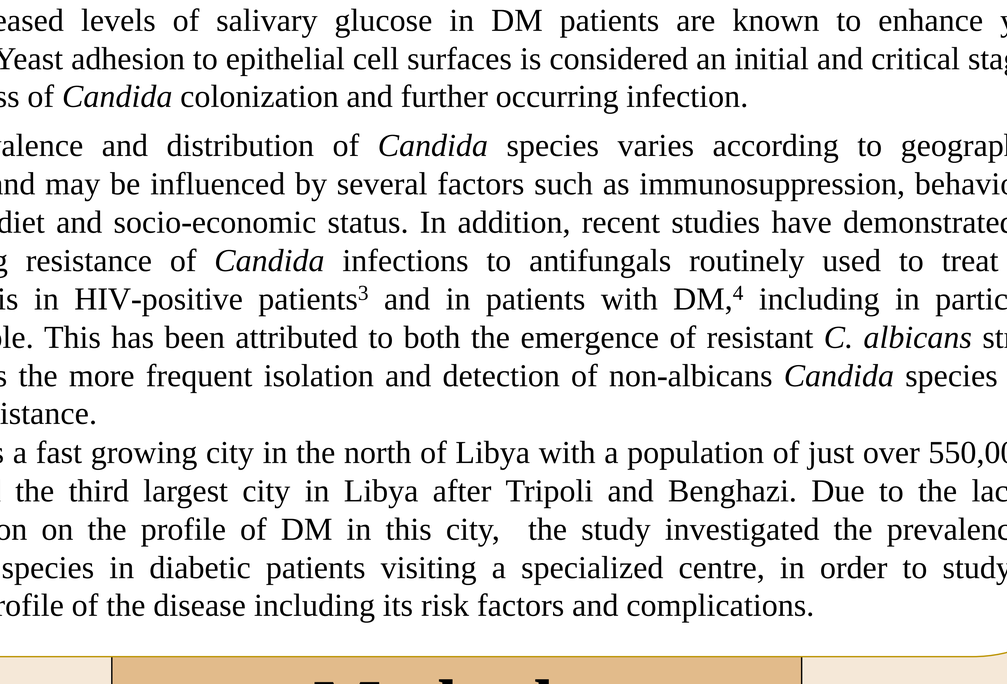

Figure 1 : Sample isolation, identification and drug susceptibility techniques used in this study.

Antifungal drug susceptibility was determined by placing 25µg fluconazole discs (Cat. no. X7148, Oxoid, UK) on yeast nitrogen base agar with glucose (YNBG) (Cat. no. 239210, Beckton, Dickinson and Company, UK) plates previously spread with a 0.5 McFarland standard suspension of the organisms and incubating at 37°C for 24 hours. Inhibition areas around the fluconazole disks were then measured and the presence of microcolonies within the susceptibility zone was noted.[5]

Results

Representatives of five rare *Candida* species, four other genera of fungi were isolated from the oral mucosa of these patients, with *Cryptococcus humicola* (previously classified as *Candida humicola*) being the most prevalent (Figure 2). These isolates were collected as part of a study on *Candida* prevalence in diabetic patients, in which 71 C. *albicans* isolates were also identified.

Fluconazole resistance was seen in *C. sake* and *C. guillermondii Candida* isolates (Table 1). While there are no established susceptibility guidelines for the other fungi isolated in this study, it was noted that all *Saprochaete capitata* demonstrated high resistance levels to fluconazole, with one being completely resistant, two showing a 0.5mm susceptibility zone, one with a 1mm susceptibility zone and one showing a 5.5mm susceptibility zone measurement. Fifteen isolates of *Cryptococcus humicola* (*Candida humicola*) showed susceptibility to fluconazole, with the remaining one having different degrees of resistance (Table 1).

Table 1 : Fluconazole susceptibility results.

Fungal species	Fluconazole susceptibility		
	S	I	R
C. sake (n=4)	1	0	3
C. magnoliae (n=2)	2	0	0
C. guilliermondii (n=1)	0	0	1
C. membranifaciens (n=1)	0	1	0
C. globosa (n=1)	1	0	0
C. humicola (n=26)	15	5	6
Saprochaete capitata (n=5)	–	–	–
Kloeckera apis/apiculata (n=2)	–	–	–
Kloeckera japonica (n=1)	–	–	–
Trichosporon mucoides (n=1)	–	–	–

- : no susceptibility guidelines available for the species.

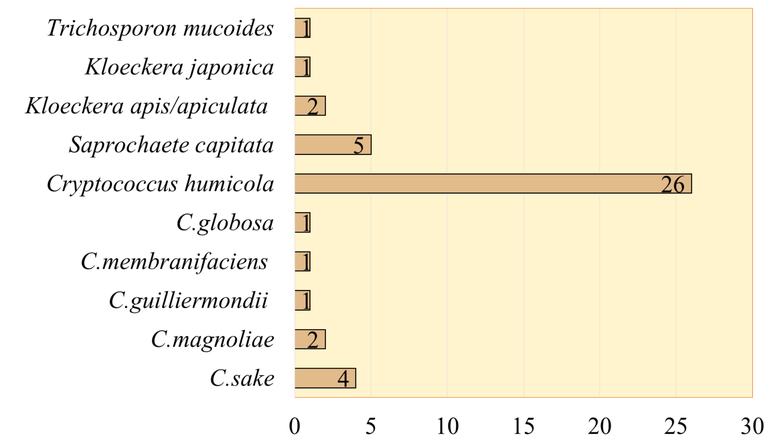

Figure 2 : Distribution of rare fungal species isolated from Libyan DM patients.

Discussion

Diabetes predisposes patients to a greater risk of being colonized by fungi. Although the fluconazole susceptibility of *C. magnolia* and *C. globosa* is promising, the resistance seen in *Saprochaete capitata*, *C. guillermondii* and *C. sake*, which can all cause life-threatening systemic infections, is an important finding.

It has been demonstrated that non - *C. neoformans*/non - *C. gattii Cryptococcus* species such as *C. humicola* do not respond well to fluconazole therapy,[6] and with recent reports of increasing *Cryptococcus humicola* infection,[7] the elevated numbers of this organism in Libyan patients and their resistance to the commonly dispensed antifungal fluconazole are a cause of concern.

Our findings stress the importance of adequate laboratory diagnostic tools in the early identification of causative agents of infections that lead to increased patient morbidity and mortality. The emergence of these uncommon fungal infections can be blamed partially on the empirical use of antifungals, which ultimately lead to the survival of these more resistant pathogenic yeasts.

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