Vitek characterisation of type 2 diabetes-associated *Candida* species

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Abstract

Background: Type 2 diabetes mellitus (T2DM) predisposes patients to opportunistic infections, such as invasive candidiasis. Treatment of candidiasis is challenged by the emerging resistance of *Candida* species. In this study, the antifungal drug resistance patterns of *Candida* species present in the oral mucosa of T2DM Libyan patients was investigated.

Methods: Seventy four (74) oral *Candida* isolates collected from T2DM patients in Misrata, Libya were characterised using the VITEK 2 Compact system.

Results: Prevalent species included *C. albicans*, *C. glabrata*, *C. dubliniensis*, *C. krusei*, *C. tropicalis*, *C. sake*, *C. kefyr*, *C. guilliermondii*, *C. parapsilopsis*, *C. membranifaciens* and *C. magnoliae*.

Drug susceptibility showed an emerging resistance across representatives of all species for which breakpoints were available, with the exception of *C. parapsilopsis*. Although there are no established interpretative breakpoints for these species, three *C. sake* isolates and the *C. membranifaciens* isolate also had high MIC values for fluconazole. The tested isolates were found to be largely susceptible to caspofungin and micafungin. All *C. albicans* isolates were susceptible to the echinocandins, amphotericin B and 5-flucytosine. Resistance to more than one drug class was seen in *C. dubliniensis*, *C. glabrata* and *C. krusei* isolates.

Conclusion: Although the susceptibility results for the echinocandins were encouraging, resistance against the azoles was apparent and should not be ignored. This was especially so in the case of fluconazole, which is often the only locally available antifungal drug for the treatment of disseminated candidiasis.

Introduction

T2DM patients are more vulnerable to fungal infection, particularly *Candida* infections of the oral cavity[1,2], due to increased salivary glucose[3] and the heightened availability of *Candida* receptors[4,5] in these subjects, with colonization by potentially pathogenic *Candida* strains being further enhanced by the hyposalivation associated with DM[6].

Drug classes routinely used in the fight against *Candida* infections include the routinely used triazoles (e.g. fluconazole and voriconazole), that affect ergosterol production in the fungal cell membrane; the echinocandins (e.g. micafungin and caspofungin), that inhibit β1-3 glucan synthesis in the fungal cell wall; 5-flucytosine, a fluorinated analogue pyrimidine that inhibits DNA and protein synthesis and amphotericin B, a polyene antifungal used for systemic infections that binds to ergosterol in the fungal cell membrane.

There is an absence of published data on the prevalence of *Candida* infection in T2DM in the Libyan population. This study aimed to investigate the prevalence and antifungal drug resistance patterns of *Candida* species in T2DM Libyan patients.

Methods

Ethical clearance for this project was granted by the Ethics Committee at University of Western Cape, South Africa and authorisation for sample collection was obtained from the Ministry of Health in Libya. Samples from T2DM patients were collected by scraping the oral mucosa and tongue with a sterile cotton swab, followed by culture on Sabouraud dextrose agar and incubation at 37˚C for 24 hours.

Confirmation of *Candida* species was achieved using microscopy, Gram staining, and the germ tube test, while Fluka chromogenic *Candida* identification agar (Cat. no. 94382; Sigma-Aldrich, St. Louis, MI, USA) and Oxoid chromogenic *Candida* agar (Cat. no. CM1002A; Oxoid, Hampshire, UK) and the API ID 32 C system (bioMérieux, Marcy l'Etoile, France) were used for presumptive species identification. The susceptibility to 6 antifungal drugs (amphotericin B, caspofungin, micafungin, fluconazole, voriconazole and flucytosine) was tested using the Vitek 2 Compact system (bioMérieux, Marcy l'Etoile, France).

Nine *Candida* type strains were used as quality control organisms for the species identification and antifungal drug susceptibility testing, namely *C. albicans* (ATCC 90028 and NCPF 3281), *C. tropicalis* (ATCC 950), *C. dubliniensis* (NCPF 3949a), *C. glabrata* (ATCC 26512), *C. krusei* (ATCC 2159), *C. parapsilosis* (ATCC 22019), *C. kefyr* (ATCC 4135) and *C. lusitaniae* (ATCC 3449).

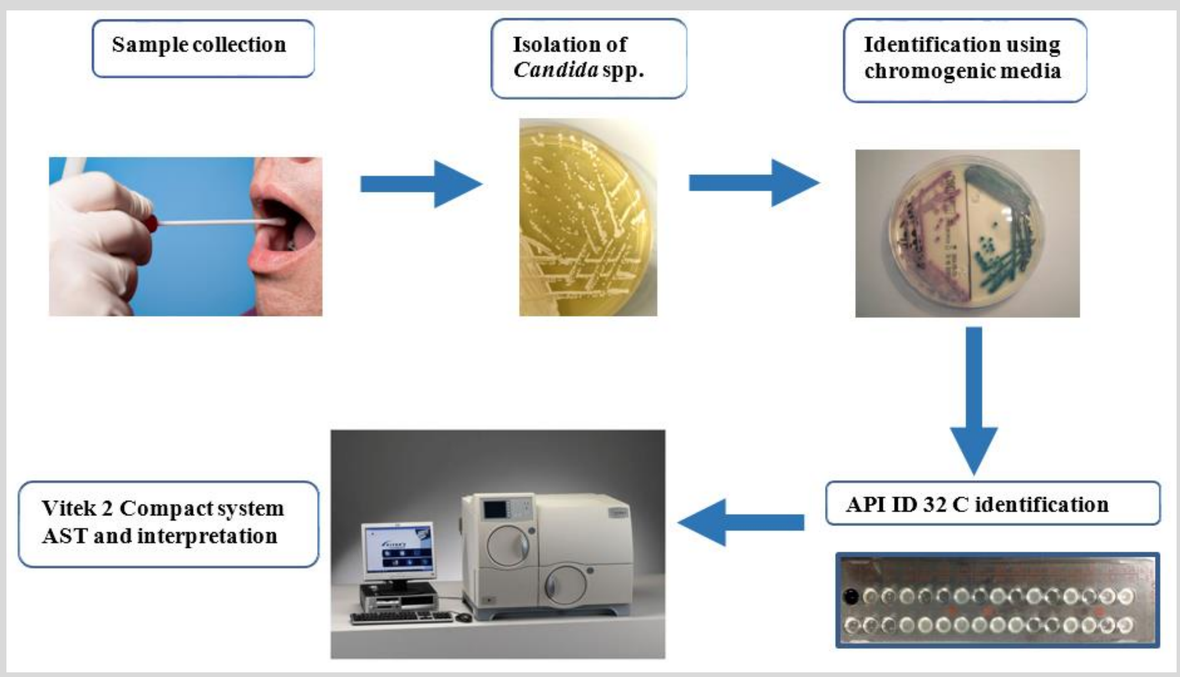

Figure 1: Sample collection, isolation, identification and drug susceptibility techniques used in this study.

Results

Eleven *Candida* species were identified and tested for their resistance to antifungals. These included *C. albicans* (20 isolates), *C. glabrata* (22 isolates), *C. dubliniensis* (13 isolates), *C. krusei* (5 isolates), *C. tropicalis* (4 isolates), *C. sake* (4 isolates), *C. kefyr* (2 isolates), *C. guilliermondii* (1 isolate), *C. parapsilopsis* (1 isolate), *C. magnoliae* (1 isolate), and *C. membranifaciens* (1 isolate).

Table 1: *Candida* species distribution and susceptibility results (only species for which interpretative breakpoints are available are shown)

Antifungal Drugs	Interpretation	*C. albicans* n=20	*C. glabrata* n=22	*C. dubliniensis* n=13	*C. krusei* n=5	*C. tropicalis* n=4	*C. kefyr* n=2	*C. guilliermondii* n=1	*C. parapsilosis* n=1
Amphotericin B	Susceptible	20	19	13	4	4	2	1	1
	Intermediate	0	0	0	0	0	0	0	0
	Resistant	0	3	0	1	0	0	0	0
5-Flucytosine	Susceptible	20	22	12	0	4	2	1	1
	Intermediate	0	0	0	0	0	0	0	0
	Resistant	0	0	1	5	0	0	0	0
Caspofungin	Susceptible	20	0	13	5	4	1	1	1
	Intermediate	0	22	0	0	0	1	0	0
	Resistant	0	0	0	0	0	0	0	0
Micafungin	Susceptible	20	22	12	5	4	2	1	1
	Intermediate	0	0	1	0	0	0	0	0
	Resistant	0	0	0	0	0	0	0	0
Fluconazole	Susceptible	16	0	10	0	2	1	0	1
	Intermediate	2	22	1	0	2	1	1	0
	Resistant	2	0	2	5	0	0	0	0
Voriconazole	Susceptible	19	22	11	5	4	2	1	1
	Intermediate	0	0	0	0	0	0	0	0
	Resistant	1	0	2	1	0	0	0	0

Of the three rarer species for which no interpretative breakpoints have been established, 3 *C. sake* isolates and the *C. membranifaciens* isolate showed high MIC values when exposed to fluconazole (with MICs ranging between 4µg/ml and 8µg/ml). All of these rarer species demonstrated low MIC values to the other antifungal drugs.

Discussion

The laboratory identification and antimicrobial susceptibility testing of *Candida* infections is not commonly preformed in Libya, with patients being treated according to their clinical symptoms.

The susceptibility of the majority of isolates to the different classes of antifungal drugs is encouraging, especially in the case of the echinocandins. However, the variety of *Candida* species seen in this population, including species that are inherently resistant to fluconazole, is a novel finding, as is the resistance of all *C. krusei* isolates to 5-flucytosine.

The monitoring of regional *Candida* species prevalence and drug susceptibility in Libyan diabetic patients is imperative, as the empirical treatment of these infections might be exacerbating the development of drug resistance.

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References

1. Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanaum L, Baka D, *et al*. 2005. Candidal overgrowth in diabetic patients: potential predisposing factors. *Mycoses*. 48**,** 192-6.

2. Khosravi A, Yarahmadi S, Baiat M, Shokri H and Pourkabireh M. 2008. Factors affecting the prevalence of yeasts in the oral cavity of patients with diabetes mellitus. *J Med Mycol*. 18**,** 83-8.

3. Sashikumar R and Kannan R. 2010. Salivary glucose levels and oral candidal carriage in type II diabetics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 109**,** 706-11.

4. Menezes EA, Augusto KL, Freire CCF, Cunha FA, Montenegro RM and Montenegro Júnior RM. 2007. Frequency and enzymatic activity of *Candida* spp. oral cavity of diabetic patients of the service of endocrinology of a hospital of Fortaleza-CE. *J Bras Patol Med Lab*. 43**,** 241-4.

5. Casqueiro J, Casqueiro J and Alves C. 2012. Infections in patients with diabetes mellitus: A review of pathogenesis. *Indian J Endocrinol Metab*. 16**,** S27-36.

6. Kadir T, Pisiriciler R, Akyüz S, Yarat A, Emekli N and Ipbüker A. 2002. Mycological and cytological examination of oral candidal carriage in diabetic patients and non-diabetic control subjects: thorough analysis of local aetiologic and systemic factors. *J Oral Rehabil*. 29**,** 452-7.