Fungal profile and antifungal susceptibility pattern in patients with oral candidiasis

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SUMMARY

Oral candidiasis is a common fungal infection, affecting the oral mucosa. The aim of this study was to investigate the epidemiology and antifungal susceptibility of *Candida* species isolated from the oral cavity of patients affected by oral candidiasis. Oral swabs were taken from 34 patients and were inoculated on to Sabouraud Dextrose Agar (SDA). The yeasts were preliminarily evaluated according to the growth (human serum) germ tube, chlamydospore formation, reproduction at 45°C and colony characteristics on SDA medium. The commercial method Phoenix (Becton Dickinson, USA) was used for identification. Clinical and Laboratory Standards Institute (CLSI) reference M27-A3 microdilution method was applied for fluconazole (FLC), voriconazole (VRC), amphotericin B (AMB), ketoconazole (KTC), nystatin (NYT) antifungal susceptibility testing. A total of 34 *Candida* species were isolated and these species were identified as follows: 14 (41.2%) *Candida albicans*, 8 (23.5%) *Candida glabrata*, 8 (23.5%) *Candida parapsilosis*, 4 (11.8%) *Candida tropicalis*. The geometric mean (GM) of the Minimum Inhibitory Concentration (MIC) for FLC, NYT, VRC, AMB, and KTC was 13.09 µg/mL, 4.77 µg/mL, 0.23 µg/mL, 0.20 µg/mL, 0.08 µg/mL, respectively. The most commonly isolated species was *C. albicans*. KTZ showed the lowest MIC value. NYT MIC values for non-albicans species were higher than for *C. albicans* ones.

Keywords: *Candida* species, oral cavity, antifungal, epidemiology.

INTRODUCTION

Candida species are fungal elements of the oral microbiota that can be responsible of opportunistic infections under appropriate conditions. The predisposing factors to oral candidiasis increase with host-related factors such as malnutrition, immunosuppression, diseases related to the endocrine system such as diabetes mellitus, cancer, autoimmune diseases, changes in the amount of saliva, changes in the epithelial cell layer, habit of eating carbohydrate-rich foods, age, poor oral hygiene, and the use of steroids, chemotherapeutic drugs, prosthesis, and cigarette smoking [1]. The most common agent of oral candidiasis is *Candida albicans*; however, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, and *C. dubliniensis* have also been reported [2-4].

Amphotericin B (AMB) and nystatin (NYT) are currently the drugs of choice for the treatment of the superficial fungal infections caused by *Candida* spp. AMB is traditionally used topically, although it may be administered systemically for the treatment of systemic infections in hospitalized patients [5]. Fluconazole (FLC) has been suggested as an antifungal agent in prophylaxis and treatment of oral candidiasis. However, a significant degree of FLC resistance has been detected in non-albicans isolates [6]. Overall, voriconazole (VRC) showed more *in vitro* potency than fluconazole or itraconazole against most *Candida* isolates

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[7]. Ketokonazole (KTC) is also an azole derivative and it is available in topical and oral formulation. It may present gastro-intestinal side as all other azoles [8].
In the current study, we aimed to determine the epidemiology of *Candida* species isolated from the patients suspected with an oral candidiasis and to investigate the susceptibility pattern of these species to antifungal agents.

PATIENTS AND METHODS

The samples were taken by sterile swabs from 34 adult patients affected by oral candidiasis observed during the period June 2016 - July 2018 at the 1200 beds Kayseri City Hospital, Turkey. Samples were submitted to the clinical mycology laboratory, preliminarily stored in 10% KOH and soon after inoculated to Sabouraud Dextrose Agar (SDA) (Oxoid, England) medium. Underlying diseases and oral lesions were noted.

Mycological identification
For the identification of the isolates, we used conventional procedures such as germ tube production, microscopic morphology on corn meal Tween 80 agar, as well as commercial methods such as CHROM agar Candida medium (Oxoid Brilliance™ Candida agar, England) and Phoenix (Becton Dickinson, USA).

Antifungal susceptibility test
The strains were inoculated in SDA medium before the susceptibility test and 24-hour-incubated isolates were used. Clinical and Laboratory Standards Institute (CLSI) M27-A3 microdilution method was applied [9]. FLC (Pfizer Pharmaceuticals Group, New York, NY, USA), VRC, KTC, AMB, NYT (Sigma-Aldrich, St. Louis, Louis, Missouri, USA) antifungals and RPMI 1640 (Sigma Chemical Co, St Louis, Mo, USA) with L-glutamine without bicarbonate, were used. Antifungal stock solutions were determined as 1280 µg/mL for FLC and KTC, as 1600 µg/mL for AMB, VRC and NYT according to reference method. The final concentration was determined as 64-0.125 µg/mL for KTC and FLC; 16-0.031 µg/mL for AMB; 8-0.016 µg/mL for VRC and NYT. Microorganism suspensions to be inoculated were adjusted as 0.5-2.5x10^3 CFU/mL spectrophotometrically. Suspension was inoculated after preparation of antifungal stock solution dilutions in U-based microplates. Plates incubated at 35°C, and the minimum inhibitory concentrations (MICs) were read at 24 h. The lowest concentration that inhibit the growth completely for AMB, NYT and the lowest concentration that shows ~%50 decrease according to growth control well for FLC, VRC, and KTC were accepted as MICs. Quality control was performed in tests in accordance with the CLSI document M27-A3 using *C. krusei* ATTC 6258 and *C. parapsilosis* ATCC 22019.

RESULTS

Totally, growth was detected in the samples of 34 patients. Isolated *Candida* species were 14 *C. albicans* (41.2%), 8 *C. glabrata* (23.5%), 8 *C. parapsilosis* (23.5%), and 4 *C. tropicalis* (11.8%). MIC geometric means of antifungals by microdilution method

Table 1 - The MIC range and MIC_{90} of five antifungals against oral Candida isolates.

Antifungal (µg/mL)	*Amphotericin B*		*Nystatin*		*Voriconazole*		*Ketoconazole*		*Fluconazole*	
Species	MIC_{90}	*MIC range*	MIC_{90}	*MIC range*	MIC_{90}	*MIC range*	MIC_{90}	*MIC range*	MIC_{90}	*MIC range*
C. *albicans* (14)	0.25	0.03-0.5	8	2-8	0.5	0.03-4	0.06	0.015-0.5	4	1-4
C. *glabrata* (8)	0.5	0.06-0.5	8	0.25-8	0.5	0.03-0.5	0.5	0.03-0.5	64	8-64
C. *parapsilosis* (8)	0.5	0.03-1	8	1-8	0.125	0,03-0.125	0.06	0.015-0.125	8	2-32
C. *tropicalis* (4)	–	0.06-0.5	–	2-8	–	0.015-0.3	–	0.015-0.03	–	1-8

MIC = Minimum Inhibitory Concentrations.

were the following: 13.09 µg/mL for FLC, 4.77 µg/mL for NYT, 0.23 µg/mL for VRC, 0.20 µg/mL for AMB, 0.08 µg/mL for KTC. For *C. albicans* species, MIC geometric means were found as 0.08 µg/mL, 4.1 µg/mL, 0.06 µg/mL, 0.03 µg/mL, 2.97 µg/mL for AMB, NYT, VRC, KTC and FLC, respectively. For non-albicans species, antifungal geometric means were 0.15 µg/mL, 5.45 µg/mL, 0.07 µg/mL, 0.05 µg/mL, 8 µg/mL for AMB, NYT, VRC, KTC, and FLC, respectively. Four *C. glabrata* strains were resistant to FLC. The resistant strains were susceptible to VRC. One *C. parapsilosis* strain was dose-dependent susceptible to FLC. Underlying diseases of patients were diabetes mellitus (10), oral candidiasis (5), steroid using (4), poor oral hygiene (4), mucositis (3), immune defensive patients (2), systemic lupus erythematosus (2), haematological malignancy (1), antibiotic usage (1), acquired immunodeficiency syndrome (AIDS) (1), simplex chronic lichen (1). The MIC range and MIC_{90} of five antifungals against *Candida* isolates are summarized in Table 1.

DISCUSSION

Among all *Candida* species, *Candida albicans* is regarded as the most pathogenic and most common isolated strain in patients with oral candidiasis [10, 11]. In our study, *Candida* species was the most frequently isolate from the oral cavity of patients with diabetes mellitus. These isolates were identified as *C. albicans* in 14 samples (41.2%), *C. glabrata* in 8 samples (23.5%), *C. parapsilosis* in 8 (23.5%), and *C. tropicalis* in 4 patients (11.8%). This study is a single-centre study, and our findings may vary according to different centres and countries.

NYT, which is the first-choice polyene antifungal, may be an effective *Candida* species in mucosal and superficial *Candida* infections [12, 13]. The World Health Organization has suggested that NYT treatment may be an alternative to FLC for the treatment of oropharyngeal candidiasis in HIV-positive children and adults [14]. NYT was detected as the most effective antifungal against *C. tropicalis* and *C. krusei* isolated from HIV-positive patients by disc diffusion method [10]. In a different study, the susceptibility to NYS of several agents responsible for oral candidiasis (114 *C. glabrata* and 97 *C. parapsilosis* strains were investigated) and no resistance was detected among these species [4]. In this study, *Candida* spp. showed MICs ranges of 0.25-8 µg/ml to NYT, and GM MICs values of NYT were higher in non-albicans *Candida* (GM MIC=5.45 µg/mL) species as compared to *C. albicans* (GM MIC =4.1 µg/mL) isolates.

FLC is a common antifungal agent used for most oral candidiasis and has been also used for systemic *Candida* infections due to its reduced toxicity, efficacy and good tolerance [15]. The efficacy of FLC has been reported in different studies. Resistance against FLC was detected only at the rate of 1% of 101 *Candida* strains isolated from HIV-positive patients [16]. In another study, resistance against FLC was detected as 11.7% in 120 *Candida* isolates. The highest resistance to FLC was detected in *C. albicans* strains at a rate of 16.7% [17]. In our study, only four *C. glabrata* strains were found to be resistant to fluconazole and the one *C. parapsilosis* strain was dose-dependent, susceptible to FLC. It seems that the widespread use of fluconazole for treatment or prophylaxis in candidiasis may be responsible for the high fluconazole resistance rates.

Multiple formulations have proved effective in treating oral candidiasis, including creams and tablets [18]. From HIV-positive patients, KTC resistance was not detected in *C. albicans* but was detected at the rate of 7.7% (7/90) in non-albicans species [19]. We found that antifungal activity increased in the following order: FLC, NYT, VRC, AMB, KTC (GM MICs: 13.09, 4.77, 0.23, 0.20 and 0.08 µg/mL, respectively). KTC was the most effective antifungal drug when compared to four other antifungal drugs against oral *Candida* isolates.

VRC is one of the novel azole groups and its local formulation is not available; it is especially used in the systemic treatment of FLC-resistant oral candidiasis [20]. Ally et al. compared the efficacy of VRC and FLC in the treatment of oesophagus candidiasis. The success rate was 98.3% for VRC and 95.1% for FLU. The results showed that VRC was as effective as FLC in the treatment of candidiasis [21]. In our study, the MIC_{90} values of VOR for all species were <1 µg/mL and no resistant strain was detected. In addition, four FLC-resistant *C. glabrata* isolates were found to be susceptible to VRC.

Topical AMB is suggested as a potentially efficient choice for the treatment of oropharyngeal *Candida*

infections according to the IDSA (Infectious Diseases Society of America) guidelines [22]. In certain studies, AMB is effective on all *Candida* species, including non-albicans isolated from the mouth [23, 24]. AMB treatment becomes useful especially when resistance to azole is detected [25]. All *Candida* isolates yielded a narrow range of MICs (0.03-1.0 µg/mL) for amphotericinB. It seems that AMB remains an effective antifungal agent against yeast species in spite of its widespread application in our hospital.

As a result, in our hospital, *C albicans* is the most commonly isolated agent responsible of oral candidiasis. KTC was found to be the most effective antifungal against isolated *Candida* species. A low degree of FLC-resistance was detected. Generally, a significant resistance pattern to antifungals was not detected. *In vitro* antifungal susceptibility tests should be performed to select the appropriate and effective antifungal therapy and monitor the development of resistance.

Conflicts of interest

The authors declare no conflict of interest.

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