Research Paper

Screening for antifungal susceptibility of oral Candida albicans and non-albicans Candida species

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ABSTRACT

Oral candidiasis is a frequent opportunistic infection in various groups of patients, especially among denture wearers. Common empirical use of antifungal agents contributed to the development of drug-resistant Candida isolates. The purpose of this study was to determine the isolation rate of Candida species from oral infections and to analyse their susceptibility to antifungal agents. Samples were taken from oral cavity from patients with symptoms of oral infections. All yeast isolates were identified biochemical ly and those classified as Candida spp. were tested for their susceptibility to nystatin, fluconazole, ketoconazole, miconazole and econazole. A total of nine hundred and fifty-seven (957) fungal isolates were obtained and among them Candida albicans (74.7%), Candida glabrata (8.9%), Candida tropicalis (6.5%), Candida krusei (3.3%), Candida kefyr (2.1%), Candida guilliermondii (1.6%), Candida lipolytica (1.4%), Candida lusitaniae (0.8%), Candida parapsilosis (0.6%) and Candida famata (0.1%). Nearly all Candida spp. were susceptible to nystatin, 98.6% to ketoconazole, 98.2% to econazole, 89% to miconazole and 80.8% (68.7% with naturally unsusceptible species) to fluconazole. C. albicans was the most common aetiologi cal factor of oral candidiasis. However, the resistance of non-albicans Candida spp. (NAC) (especially, C. glabrata and C. tropicalis) to azole antifungals fluconazole and miconazole (p<0.001) should be monitored thoroughly as these isolates might be resistant more often than C. albicans.

Key words: Antimycotics, Candida albicans, non-albicans Candida, susceptibility.

INTRODUCTION

Oral candidiasis is one of the most common opportunistic infections in some groups of patients (Kuryiama et al., 2005; Mattos et al., 2009; Coelho et al., 2012; Miskiewicz et al., 2013). It is estimated that 20 to 75% of healthy individuals may be colonized by Candida species, and under optimal conditions, these fungi can become pathogenic (Mushi et al., 2017). The pathogenesis of oral candidiasis is complex and may involve both direct and indirect factors. The principal risk factors are long-term antimicrobial therapy, anticancer chemotherapy and immunosuppressive treatment. Denture wearers (especially acrylic denture users) are also predisposed to oral candidiasis. Candida spp. can easily adhere to acrylic surfaces of the dentures which become a primary reservoir for oral infections (Altieri et al., 2013). In some cases, the fungi colonizing oral cavity (by disseminating in various parts of human body) are able to cause non-oral infections and invasive fungal diseases (Bulik et al., 2009; Nasution, 2013).

A growing incidence of candidiasis was reported by several authors, and this infection still constitutes an important medical problem (Badiee and Alborzi, 2011; Godoy et al., 2012; Nawrot et al., 2013). In some patients with candidiasis, inappropriate use of antifungal agents may contribute to the treatment failure and development of multi-drug resistance. Common empirical use of antimycotics resulted in the development of drug resistance...
in previously susceptible *Candida* strains. Several mechanisms of *Candida* resistance to antifungal agents have been identified, for example, overexpression of membrane transporters responsible for the removal of the drug from fungal cell or efflux pumps (Cowen, 2008). Hence, to increase the likelihood of therapeutic success and to avoid the development of drug resistance, the choice of antifungal treatment should be preceded by testing of clinical *Candida* isolates for their susceptibility to antifungal agents including nystatin (100 UI), ketoconazole (50 µg), miconazole (50 µg), econazole (50 µg) and fluconazole (25 µg) (Bio-Rad, France) were tested by agar disc diffusion method. Yeast-like fungi inoculum (10⁵ CFU/ml) in distilled sterile water solution was spread on the surface of Casitone-Agar and Mueller Hinton agar with glucose and methylene blue (Bio-Rad, France). Sterile paper discs with antifungal agents earlier mentioned were placed on the inoculated plates. The plates were incubated for 24 h at 30°C and sizes of inhibition zone measured according to Clinical and Laboratory Standards Institute (CLSI) M44-A document. *Candida albicans* ATCC 10231 was used as control strains.

**MATERIALS AND METHODS**

A total of nine hundred and fifty-seven (957) oral *Candida* isolates originated from patients with symptoms of oral infections treated at the Denture and Implant Clinic, University Dental Centre at Medical University of Gdansk. The material was collected routinely during the course of infection treatment or control visit. Among them eight hundred and forty-seven (847) patients were denture wearers, while five hundred and twenty-four (524) were non-wearers. Collection of swabs for laboratory testing was a part of routine clinical practices. Oral swabs were cultured on Sabouraud dextrose agar plates (Becton Dickinson, USA) and on CHROMagar medium (Becton Dickinson, USA); incubated aerobically for 48 to 72h at 37°C. The identification was based on biochemical tests using API 20C AUX (bioMerieux, France) in accordance with the manufacturer’s instruction. The activity of antifungal agents including nystatin (100 UI), ketoconazole (50 µg), miconazole (50 µg), econazole (50 µg) and fluconazole (25 µg) (Bio-Rad, France) were tested by agar disc diffusion method. Yeast-like fungi inoculum (10⁵ CFU/ml) in distilled sterile water solution was spread on the surface of Casitone-Agar and Mueller Hinton agar with glucose and methylene blue (Bio-Rad, France). Sterile paper discs with antifungal agents earlier mentioned were placed on the inoculated plates. The plates were incubated for 24 h at 30°C and sizes of inhibition zone measured according to Clinical and Laboratory Standards Institute (CLSI) M44-A document. *Candida albicans* ATCC 10231 was used as control strains.

**Statistical analysis**

Distributions of the study variables were presented as numbers and percentages, and compared between groups with Pearson chi-squared test or Fisher exact test. All calculations were carried out with Statistica 10 software (StatSoft, USA), with the threshold of statistical significance set at p<0.001.

**RESULTS**

Among oral samples taken from one thousand three hundred and seventy-one (1,371) patients with symptoms of oral infections, nine hundred and fifty-seven (957) (69.8%) *Candida* strains were isolated. The most commonly isolated *Candida* species was *C. albicans* (n=715). Other identified species were *Candida glabrata* (n=85), *Candida tropicalis* (n=62), *Candida krusei* (n=32), *Candida kefyr* (n=20), *Candida guilliermondii* (n=15), *Candida lipolytica* (n=13), *Candida lusitaniae* (n=8), *Candida parapsilosis* (n=6) and *Candida famata* (n=1) belonging to non-\*albicans Candida* (NAC) group (Figure 1 and Table 1). Nearly all
Candida spp., with exception of C. lusitaniae isolate, were susceptible to nystatin. Azole antifungals turned out to be less active against Candida spp., with 98.6% of the isolates susceptible to ketoconazole, 98.2% to econazole, 89% to miconazole and 80.8% (68.7% with naturally resistant species) to fluconazole (Table 1). The proportion of fluconazole-susceptible NAC isolates was slightly lower than the proportion of fluconazole-susceptible C. albicans isolates (71.2% vs. 79.3%, p=0.057) (Table 2). Two of NAC species, C. kefyr and C. tropicalis, with susceptibility rates of 70 and 62.9%, respectively, seemed to be more resistant to fluconazole than the remaining non-albicans isolates (Table 1).

The susceptibility to miconazole turned out to be significantly less common among NAC isolates than in C. albicans (69.4% vs. 95.7%, p<0.001). The resistance to miconazole was particularly evident in the case of C. glabrata (37.6% of susceptible isolates). The proportion of other NAC isolates showing susceptibility to miconazole varied between 81.3 and 95%, respectively (Tables 1 and 2).

Most Candida (98.2%) isolates were sensitive to econazole. Resistance to this antifungal agent was observed only in some individual isolates of C. albicans, C. glabrata, C. tropicalis, C. krusei, C. lipolytica and C. lusitaniae (Table 1). Ketoconazole was the most effective of all analysed azole antifungals, as resistance to this agent was observed only in some single isolates of C. albicans, C. glabrata, C. kefyr and C. lusitaniae (Table 1).

**DISCUSSION**

Many previous studies demonstrated that C. albicans was the most frequent aetiologic factor involved in all types of fungal infections diagnosed worldwide (Badiee and Alborzi, 2011; Biernasiuk et al., 2013; Guo et al., 2013). The isolation rate of C. albicans from our patients with oral candidiasis (74.7%) was within the range reported by other authors (Mattos et al., 2009; Miś et al., 2011). Depending on the study methodology, examined patient population, time and place of sampling, the isolation rates of C. albicans in previous studies varied from 30 up to 84.3%, respectively (Nawrot et al., 2013; Khan and Baqai, 2010; Łukaszuk et al., 2017; Mahmoudabadi et al., 2013). The second most
frequently isolated fungal species in our study was *C. glabrata* (8.9%). The isolation rate of this species was lower than in previous studies (13.4, 16.1 and 26.8%), respectively which might be associated with different characteristics of examined patient populations (Badiée and Alborzi, 2011; Godoy et al., 2012; Mahmoudabadi et al., 2013). The isolation rates of other *Candida* spp., both in our present study and in previous experiments, were equally low (Mahmoudabadi et al., 2013; Toll et al., 2011).

In this study, we examined the susceptibility of *Candida* spp. isolates to antifungal agents with the disc diffusion method. This is a quick and inexpensive method providing reproducible results which are generally comparable with those obtained with the reference broth microdilution method (Pfaller et al., 2003).

Although triazoles are still widely used in the treatment of candidiasis, frequent and prolonged administration of some agents from this group contributed to their lesser antifungal activities. Azoles inhibit C14a demethylation of lanosterol, which interferes with the synthesis of ergosterol in fungal cell membrane. The agents from this group vary in terms of their affinity to the target sites, which might be reflected by their different activity spectra and hence, different primary resistance profiles of various fungi.

Furthermore, variations in the structure ofazole antifungal agents are postulated to contribute to cross-resistance observed in *Candida* spp. (Kanafani et al., 2008). Due to its high efficacy and relatively lower toxicity, fluconazole became the most popularazole agent used in either prevention or treatment of candidiasis (Guo et al., 2013). Unfortunately, this has also contributed to a relatively frequently reported resistance to this agent (Huei-Fung et al., 2006; Gulshan and Moye-Rowley, 2007). Since fluconazole was not effective against *C. glabrata* and *C. krusei* we did not include these species in the comparative analysis of susceptibility to this agent (Pfaller et al., 2003).

Most *Candida* isolates obtained from our patients turned out to be susceptible to fluconazole. Our findings suggest that NAC isolates were less susceptible to fluconazole than *C. albicans*. Moreover, NAC group included two species, *C. tropicalis* (37.1%) and *C. kefyr* (30%), that seemed to be markedly more resistant to fluconazole than the remaining non-*albicans* isolates. Our findings are consistent with the results published by Kędzia et al. (2007) and Toll et al. (2011) according to whom fluconazole-resistance rates among NAC isolates were 26.3 and 20.6%, respectively. Similar to our study, Osaigbovo et al. (2017) demonstrated that the proportion offluconazole-resistant oral NAC strains was higher than the proportion of *C. albicans* showing resistance to this antifungal agent.

Previous studies documented miconazole activity against fluconazole-resistant yeast-like fungi (Isham and Ghannoun, 2009). Indeed, our *Candida* isolates showed higher susceptibility to miconazole than to fluconazole; the only exception pertained to *C. glabrata* isolates which were relatively more often resistant to this agent (37.6% of susceptible isolates only). Equally high resistance rates of *C. glabrata* to miconazole were previously reported by Kędzia et al. (2007). The remaining NAC and *C. albicans* isolates were more susceptible (81.3 to 100%). However, the proportions of *C. albicans* resistant strains identified in other studies varied considerably from 17.2 to 88.1%, respectively (Coelho et al., 2012; Toll et al., 2011; Carvalhinho et al., 2012). A retrospective study conducted by Łukaszuk et al. (2017) demonstrated that the resistance of *C. albicans* strains to miconazole increased over time, but among NAC isolates variable susceptibility was noticed.

In this study, most of the isolates had a similarly very low resistance against ketoconazole and econazole (1.4 and 1.8%, respectively). *C. albicans* occurred slightly more susceptible to both of these antifungics than NAC isolates. Among NAC group *C. lusitaniae* was more often resistant (12.5%). Mahmoudabadi et al. (2013) reported lack of resistant fungi. There were studies which noticed variable lower susceptibility to ketoconazole (Biernasiuk et al., 2013; Kędzia et al., 2007) or high activity against candida oral isolates (Kuriyama et al., 2005). In the case of econazole, Miśkiewicz et al. (2013) obtained similar results to ours, but ketoconazole inhibited tested species much less (16.7% susceptibility). Nystatin, a polyene, has been widely used as an agent of choice in topical treatment of oral candidiasis (Barão de Aguiar et al., 2010) in several countries including Poland as reported by Maroszyńska et al. (2013).

Nystatin is mostly well tolerated and has a low toxicity ratio. Most *Candida* spp. analysed in the present study with exception of *C. lusitaniae* isolate (0.1%), turned out to be highly susceptible to nystatin. Previous studies demonstrated that nystatin was a potent antifungal agent, inhibiting the growth of nearly all tested strains (93 to 99.9%) (Mahmoudabadi et al., 2013; Maroszyńska et al., 2013). According to Kuriyama et al. (2005) and Godoy et al. (2012), *Candida* spp. did not show resistance to nystatin and another study demonstrated susceptibility to this agent in all *C. albicans* isolates (Miśkiewicz et al., 2013; Carvalhinho et al., 2012).

**Conclusions**

*C. albicans* is the most common aetiologic factor of oral candidiasis. However, the resistance of non-*albicans Candida* spp. (especially *C. glabrata* and *C. tropicalis*) toazole antifungals (fluconazole and miconazole) should be monitored thoroughly as these isolates might be resistant more often than *C. albicans*.

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Candida glabrata


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