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IN VITRO SUSCEPTIBILITY OF ORAL *CANDIDA ALBICANS* ISOLATES TO CHLORHEXIDINE

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Abstract: Fungal infections are an important medical problem in patients from different risk groups. The majority of these infections are caused by *Candida* spp., with over 50% due to *C. albicans*. The purpose of the study was to evaluate *in vitro* chlorhexidine effect on *C. albicans* colonizing the mouth and throat isolated from 5 population groups. The study material included the reference strains of *C. albicans* ATCC 2091 and *C. albicans* ATCC 10231, routinely used for evaluation of antimicrobials, and 120 clinical isolates of *C. albicans* from: hospitalized cancer patients (30 isolates), chronic HCV patients (31 isolates), immunocompromised patients (28 isolates), healthy school-age children (31 isolates), elderly people – aged 65 years or older (30 isolates). Chlorhexidine inhibited the growth of *C. albicans* at the concentrations of 0.625-5 µg/mL (in particular, 2.5 µg/mL solution was effective against strains from immunocompromised patients and 5 µg/mL – against the remaining isolates). The yeasts were also killed by 2.5-20 µg/ml chlorhexidine solutions. The concentration of 5 µg/mL was particularly active against the strains isolated from the elderly, immunocompromised and lung cancer patients, while 10 µg/mL inhibited the growth of the strains from the remaining two groups. Moreover, *C. albicans* isolates from hepatitis C patients and healthy children comparing to strains from the elderly were less sensitive to chlorhexidine fungicidal effect and these differences were statistically significant. According to our studies, the fungicidal effect of chlorhexidine seems to depend on the origin of the tested oral *C. albicans* strains from various patient groups.

Keywords: antifungal agents, *Candida albicans*, chlorhexidine

Fungal infections are an important medical problem in patients of different risk groups. Most of these infections are caused by *Candida* spp., with over 50% due to *Candida albicans*. *C. albicans* is considered a part of the normal oral cavity flora, which does not cause a disease, but under unfavorable local conditions, such as poor oral hygiene, wearing of dentures and general predisposing factors, such as immunodeficiency, immune-suppression, organ transplantation, HIV/AIDS, diabetes, leukemia, cancer, chemotherapy, genetic disorders, corticosteroid therapy, senility, radiotherapy, high carbohydrate diet, malnutrition or smoking, may cause candidiasis (1-7).

An increased frequency of candidal infections in recent years is also associated with a widespread use of antibiotics, which destroy natural bacterial flora thus creating an environment for fungal growth, and an inappropriate use of antifungal drugs. This is a major cause of the development of resistant or multi-drug resistant strains, which may lead to many therapeutic failures (2, 5, 6). An increase in the resistance of *Candida* to some antifungal agents has been observed. This has caused concern due to the eukaryotic character of both *Candida* and host cells; moreover, compared to antibiotics, fewer antifungal drugs are available (4). Even up to 90% of patients, especially those with compromised immune function and hospitalized with serious underlying diseases, suffer from superficial and systemic candidiasis, causing their morbidity and mortality (8). Interestingly, oral candidiasis (among others pseudomembranous, erythematous and angular cheilitis) is considered the most common oral manifestation in human immunodeficiency virus (HIV) infection, and *C. albicans* the most frequently found opportunistic pathogen (1, 3). The disease may cause oral discomfort, pain, loss of

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taste, thus affecting quality of life. Moreover, without treatment, the lesion may spread to the esophagus, causing invasive esophageal (3).

The use of mouthwashes, widespread in dentistry, is a common way to control the *C. albicans* population in the mouth. Mouthwashes have been recommended for the prevention and control of oral diseases, especially for the control of oral microorganisms. They usually contain some active components, such as antibiotics, antifungal and anti-inflammatory substances. The mouthwashes have been found to enhance the removal process and elimination of microorganisms (7).

In the prophylaxis and treatment of oral candidiasis both antifungals and antiseptics are currently used. An increase in the number of opportunistic infections caused by fungi and the great number of strains that have become resistant to the common antifungals encouraged new research focused on alternative treatments of such infections, among which the use of chlorhexidine is recommended (7, 9). Chlorhexidine is an effective chemical compound and one of the most frequently used antiseptics in dentistry. It is used in prevention and treatment of gingivitis and periodontitis. Its strong antimicrobial effect and capacity inhibit the accumulation of periodontal plaque (10-12). Agents containing high concentrations of this compound are used in periodontic and endodontic clinical practice (13). Chlorhexidine is safely applied at very low concentrations in several commercial dental hygiene products that prevent or inhibit the development of caries, including mouthwashes, gels, varnishes or sprays (10, 12, 13). The substance is also successfully used in root canal treatment as a drug applied between visits or a root canal irrigant, in the therapy of oral cavity candidiasis, as well as to decontaminate dental impressions and dental prostheses (10, 14). Chlorhexidine is available in various forms, such as digluconate, acetate and hydrochloride salts, which are sparingly soluble in water (12).

Chlorhexidine is the most active agent, due to its wide spectrum of antimicrobial activity against various organisms, such as bacteria, viruses and fungi including *C. albicans* (7, 12). The mode of action of this substance is not entirely understood, but it is known that it acts as a fungicide and has a fungistatic function, leading to the coagulation of nucleoproteins and changes in cell walls allowing the possible escape of cytoplasmic components through the plasmalemma (7, 9). Their activity is thought to be due to microbial membrane disruption, which leads to increased cell permeability and leakage of intracellular ions (Na+, K) (12, 13).

The purpose of the present work was to evaluate the chlorhexidine activity *in vitro* against 120 isolates of *C. albicans* colonizing the mucous membranes of the mouth and throat, isolated from persons from 5 selected population groups. Some of them are predisposed to opportunistic infections caused by microorganisms of normal microflora, *e.g.*, fungal infections caused especially by yeast species belonging to *Candida* spp., mainly *Candida albicans*.

EXPERIMENTAL

Chlorhexidine was screened *in vitro* for antifungal activities using the broth microdilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (15) and the Clinical and Laboratory Standards Institute guidelines (16) against *Candida albicans* strains. The investigated strains included the reference strains of *C. albicans* ATCC 2091 and *C. albicans* ATCC 10231, which came from the American Type Culture Collection, routinely used for the evaluation of antimicrobials, and 120 clinical isolates of *C. albicans*. These fungi were isolated from the mucous membranes of the mouth and throat of:

1. hospitalized patients 37 – 73 years old suffering from cancer (*i.e.*, with non-small cell lung cancer or hematological malignancies). Some patients underwent pre- or post-operative chemotherapy (were treated with Vepesid or *cis*-platin given in doses according to the standard procedures).– 30 isolates;
2. patients with chronic HCV infection 30 – 65 years old – hepatitis C caused by the hepatitis C virus (undergoing peginterferon and ribavirin therapy or without antiviral therapy – 31 isolates;
3. patients with compromised immune systems, 43 – 80 years old – 28 isolates;
4. healthy school-age children 6 – 13 years old– 31 isolates;
5. elderly people 65 – 97 years old , living in closed communities (care centers), and people staying outside the home care – 30 isolates.

All strains were grown from swabs and identified by the standard diagnostic methods: microscopic, macroscopic and biochemical microtest, e.g., API 20 C AUX, ID 32 C, API Candida (bioMérieux) on the basis of assimilation of various substrates.

All of the used microbial cultures were first subcultured on Sabouraud agar at 30^{o}C for 24-48 h. The surface of RPMI 1640 with MOPS was inoculated with the suspensions of fungal species.

Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of McFarland standard scale 0.5 – approximately 5 x 10^5 CFU/mL (Colony Forming Units/mL). Samples containing 5 mg of chlorhexidine were dissolved in 1 ml dimethylsulphoxide (DMSO).

Subsequently, the Minimal Inhibitory Concentration (MIC) of chlorhexidine was examined by the microdilution broth method, using their two-fold dilutions in RPMI 1640 broth with MOPS prepared in 96-well polystyrene plates. The final concentrations of the antiseptic ranged from 40 to 0.312 µg/mL. Next 2 µL of each fungal suspension was added per each well containing 200 µL broth and various concentrations of chlorhexidine. After incubation (37°C, 24 h) the MIC was assessed spectrophotometrically as the lowest concentration of the samples showing complete fungal growth inhibition. The DMSO, growth and sterile controls were carried out. The medium without tested substances was used as control. The inhibition of microorganisms growth was evaluated by comparison with a control culture prepared without any sample tested (17, 18). The Minimal Fungicidal Concentration (MFC) is defined as the lowest concentration of a given compound necessary to kill a fungal species. MFC was determined by removing 20 µL of the culture used to determine MIC from each well and spotting onto the appropriate agar medium. Then, the plates were incubated in the appropriate conditions. The lowest concentration of the compound with no visible growth observed was assessed as a fungicidal concentration. All the experiments were repeated three times, and the reported data are representative (17, 18).

In addition, the MIC_{50} or MIC_{90} values for chlorhexidine were also calculated, defined as the MIC inhibiting 50% or 90% of the isolates, respectively. In turn, MFC_{50} and MFC_{90} values were described as the lowest concentrations required to kill 50% or 90% of fungal isolates. The MFC/MIC ratios were also calculated in order to determine fungicidal (MFC/MIC = 4) or fungistatic (MFC/MIC > 4) effect of the tested compounds (17, 18).

Statistical analysis

Data processing and analysis were performed using STATISTICA version 10 (StatSoft. Inc.). The difference in fungicidal or fungistatic effect of chlorhexidine was statistically analyzed in different groups by Fisher exact test. Relative risk (RR) and their 95% confidence intervals (CI) were calculated. Statistical significance was set at $p < 0.05$.

Ethics

The Ethical Committee of the Medical University of Lublin approved the study protocol (No. KE-0254/75/2011).

RESULTS

The present study, as well as other research (19), found a very strong bioactivity of both synthetic or naturally occurring compounds at MIC < 10 µg/mL. Our data (Table 1) showed a very strong antifungal effect of chlorhexidine against *C. albicans* clinical strains isolated from mucous membranes of the oral cavity of the selected groups of patients. The minimum concentrations of this compound that inhibited the growth of the yeasts were very high: 0.625-5 µg/ml. The MIC values slightly varied for different *C. albicans* strains.

The minimum concentrations of chlorhexidine that inhibited the growth of *C. albicans* isolates from patients with lung cancer were 1.25-5 µg/mL. Among them, the growth of 12 (40%) and 11 (37%) isolates was inhibited at the concentration of 5 µg/mL and 2.5 µg/mL, respectively. The antiseptic exhibited also an especially high activity against strains isolated from immunocompromised patients, with MIC ranging from 0.625 to 5 µg/mL. The 2.5 µg/mL solution showed anticandidal effect against as many as 20 (71%) isolates. The strains of *C. albicans* obtained from patients with chronic HCV infection, healthy children and elderly people were susceptible to chlorhexidine at the concentration range of 2.5-5 µg/mL. Most yeasts were inhibited by the antiseptic at the concentration of 5 µg/mL – 26 (84%), 22 (71%), and 26 (87%) strains isolated from patients with hepatitis C, healthy children, and elderly people, respectively (Table 1).

Most strains of *C. albicans* were inhibited by chlorhexidine at the minimum concentration of 5 µg/ml, except for the isolates from immunocompromised patients (2.5 µg/mL).

The obtained results indicate that the lowest concentration of chlorhexidine at which no growth was observed in all studied yeasts ranges from 2.5 µg/mL to 20 µg/mL, except in the case of isolates obtained from elderly people, where MFC = 5-10 µg/mL. The minimal fungicidal concentration was 5 µg/mL for 13 (46%), 17 (57%) and 28 (93.5%) strains of *C. albicans* isolated from patients with lung cancer, those with compromised immune systems and elderly people, respectively. Moreover, the lowest concentration of the antiseptic that was required to kill 14 (45%) isolates in both remaining groups of strains was 10 µg/mL (Table 1).

Table 1. Chlorhexidine activity, expressed as MIC and [MFC], against the *C. albicans* isolates from the studied groups.

The examined isolates	The number (percentage) of isolates with MIC and [MFC]						MIC_{50} and [MFC_{50}] (µg/mL)	MIC_{90} and [MFC_{90}] (µg/mL)
	20 µg/mL	10 µg/mL	5 µg/mL	2.5 µg/mL	1.25 µg/mL	0.625 µg/mL		
Patients with lung cancer	0 (0) [2 (7)]	0 (0)[10 (33)]	**12 (40)** **[17 (57)]**	11 (37) [1 (3)]	7 (23) [0 (0)]	0 (0)[0 (0)]	2.5 [5]	5 [10]
Immunocompromised patients	0 (0)[1 (4)]	0 (0)[11 (39)]	2 (7) **[13 (46)]**	**20 (71)** [3 (11)]	3 (11) [0 (0)]	3 (11) [0 (0)]	2.5 [5]	2.5 [10]
Patients with hepatitis C	0 (0)[8 (26)]	0 (0)**[14 (45]**	**26 (84)** [8 (26)]	5 (16) [1 (3)]	0 (0)[0 (0)]	0 (0)[0 (0)]	5 [10]	5 [20]
Healthy children	0 (0)[4 (13)]	0 (0)**[14 (45)]**	**22 (71)** [9 (29)]	9 (22) [4 (13)]	0 (0)[0 (0)]	0 (0)[0 (0)]	5 [10]	5 [20]
Elderly people	0 (0)[0 (0)]	0 (0)[2 (6.5)]	**26 (87)** **[28 (93.5)]**	4 (13) [0 (0)]	0 (0)[0 (0)]	0 (0)[0 (0)]	5 [5]	5 [10]

According to the data presented in Table 1, in the case of *C. albicans* isolates from patients with hepatitis C, healthy children and elderly people, which represented 50% or 90% of the studied isolates, MIC_{50} = MIC_{90} = 5 µg/mL. Both values for the strains isolated from immunocompromised patients were 2.5 µg/mL. In turn, MIC_{50} was 2.5 µg/ml and MIC_{90} was 5 µg/mL for isolates obtained from patients with lung cancer.

Our data also show that MFC_{50} was 10 µg/mL and MFC_{90} was 20 µg/mL for *C. albicans* isolates from patients with hepatitis C and healthy children. For all the remaining strains these values were similar: MFC_{50}= 5 µg/mL and MFC_{90} = 10 µg/mL.

Analyzing the results, we observed that chlorhexidine showed mainly fungicidal activity (MFC/MIC = 4) against *C. albicans* strains obtained from different population groups. The number of isolates on which the antiseptic had fungicidal effect ranged from 23 (74%) strains isolated from patients with hepatitis C to 30 (100%) isolates obtained from elderly people. The fungistatic activity (MFC/MIC > 4) was observed in a small number of *C. albicans* isolates (Table 2). As shown in Table 2, regarding the origin of *C. albicans* isolates, the statistically significant differences in fungicidal effect of chlorhexidine were found. It concerns the strains isolated from patients with hepatitis C ($p = 0.0047$) and healthy children ($p = 0.024$) which were somewhat less sensitive in comparison to the strains isolated from eldery people. However, in the case of isolates from remaining group of people, no statistically significant differences were found.

Chlorhexidine showed also a similar fungistatic or fungicidal effect on the reference strains of *C. albicans* ATCC 2091 and *C. albicans* ATCC 10231 (MFC/MIC = 4-8). The activity against these isolates was also very strong (MIC = 2.5 µg/mL, MFC = 10-20 µg/mL).

DISCUSSION

Different types of medicines are currently used in the treatment of oral problems. Most of these substances are chemical-based and show many side effects. Numerous studies have indicated that the use of a mouthwash in patients with superficial and systemic infection may decrease fungal and bacterial colonization in the oral cavity, and is a component of a complete oral hygiene regimen. Mouthwashes are a convenient and accepted method of oral hygiene (7). In many clinical studies chlorhexidine-based oral rinses were used with good results as an alternative to antifungal agents (4).

Other studies confirmed that chlorhexidine mouthrinse may be useful in treating and preventing oral candidiasis. Adhesion of *Candida* to the mucosal surfaces is a vital prerequisite for successful colonization and infection. Chlorhexidine is capable of inhibiting candidal adhesion to the surfaces (1, 3). Chlorhexidine mouthwash (0.12-0.2%) has been found useful in the prevention and treatment of oral candidiasis, reducing also the recurrence of the lesions (3, 12). The antifungal effect of chlorhexidine has been demonstrated in several *in vivo* and *in vitro* trials, including some related to *Candida* infections (3).

Our results indicate that chlorhexidine shows a very strong activity against different groups of *C. albicans* isolates obtained from mucous membranes of the oral cavity of the selected population groups (MIC = 0.625-5 µg/mL; MFC = 2.5-20 µg/mL). Similar results were obtained by many other authors (20-22). According to their data, chlorhexidine showed activity against *C. albicans* at MIC = 5 µg/mL. In turn, Amorim et al. and Park et al. found slightly higher values of MIC: 4 µg/mL and 4.88 µg/mL, respectively (23, 24).

The results of Teixeira et al. proved that the minimum concentration of chlorhexidine that inhibited *C. albicans* growth was 2 µg/mL (13). The strongest effect of the antiseptic was found by Ferguson et al. (MIC < 0.63 µg/mL) (25), while Downs et al. showed chlorhexidine activity against *C. albicans* at MIC = 9.4 µg/mL (26). According to Hwang et al., the values of MIC and MFC for the compound were the same: 32 µg/mL (27). Other authors indicated a little lower MIC: 40 µg/mL (28) and 50 µg/mL (29).

At the same time, Keijser et al. showed a broad range of activity of chlorhexidine at MIC = 3.5-60 g/mL against *C. albicans* isolated from 80 volunteers (40 females, 40 males; mean age 25.7 years) (30). Calamari et al. also showed a wide range of MIC for the antiseptic, from 12.5 µg/mL to 50 µg/mL, against *C. albicans* isolated from the saliva of 10 patients with oral candidiasis, wearing complete or partial dentures (4). The widest range (2.67-80 µg/mL) of chlorhexidine activity relative to *C. albicans* strains was found by Amorim et al. (23). Morrissey et al. confirmed that the lowest concentrations of chlorhexidine inhibiting the growth of *C. albicans* obtained from patients with hospital-acquired infections varied from 0.5 to 20 µg/mL (mainly 5-6 µg/mL). In turn, the minimum concentration at which the absence of fungus growth was observed was 3-30 µg/mL, with particular activity at the concentration of 8 µg/mL (31).

The approximate values of MIC against *C. albicans* strains, indicated by other authors, were: 1-20 µg/mL (32) and 1-16 µg/mL (33). Buxbaum et al. isolated *C. albicans* strains from patients with documented infections in hospitals located in Austria (33). On the other hand, Abbaszadegan et al. found much higher values of MIC and MFC (200 µg/mL) against *C. albicans* strains (34).

Our data confirm that chlorhexidine inhibited the growth of *C. albicans* isolated from healthy children at MIC = 2.5-5 µg/mL, while MFC varied from 2.5 to 20 µg/mL. The study by Smith et al. showed a similar activity of this compound against *C. albicans* obtained from 24 healthy volunteers (35).

In turn, Semprebom et al. evaluated the inhibitory performance of chlorhexidine digluconate under normoxic and anoxic conditions against 16 strains of *C. albicans* from periodontal pockets and other 20 from the oral mucosa. Strains were grown in normoxia and anoxia to adapt themselves to the different atmospheric conditions. The normoxic MIC of chlorhexidine varied broadly from 150 to

Table 2. Number (percentage) of *C. albicans* isolates with fungicidal or fungistatic effect of chlorhexidine.

The examined isolates	Number (percentage) of isolates with		Statistical analysis	
	Fungicidal activity	Fungistatic activity	RR (95%CI)	P value
Patients with lung cancer	29 (96)	1 (4)	0.97 (0.9-1.03)	1.0
Immunocompromised patients	25 (89)	3 (11)	0.89 (0.79-1.02)	0.11
Patients with hepatitis C	23 (74)	8 (26)	0.74 (0.6-0.91)	0.0047
Healthy children	25 (80)	6 (20)	0.81 (0.68-0.96)	0.024
Elderly people	30 (100)	0	referent	

RR, relative risk; 95%CI, 95% confidence interval

1200 µg/mL, whereas its anoxic MIC varied narrower from 2.34 to 37.5 µg/mL. Regarding the origins of strains, no statistically significant differences ($p > 0.05$) were found. These results indicate that anoxic environmental conditions, compatible with periodontal pockets, tend to enhance *C. albicans* susceptibility to chlorhexidine (36).

Furthermore, the results obtained by Salim et al. also indicated high anticandidal effect of chlorhexidine. The minimum inhibitory concentration of the antiseptic that inhibited the growth of 79 isolates comprising 8 different species associated with oral candidiasis ranged from 6.25 to 0.78 µg/mL, an average of 3.03 µg/mL, whereas the MIC for fluconazole was = 0.125-256 µg/mL, mainly 19.12 µg/mL. Among these isolates, 14 (18%) strains were resistant to fluconazole (MIC = 64 µg/mL), while all the strains were effectively inhibited by = 6.25 µg/mL solution of chlorhexidine (37). As our study shows, chlorhexidine exhibits also a very strong activity against *C. albicans* obtained from the oral cavity mucous membranes in immunocompromised patients (MIC = 0.625-5 µg/mL, mainly 2.5 µg/mL; MFC = 2.5-20 µg/mL). According Patel and Coogan, MIC of the antiseptic against 41 *C. albicans* strains (20 isolated from HIV-positive patients, 20 from HIV-negative ones, and *C. albicans* ATCC 90028) was 8-160 µg/mL (38). Traboulsi et al. found the activity of chlorhexidine against *Candida* spp. isolated from the oral cavity of HIV-infected patients with MIC ranging from 0.5 to 16 µg/mL (mostly 4 µg/mL). According to these data the values of MIC_{50} and MIC_{90} were 4 µg/mL and 8 µg/mL, respectively (39). Moreover, the data of Thurmond et al. showed that the MIC of chlorhexidine towards isolates obtained from the tongue and buccal mucosa of bone marrow transplant recipients ranged from 2.5 to 20 µg/mL (primarily 8.5 µg/mL) (40).

According to our data, the minimum concentration which inhibited the growth of *C. albicans* isolates obtained from patients with chronic HCV infection ranged from 2.5 to 5 µg/mL, while MFC was 2.5-20 µg/mL. In the case of strains isolated from cancer patients, both MIC and MFC were 1.25-5 µg/mL. Lafleur and Lewis indicated that MIC for chlorhexidine against 131 strains of *C. albicans* obtained from cancer patients ranged from 1.5 to 6 µg/mL, being 3.125 µg/mL against 50% of these strains. These isolates were obtained from 46 cancer patients, including 4 patients with hematological malignancies and 42 patients with solid tumors, from two affiliated teaching hospitals and a cancer hospital of the Shandong University Medical School. The isolates were selected for the cited study and sampled for oral *Candida* carriage. The patients' ages ranged from 21 to 76 years, and the average age was 48.8 years (41).

In addition, Bueno et al. reported that chlorhexidine inhibited biofilm formation at a high concentration of 64 µg/mL (42).

According to our results, chlorhexidine showed a very strong activity, with special fungicidal effect, against the majority of the tested *C. albicans* strains isolated from the studied patient groups. Interestingly, taking into account the origin of *C. albicans* strains, the isolates from patients with chronic HCV infections and healthy children were somewhat less sensitive to fungicidal effect of chlorhexidine in comparison to strains from elderly people with the statistically significant differences $p = 0.0047$ and $p = 0.024$, respectively. In turn, in the case of the isolates from other group of people, no statistically significant diferences were found.

In the case of *C. albicans* ATCC 2091 and *C. albicans* ATCC 10231, chlorhexidine also showed similar fungistatic or fungicidal effects. The antifungal effect was also very strong at MIC = 2.5 µg/mL and MFC = 10-20 µg/mL. According Nabavizadeh et al., MIC of this antiseptic for reference *C. albicans* strains was considerably lower: 0.02 µg/mL, whereas MFC was 10 µg/mL (43). The results of Talebi et al. indicated that both MIC and MFC of chlorhexidine against ATTC strains were 15 µg/mL (7). Similarly, according to Hendry et al., MIC was nearly the same against the reference strains of *C. albicans* – 16 µg/mL (44).

Furthermore, a number of clinical studies confirm the efficiency of *in vivo* chlorhexidine application, indicating that the use of a mouthwash in patients with systemic infection could decrease fungal and bacterial colonization in the mouth cavity. The studies on the effect of chlorohexidine gluconate on *Candida* adhesion to acrylic denture also found that fungal colonization decreases by using chlorhexidine. Other results indicated a considerable decrease in the colonization of adhesive *Candida* to epithelial cells in individuals using chlorhexidine (7). Nayak et al., having investigated the effect of chlorhexidine on dental plaque, concluded that chlorhexidine is an effective agent reducing dental plaque and the colonization of microorganisms in plaque (11).

Chlorhexidine-containing mouth-rinse has been shown to possess antifungal activity both *in vitro* and *in vivo* (1, 3). For this reason, chlorhexidine may be an alternative to conventional antifungals in the management of oral candidiasis. Moreover, the antiseptic can be used in the treatment

of this infection instead of antibiotics, to reduce the emergence of antibiotic resistance.

CONCLUSIONS

Chlorhexidine inhibited the growth of *C. albicans* at the concentrations of 0.625-5 µg/mL (in particular, 2.5 µg/mL chlorhexidine solution was effective against strains isolated from immunocompromised patients, and 5 µg/mL against the remaining isolates). The yeasts were also killed by 2.5-20 µg/mL chlorhexidine solutions. The concentration of 5 µg/mL was particularly active against the strains isolated from the elderly, immunocompromised and lung cancer patients, while 10 µg/mL was effective against the strains from the remaining two groups of isolates.

In conclusion, chlorhexidine showed a very strong activity, with particular fungicidal effect, against the majority of the tested *C. albicans* strains. This data and those from literature confirm their usefulness in prevention of oral candidiases in various groups of patients alone or in combination with other antifungal agents.

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Authors' statement

The authors declare that they have no competing interests.

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