Ann Med Res 2020;27(8):2041-6

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.05.478

Original Article

Investigation of antifungal, antibiofilm and anti-filamentation activities of biocides against *Candida* isolates

Ali Ozturk[1], Olkar Abdulmajed[2], Merve Aydin[3]

[1]Department of Medical Microbiology, Faculty of Medicine, Nigde Omer Halisdemir University, Nigde, Turkey
[2]Department of Medical Microbiology, Faculty of Medicine, Yuksek Ihtisas University, Ankara, Turkey
[3]Department of Medical Microbiology, Faculty of Medicine, KTO Karatay University, Konya, Turkey

Abstract

Aim: This study was aimed to evaluate the antifungal, antibiofilm, and anti-filamentation activities of three commonly used biocides against clinical *Candida* isolates.

Material and Methods: The in vitro activities of benzalkonium chloride (BZC), chlorhexidine digluconate (CHX), and triclosan (TRC) were studied against three *Candida (C. albicans, C. parapsilosis, and C. glabrata)* isolates. The isolates were identified, using the germ tube test, Cornmeal agar, and ID32 C (bioMerieux, France) kit. Antifungal susceptibility of these isolates was determined using the broth microdilution method. Antibiofilm activity of biocides were assessed with a colorimetric method based on crystal violet. The effect of biocides on the ability of C. albicans to form hyphae was examined microscopically.

Results: The antifungal, antibiofilm, and anti-filamentation activities of biocides used were determined against *Candida* isolates. Antifungal activities of biocides against *Candida* isolates ranged from 1–4 µg/ml. The potent fungistatic effect of biocides tested was detected against *Candida* spp. at 1 µg/ml. All tested biocides showed good antifungal activity against *Candida* isolates. The results of the antibiofilm activity assay showed that the highest biofilm inhibition ratios were reported in BZC, CHX, and TRC (66%, 60.3%, and 58%, respectively) at 16 and 32 µg/ml. The lowest biofilm inhibition ratios were reported at MIC values for each biocide between 6% and 12%. In addition, the anti-filamentation activity of biocides tested was screened against *C. albicans* at concentrations of 1, 2, and 4 µg/ml of biocide and the inhibition rates varied from 10% to 59%.

Conclusions: Important information has been obtained about the fungistatic/fungicidal, antibiofilm activities, and effects on *C. albicans* micromorphology of the biocides used in this study.

Keywords: Antifungal; antibiofilm; anti-filamentation; biocides; *candida*

INTRODUCTION

In recent years, the increase in the number of immunocompromised patients and hospitalized patients has led to a dramatic increase in the incidence of fungal infections (1,2). Candidiasis is one of the most common fungal infections worldwide. *Candida* species are opportunistic fungal pathogens leading to various diseases that found in normal microbiota in the gastrointestinal tract, respiratory tract, genital region, and mouth (2-4). Although *Candida albicans (C. albicans)* is the most common cause of mucocutaneous and systemic infections, the incidence of non-albicans *Candida* (NACA) species, such as *C. tropicalis, C. glabrata and C. parapsilosis* related disease are also increased (4,5).

Candida species have various virulence factors that enabling avoidance of host defence mechanisms and invasion of host tissues (6). One of the most important virulence factors of *Candida* spp. is that it adheres to the surfaces of medical devices such as catheters, prosthetic heart valves, and artificial joints forming biofilms. Biofilm formation causes negative consequences as it leads to the development of resistance to antifungal agents that are most frequently used in the clinical practice (7,8). Among *Candida* spp. biofilm production is most commonly observed in *C. albicans*. Biofilm production has also been reported to be a major virulence factor for *C. parapsilosis*, which plays a significant role in catheter-related infections. In addition, it has been showed that C. *tropicalis*, *C. parapsilosis*, and C. *glabrata* are producing biofilms (9-11).

Received: 15.05.2020 **Accepted:** 05.08.2020 **Available online:** 24.08.2020
Corresponding Author: Ali Ozturk, Department of Medical Microbiology, Faculty of Medicine, Nigde Omer Halisdemir University, Nigde, Turkey, **E-mail:** ali.81ozturk@gmail.com

The pseudohyphae formation of *C. albicans* is a cell dimorphism that depends on environmental conditions (12). The transition from the yeast cells to filamentous forms (pseudohyphae or hyphae) represents a major virulence factor associated with fungal cells escaping from the phagocytosis of macrophages, invading host tissues and causing greater damage. Furthermore, filamentation is crucial for the development of robust biofilm which further complicates treatment (13-15).

Biocides have been used for many years in a wide variety of commonly used compounds, including disinfectants, antiseptics, food preservatives, toothpaste, home-used detergents, and pesticides (16,17). They have also an essential role in the prevention and control of nosocomial infections (18). Benzalkonium chloride (BZC), chlorhexidine digluconate (CHX) and triclosan (TRC) are the most commonly used biocides in healthcare (19). BZC is a quaternary ammonium compound that has been in clinical use for many years. It is used as a disinfectant and antiseptic in food industry, healthcare institutions and households as well as as antimicrobial preservatives in drugs (20,21). CHX is a cationic bisbiguanide with broad antimicrobial activity. It is widely used as a disinfectant and preservative. Due to its antiseptic feature, it is also found in hand washing and oral products (22,23). TRC (Irgasan), is a non-ionizing, synthetic, and broad-spectrum antimicrobial agent. In clinical practice, TRC is used as a disinfectant and an antiseptic in implants, surgical sutures, scrubs, and medical devices. TRC is available as a preservative in various consumer products such as cosmetics, soaps, shower gels, antiperspirants, hand lotions, hand creams, toothpastes, plastics and toys (24,25).

Widespread use of biocides in many areas, concerns about their effects on human health, and their impact on biocide resistance in microorganisms have increased (17). It is believed that the mechanism of action of biocides, which have fungicidal and fungistatic activity on fungi, is similar to the antibacterial activity. In addition, there are a limited number of studies evaluating the effectiveness of biocides against fungi (26). The aim of this study was to investigate the fungistatic/fungicidal and antibiofilm activities of BZC, CHX and TRC against clinical *C. albicans* and NACA isolates, and also to evaluate its effect on *C. albicans* filamentation.

MATERIAL and METHODS

Fungal isolates

Three *Candida* (*C. albicans*, C. *glabrata*, and *C. parapsilosis*) isolates were selected from the Gazi University Medical Mycology Laboratory culture collection. These isolates were cultured on Sabouraud dextrose agar (SDA) (OXOID, Italy) and incubated at 37°C for 24–48 hours under aerobic conditions. All isolates were identified at the species level using macroscopic and microscopic observations of cell morphology (germ tube and cornmeal agar) and the ID32 C (bioMerieux, France) kit was used for confirmation.

Biocides

Biocides were supplied from the manufacturers. The concentrations of biocides were determined through a preliminary study. The highest concentrations of BZC (Sigma, Denmark), CHX (Sigma, Spain) and TRC (Sigma, India) were determined as 256 µg/ml, 64 µg/ml, and 16 µg/ml, respectively.

Antifungal susceptibility assay

In this study, the *in vitro* susceptibility test with microdilution method was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) M27-A3 guidelines (27). Serial dilutions were performed 10 times for each biocide starting from 256 µg/ml, 64 µg/ml and 16 µg/ml concentrations for BZC, CHX, and TRC, respectively. The yeast suspension was prepared to be 1.5×10^3 CFU/ml and 10 µL of the prepared yeast suspension was transferred to microplate wells containing different biocide concentrations. Positive and negative control was included in each assay. The microplates were incubated at 35°C for 24 hours and the presence or inhibition of microbial activity was determined visually. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were determined for each isolate. To determine the MFC value, 10 µL of aliquots of the wells that equal and two- and four-fold of the MIC value were transferred to SDA plates. Following the incubation at 35°C for 48 hours, the plates were examined for colony formation. The concentration without fungal growth was determined as MFC value. In the experiment, fluconazole (FLC) was used as the reference antifungal agent.

Biofilm formation assay

Antibiofilm activity of the biocides was tested using a 96-well flat-bottom microplate. Four different concentrations (MIC, 2 × MIC, 4 × MIC, and 8 × MIC) prepared from each biocides were tested. Briefly, 0.5 McFarland of yeast suspensions were prepared in Sabouraud dextrose Broth (SDB) and 100 µL from yeast suspension was added to each well of the microplate. Microplates were incubated at 37°C for 24 hours in shaker incubator (at 180 rpm). Following the incubation, the microplates were washed three times with phosphate-buffered saline (PBS) to remove unbound cells. 100 µL of the prepared concentrations of biocide was added to each well and incubated with shaking at 37°C for 24 hours. Following the incubation, the microplates were washed three times with PBS and 100 µL of 0.1% crystal violet was added to each well. After waiting for five minutes at room temperature, the microplates were washed again three times with PBS. Then, 150 µL of 0.04 N HCl-isopropanol and 50 µL of 0.25% sodium dodecyl sulfate (SDS) were added to each well. Then the optical density (OD) of each well was read at 590 nm. The wells identified as positive controls in the experiment were those containing only SDB and yeast suspensions. The rate of biofilm inhibition was calculated using the following formula. The biofilm inhibition rates were expressed as percent (%) by comparison with positive controls (28).

Biofilm inhibition rate = (OD control-OD sample / OD control) × 100

Inhibitory effect of the biocides on C. albicans filamentation

The ability of *C. albicans* to form pseudohyphae at 37°C is one of the virulence factors and is the first step in the adhesion to surfaces and colonization. This experiment aimed to investigate the effect of biocides on the ability of *C. albicans* to form hyphae. The experiment was carried out as defined by Romo JA. et al. with some modifications (29). Briefly, different dilutions of biocides (MIC, 2 × MIC, 4 × MIC) were prepared on Yeast Extract Peptone Dextrose (YPD) medium containing 10% fetal bovine serum and the prepared dilutions were inoculated with 10 µL of yeast suspension and incubated at 37°C for six hours with shaking. After incubation, the samples were centrifuged at 300 rpm for four minutes and samples were prepared by using the pellets formed. Samples without biocide were used as positive controls in the experiment. Per sample, 50 cells were randomly selected and the number of *C. albicans* cells with filamentation was noted. Morphological changes were compared with positive control and examined microscopically.

RESULTS

Fungistatic and fungicidal effects of biocides

Broth microdilution method was used to examine the antifungal activities of the biocides tested. FLC (128 µg/ml) was used as reference antifungal agent. The MIC and MFC values of the biocides against *C. albicans*, C. *glabrata*, and *C. parapsilosis* are given in Table 1. The MIC range values for biocides tested were 1-4 µg/ml for BZC and 1-2 µg/ml for CHX and TRC in the test. The potent fungistatic effect was detected against *Candida* isolates at 1 µg/ml concentration. As for the standard antifungal drug, MIC values of FLC against

C. albicans and NACA were found to be 4–16 µg/ml. Comparison of the MIC values of biocides with the reference drug showed that the fungistatic effect of biocides against *Candida* isolates was more effective than FLU. The MFC range values for biocides tested found to be 4-16 µg/ml for BZC, 4-8 µg/ml for CHX and 4-16 µg/ml for TRC. MFC values of FLC were found to be higher than from MFC values of biocides (Table 1). Thus, it was concluded that all biocides tested had fungicidal activity against *Candida* isolates at low concentrations.

Table 1. Antifungal activity of biocides and fluconazole against clinical Candida isolates

Isolates	BZC		CHX		TRC		FLC	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans	1	8	1	8	1	4	4	16
C. glabrata	4	16	2	8	2	16	16	64
C. parapsilosis	1	4	1	4	2	8	8	16

BZC: Benzalkonium chloride; CHX: Chlorhexidine digluconate; TRC: Triclosan; FLC: Fluconazole. Values are expressed in µg/ml

Antibiofilm activity assay

The antibiofilm activity of used biocides was evaluated via crystal violet method by exposing *Candida* isolates to four different dilutions (MIC, 2×MIC, 4×MIC, and 8×MIC) of each biocide. Figure 1 provides the biofilm inhibition rate (%) for each biocide. As for preformed biofilms, exposure to 1–32 µg/ml of biocides tested could only reduce the viability of mature biofilms by 6%-66%, as compared to positive controls. The highest biofilm inhibition rates were determined at 8–32 µg/ml (above MIC values). Therefore, the highest biofilm inhibition ratios were reported for BZC, CHX and TRC (66%, 60.3% and 58%, respectively) at 16 and 32 µg/ml. The lowest biofilm inhibition ratios were reported at MIC values for each biocide between 6% and 12% at MIC values. Most powerful antibiofilm activity was observed with 66% inhibition rate in BZC at 32 µg/ml against C. *glabrata* isolate. On the other hand, the weakest antibiofilm activity of biocides was observed in CHX against *C. albicans* at 1 µg/ml with an inhibition rate 6%. The results showed that the antibiofilm activity of the biocides was dose-dependent.

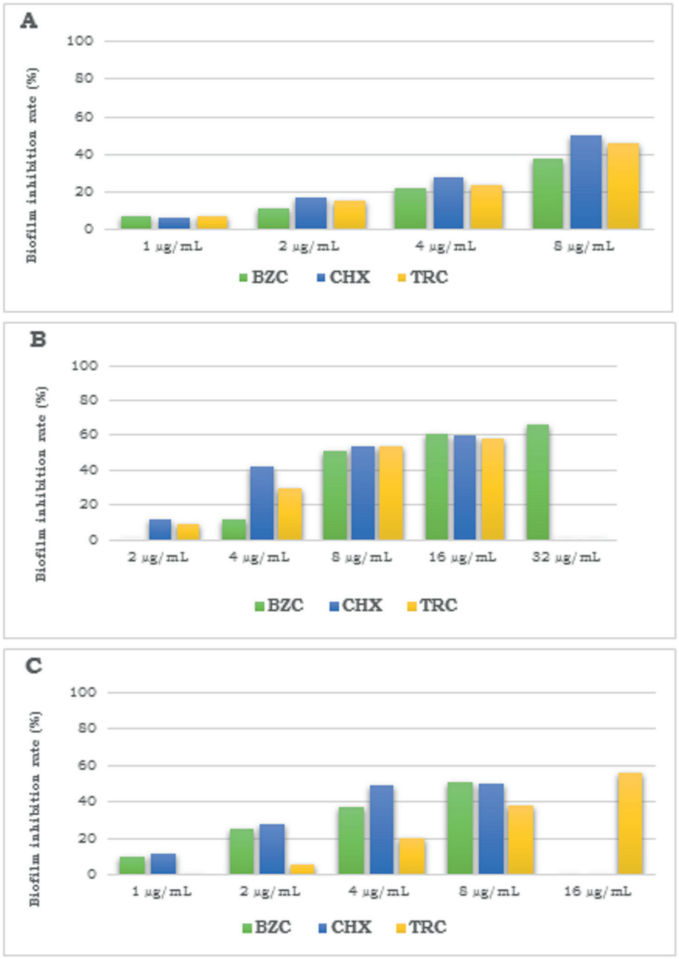

Figure 1. Biofilm inhibition rates of biocides against C. albicans (A), C. glabrata (B) and C. parapsilosis (C) isolates. Four different dilutions (MIC, 2×MIC, 4×MIC, and 8×MIC) of each biocide was used to examine the antibiofilm activities of the biocides tested

Anti-filamentation activity of biocides

In the present study, the effects of biocides tested on pseudohyphae/hyphae formation in clinical isolate of *C. albicans* have been investigated. As shown in Figure 2, increasing the biocide concentration resulted in the reduction of the hyphal form. In the microscopic examination, untreated control sample showed massive hyphal growth in *C. albicans* after six hours whereas biocides treated sample were reduced the hyphal formation in a concentration-dependent manner in tested medium. In the experiment, anti-filamentation activity of biocides against *C. albicans* isolate ranged 10%–59% at concentrations of 1, 2 and 4 µg/ml of biocide. The best inhibition ratios were reported for BZC, CHX and TRC (59%, 47% and 45%, respectively) at 4 µg/ml concentration.

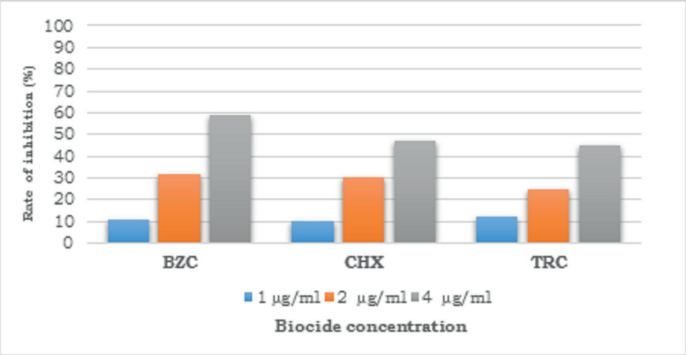

Figure 2. Inhibitory effect of biocides tested on C. albicans filamentation

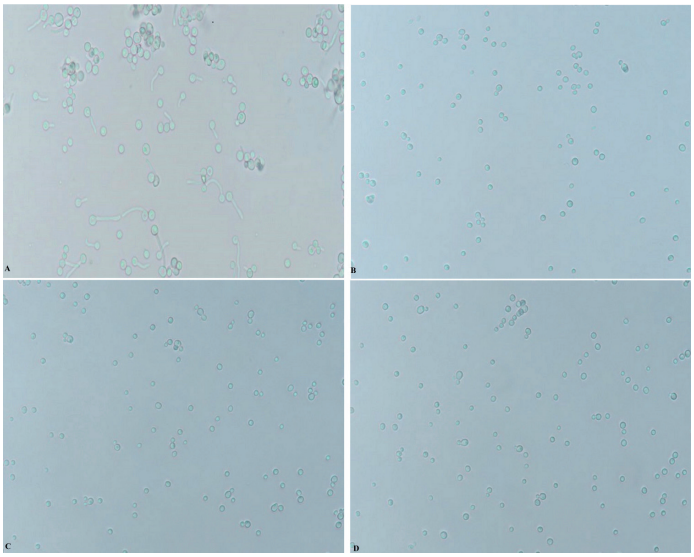

Figure 3. Microphotographs (40X) of C. albicans in the absence and presence of biocides. (a) C. albicans (in the absence of the biocides) as controls showing the presence of pseudohyphae. (b, c and d) decreased ability of the yeast to develop germ tube or pseudohyphae after exposure to BZC, CHX and TRC, respectively at a concentration of 4 µg/ml

Also biocides showed an inhibitory effect with inhibition rates ranged 10%–32% at 1 µg/ml and 2 µg/ml concentrations. At higher concentration (4 µg/ml) the yeast form prevailed so that and the filament forms were significantly reduced. It has been found that the biocides affect the morphological transition capability, preventing the formation of germ tube or pseudohyphae (Figure 3).

DISCUSSION

Biocides are antimicrobial chemicals used to protect surfaces in the hospitals setting or products in the industry from environmental contamination. Effective application of disinfection is important in the prevention and control of hospital infections. However, biocide resistance occurring in microorganisms causing hospital infection is one of the problems encountered in disinfection applications (30, 31).

As for bacteria, the mechanisms of fungal resistance to biocides can be divided into the intrinsic and the acquired mechanisms. Intrinsic mechanisms refer to a natural feature of the fungal cell, such as decreased permeability, stimulated activity of efflux pumps, repair mechanisms, phenotypic modulation, and inactivation of biocides. In parallel, acquired resistance mainly refers to the mutation or collection of genetic materials that allow cells to survive in detrimental condition (32).

In the present study, we selected BZC, TRC and CHX biocides as they are widely used in hospitals, foods and cleaning agents for disinfectant or protective purpose. Therefore, these biocides encounter fungi at low doses for a long time.

Here, we evaluated both the antifungal and antibiofilm activities of the selected biocides against clinically isolated *C. albicans*, *C. parapsilosis*, and C. *glabrata* and further investigated the effects of these biocides on the micromorphology of *C. albicans* (ability to form hyphae).

Biocides, which were tested according to their MIC and MFC values determined via microdilution method, were found to have both fungistatic and fungicidal activity against *C. albicans* and NACA isolates at different concentrations.

The antibiofilm activity of the biocides was investigated *in vitro* using crystal violet method. Biofilm inhibition rates were calculated by adding different concentrations of the biocides tested against *Candida* spp. biofilms. Results showed that the highest biofilm inhibition ratio was in BZC which was found to reduce the biofilm formation of the C. *glabrata* isolate by 66% at 32 µg/ml. The highest antibiofilm activity of the biocides was seen at 8, 16, and 32 µg/ml, which was the highest concentrations, whereas the lowest activity was observed at MIC values. Our findings have shown that the antibiofilm activity of the biocides is dose-dependent.

Cell dimorphism (transition from the yeast form to pseudohyphal-hyphal form), which is an important virulence factor of *C. albicans*, has been investigated in the presence of biocides. Biocides significantly inhibited the production of *C. albicans* pseudohyphae/hyphae formation up to 59% at 4 µg/ml biocide concentration (Figure 2). The difference in *C. albicans* micromorphology

was found to be directly proportional to the difference in biocide concentrations. Therefore, suppression of *C. albicans*' filamentation by biocides also inhibits the adherence and virulence of *Candida*. When all the results obtained from the experiments were evaluated, biocides tested have been found to have both antifungal activities and inhibits the biofilm and filamentation of *Candida*.

Although biocide resistance on bacteria and bacterial biofilms has been investigated in detail over a number of years, a limited number of studies have been conducted on the susceptibility of fungus and fungal biofilms to biocides.

Gupta et al. reported that chlorhexidine and calcium hydroxide used in canal treatment in teeth was effective on *C. albicans* and Enterococci (33).

In another study, antifungal activity of 12 commercial essential oils, two biocides (chlorhexidine and triclosan) and two terpenes on *C. albicans* planktonic and biofilm phases was evaluated. While all compounds have antifungal activity on the planktonic phase of *C. albicans*, six of these compounds, including chlorhexidine, were also effective against *C. albicans* biofilms (34).

Theraud et al. evaluated the effectiveness of UV radiation, five antiseptics and three biocides against the cultures, yeast mixtures, and biofilms of clinical and environmental yeast isolates. Three clinical (*C. albicans*, Rhodotorula rubra and Cryptococcus neoformans) and two environmental yeast isolates (*C. albicans* and Cryptococcus uniguttulatus) were included in their study. They reported that in seven of the eight biocides tested and UV radiation, the sensitivity was not different by type, species or origin. Biocides were effective on planktonic cultures and less effective on yeast mixtures. When the effect on biofilms was examined, only one of the nine biocides was found to be effective on the biofilm. In the study, Chlorhexidine (0.5%) was found to be only agent effective on all forms (35).

Leung et al. assessed the efficacy of commonly used biocides (sodium hypochlorite (Clorox), hydrogen peroxide, ethanol and iodine solution) on different growth stages of Escherichia coli and *Candida* species. They reported that the planktonic phase was sensitive to all biocides, whereas both adhesion and late biofilm phase of biofilms were less sensitive to biocides at recommended concentrations. They stated that biocides could not completely eliminate microbial cells adhering after short exposure and forming biofilms on biocide treated surfaces (36).

The data obtained in this study are consistent with the results of studies evaluating the effectiveness of biocides on yeasts. However, the low number of yeast species and samples was limitation of our study. Further studies with larger sample size, fungus diversity and common biocides are needed to investigate the biocide resistance on fungus and fungal biofilms.

CONCLUSION

Important information has been achieved about the antifungal effectiveness, antibiofilm activity and effects on *C. albicans* micromorphology by the used biocides in the present study. *In vitro* and *in vivo* studies investigating other properties of these biocides should be performed to ensure that they can be used effectively in the prevention of fungal infections. However, should not be forgotten the improper use of such biocides can lead to the development of resistance in fungi as well as in bacteria. For this reason, it is great importance to select the appropriate biocides and determine their effectiveness against the causative agents of infection.

Conflict of interest: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: Ethics committee approval was not obtained because the tested yeast isolates were obtained from the culture collection.

REFERENCES

1. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol 2010;36:1-53.
2. Sardi JCO, Scorzoni L, Bernardi T, et al. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol 2013;62:10-24.
3. Martins N, Ferreira IC, Barros L, et al. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. Mycopathologia 2014;177:223-40.
4. Taei M, Chadeganipour M, Mohammadi R. An alarming rise of non-albicans Candida species and uncommon yeasts in the clinical samples; a combination of various molecular techniques for identification of etiologic agents. BMC Res Notes 2019;12:779.
5. Juyal D, Sharma M, Pal S, et al. Emergence of non-albicans Candida species in neonatal candidemia. N Am J Med Sci 2013;5:541-5.
6. Schaller M, Borelli C, Korting HC, et al. Hydrolytic enzymes as virulence factors of Candida albicans. Mycoses 2005;48:365-77.
7. Pierce CG, Chaturvedi AK, Lazzell AL, et al. A novel small molecule inhibitor of Candida albicans biofilm formation, filamentation and virulence with low potential for the development of resistance. NPJ Biofilms Microbiomes 2015;1. pii: 15012.
8. Gulati M, Nobile CJ. Candida albicans biofilms: development, regulation, and molecular mechanisms. Microbes Infect 2016;18:310-21.
9. Kumamoto CA. Candida biofilms. Curr Opin Microbiol 2002;5:608-11.
10. Silva S, Henriques M, Martins A, et al. Biofilms of non-Candida albicans Candida species: quantification, structure and matrix composition. Med Mycol 2009;47:681-9.

11. Cavalheiro M, Teixeira MC. Candida biofilms: threats, challenges, and promising strategies. Front Med (Lausanne) 2018;5:28.
12. Whiteway M, Bachewich C. Morphogenesis in Candida albicans. Annu Rev Microbiol 2007;61:529-53.
13. Romo JA, Pierce CG, Chaturvedi AK, et al. Development of anti-virulence approaches for Candidiasis via a novel series of small-molecule inhibitors of Candida albicans filamentation. mBio 2017;8:pii:e01991-17.
14. Chen H, Zhou X, Ren B, et al. The regulation of hyphae growth in Candida albicans. Virulence 2020;11:337-48.
15. Lee JH, Kim YG, Gupta VK. Suppression of fluconazole resistant Candida albicans biofilm formation and filamentation by methylindole derivatives. Front Microbiol 2018;9:2641.
16. De Muynck W, De Belie N, Verstraete W. Antimicrobial mortar surfaces for the improvement of hygienic conditions. Appl Microbiol 2010;108:62-72.
17. Morrissey I, Oggioni MR, Knight D, et al. Evaluation of epidemiological cut-off values indicates that biocide resistant subpopulations are uncommon in natural isolates of clinically-relevant microorganisms. PLoS One 2014;9:e86669.
18. Maillard JY. Antimicrobial biocides in the healthcare environment: efficacy, usage, policies, and perceived problems. Ther Clin Risk Manag 2005;1:307-20.
19. Chiapetta SC, de Oliveira ÉCB, Olivier BC, et al. Intralaboratory validation, comparison and application of HPLC-UV-DAD methods for simultaneous determination of benzalkonium chloride, chlorexidine digluconate and triclosan. J Braz Chem Soc 2011;22:1913-20.
20. Gerba CP. Quaternary ammonium biocides: efficacy in application. Appl Environ Microbiol. 2015;81:464-9.
21. Merchel Piovesan Pereira B, Tagkopoulos I. Benzalkonium chlorides: uses, regulatory status, and microbial resistance. Appl Environ Microbiol 2019;85:pii: e00377-19.
22. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12:147-79.
23. Zorko M, Jerala R. Alexidine and chlorhexidine bind to lipopolysaccharide and lipoteichoic acid and prevent cell activation by antibiotics. J Antimicrob Chemother 2008;62:730-7.
24. Dhillon GS, Kaur S, Pulicharla R, et al. Triclosan: current status, occurrence, environmental risks and bioaccumulation potential. Int J Environ Res Public Health 2015;12:5657-84.
25. Alfhili MA, Lee MH. Triclosan: An update on biochemical and molecular mechanisms. Oxid Med Cell Longev 2019;2019:1607304.
26. Russell AD, Furr JR. Biocides: mechanisms of antifungal action and fungal resistance. Sci Prog 1996;79:27-48.
27. CLSI. Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard-Third Edition; CLSI document M27-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2008.
28. Janek T, Łukaszewicz M, Krasowska A. Antiadhesive activity of the biosurfactant pseudofactin II secreted by the Arctic bacterium Pseudomonas fluorescens BD5. BMC Microbiol 2012;12:24.
29. Romo JA, Pierce CG, Chaturvedi AK, et al. Development of anti-virulence approaches for Candidiasis via a novel series of small-molecule inhibitors of Candida albicans filamentation. mBio 2017;8:e01991-17.
30. Gunaydin M, Esen S, Karadag A, et al. In vitro antimicrobial activity of Medilox® super-oxidized water. Ann Clin Microbiol Antimicrob 2014;13:29.
31. Bridier A, Briandet R, Thomas V, et al. Resistance of bacterial biofilms to disinfectants: a review. Biofouling 2011;27:1017-32.
32. Maillard JY. Antifungal activity of disinfectants. In: Fraise AP, Lambert PA, Maillard JY, eds. Russell, Hugo and Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization. 4th edition. Blackwell; 2008. p.205-13.
33. Gupta SP, Bhati M, Jhajharia K, et al. Evaluation of antimicrobial and antifungal efficacy of inter appointment intracanal medicaments against Enterococcus and Candida albicans: an in vitro study. J Int Oral Health 2015;7:97-102.
34. Serra E, Hidalgo-Bastida LA, Verran J, et al. Antifungal activity of commercial essential oils and biocides against Candida albicans. Pathogens 2018;7:E15.
35. Théraud M, Bédouin Y, Guiguen C, et al. Efficacy of antiseptics and disinfectants on clinical and environmental yeast isolates in planktonic and biofilm conditions. J Med Microbiol 2004;53:1013-8.
36. Leung CY, Chan YC, Samaranayake LP, et al. Biocide resistance of Candida and Escherichia coli biofilms is associated with higher antioxidative capacities. J Hosp Infect 2012;81:79-86.