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SUSCEPTIBILITY OF PLANKTONIC AND FILM FORMS OF *CANDIDA GLABRATA* AND *CANDIDA ALBICANS* TO CATIONIC SURFACTANT ANTISEPTICS

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Дослідити чутливість планктонних і плівкових форм C. albicans та C. glabrata до антисептиків на основі катіонних поверхнево-активних речовин (ПАР). Матеріали та методи дослідження. Об'єктом дослідження стали 20 клінічних штамів C. albicans та 15 C. glabrata, виділених від хворих хірургічних відділень. Чутливість планктонних форм досліджуваних штамів мікроорганізмів до антисептичних засобів вивчали за допомогою кількісного макрометоду подвійних серійних розведень у рідкому поживному середовищі Сабуро. Біоплівкоутворюючі властивості досліджуваних клінічних штамів визначали за допомогою спектрофотометричного методу за G.D. Christensen (MtP-test). Вплив антисептиків на плівкові форми Candida spp. визначали шляхом відтворення біоплівок за вищеописаною методикою з додаванням суббактеріостатичних концентрацій антисептиків. В дослідженні використовували антисептики на основі катіонних ПАР хлоргексидину біглюконат 0,05 (Хлоргексидин-КР, виробництва ПАТ «Хімфармзавод «Червона зірка», м. Харків, Україна (ХГ)) та декаметоксин 0,2 (Декасан, виробництва ТОВ «Юрія-Фарм», м. Київ, Україна (ДКМ)). Результати. За результатами досліджень встановлено нижчу чутливість штамів C. glabrata до ХГ, у порівнянні з чутливістю штамів C. albicans. Поряд з цим, активність ДКМ щодо досліджуваних представників Candida spp. достовірно не відрізнялась. Клінічні штами C. glabrata виявилися більш чутливими до ДКМ порівняно з їх чутливістю до ХГ. Штами C. albicans проявили середні плівкоутворюючі властивості, в той час як C. glabrata – високі. Досліджувані антисептики на основі катіонних ПАР володіли в однаковій мірі потужною активністю щодо плівкоутворення клінічних штамів Candida spp. Висновки. Антисептики на основі катіонних ПАР (ХГ та ДКМ) володіють протигрибковою активністю щодо планктонних і плівкових форм клінічних штамів C. albicans та C. glabrata.

Ключові слова: *Candida,* катіонні поверхнево-активні речовини, чутливість, біоплівки

The aim of the study was to investigate the sensitivity of planktonic and film forms of C. albicans and C. glabrata to cationic surfactant antiseptics. Materials and methods. The study was based on investigating 20 clinical strains of C. albicans and 15 C. glabrata isolated from surgical inpatientsю. The sensitivity of planktonic forms of investigated strains to antiseptic agents was quantitatively evaluated by two-fold serial dilutions (macrodilution) in Sabouraud liquid nutrient medium. Biofilm-forming properties of clinical strains C. albicans and C. glabrata were assessed by using the Christensen's spectrophotometric method (MtP-test "microtiter plate test"). The influence of the antiseptics on C. albicans and C. glabrata film forms was assessed by the reproduction of the biofilms according to the above-described procedure with adding antiseptics in sub-bacteriostatic concentrations and the subsequent spectrophotometric ODU assessment. In the study we used antiseptics based on cationic surfactants, chlorhexidine digluconate 0.05 (Chlorhexidine-KR, manufactured by PJSC "Khimfarmzavod "Chervona zirka"", Kharkiv, Ukraine (CHH)) and decamethoxin 0.2 (Decasan, produced by Yuria-Farm LLC ", Kyiv, Ukraine (DCM)). Results. According to the research results, lower sensitivity of C. glabrata strains to CHH was found, compared to the sensitivity of C. albicans strains. In addition, the activity of DCM in the investigated representatives of Candida spp. did not differ significantly. Clinical strains of C. glabrata were more susceptible to DCM compared to their susceptibility to CHH. C. albicans strains showed medium film-forming properties, while C. glabrata - high. The investigated cationic surfactant antiseptics possessed the same degree of activity on the film-forming properties of clinical strains of Candida spp. Conclusions. Cationic surfactant antiseptics (CHH and DCM) possess antifungal activity against planktonic and film forms of C. albicans and C. glabrata.

Key words: *Candida,* cationic surfactant antiseptics, sensitivity, biofilm.

Introduction

Yeast-like fungi that belong to the genus Candida are known to be an inseparable component of human microbiota. However, immune deficiency conditions or / and

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impaired homeostasis can contribute to the development of candidiases [1].

According to the relevant literature, until the beginning of the 21st century yeast fungi caused about 3% of post-operative infectious complications [2]. Lately, however, *Candida spp*. has been reported to as a rapidly growing cause of infectious post-operative complications. Fungal infections have been found out to result in high mortality rate, which sometimes reaches the rates of deaths due to tuberculosis and malaria [3]. Fungi of the genus *Candida* rank the sixth place among the main nosocomial pathogens and the fourth place among the dominant causative agents of nosocomial bacteria. This is due to the decline in community immunity among the population, alterations in the biological properties of pathogens and the rapid development of microbial resistance to antimicrobial agents [2, 4]. In order to improve the situation it is of great importance to reconsider and update existing treatment standards.

In the past, *C. albicans* was known as the most prevalent species of *Candida spp.* throughout the world and described as a causative agent of about two thirds of all cases of invasive candidiasis [5]. However, today there are some changes within the spectrum of etiological factors of candidiasis infection that indicate a growing shift towards the so-called non-albicans species (*C. glabrata, C. krusei, C. tropicalis*) [5-7].

While *C. albicans, C. krusei, C. tropicalis* are genetically related, *C. glabrata* does not have a close phylogenetic relationship with other members of the genus. This determines the existence of significant differences in their morphology, pathogenicity factors, sensitivity to medications and thus the pathogenesis of mycoses caused by this causative agent [8]. This requires a comprehensive in-depth study of the biological properties of *Candida spp.* within the boundaries of the genus and the search for medicines that would have the same equal antifungal effect on different representatives of the genus.

The aim

of the study was to investigate the sensitivity of planktonic and film forms of *C. albicans* and *C. glabrata* to cationic surfactant antiseptics.

Materials and methods

The study was based on investigating 20 clinical strains of *C. albicans* and 15 *C. glabrata* isolated from surgical inpatients staying at M. Sklifosovskiy Poltava Regional Clinical Hospital and M. Pyrogov Vinnytsia Regional Clinical Hospital. The research has been carried out according to Helsinki Declaration on the ethical principles for medical research involving human subjects and approved by the Bioethics Committee of M. Pyrogov Vinnytsia National Medical University and the Commission on Ethical Issues and Bioethics of Ukrainian Medical Stomatological Academy.

To investigate the microflora, the material was taken from the surface of the infected surgical wound, followed by cultivation on Sabouraud culture medium for 48 hours. The final identification was carried out in accordance with the standard methodology for morphological, tinctorial and biochemical properties and by applying automatic bacteriological analyzer Vitec - 2compact bioMérieux (France).

The sensitivity of planktonic forms of investigated strains to antiseptic agents was quantitatively evaluated by two-fold serial dilutions (macrodilution) in Sabouraud liquid nutrient medium in accordance with the Order of the Ministry of Public Health of Ukraine No. 167, April 5, 2007 "On Approval of Methodological Instructions on "Assessment of Sensitivity of Microorganisms to Antibacterial Drugs "[9]. For this purpose, a microbial suspension (inoculum) equivalent to 1.0 was prepared according to the McFarland standards, and diluted 100-fold in a saline, after that the microbial concentration obtained was 3x1010 CFU / cm^3. Then, inoculums prepared *ex tempore* and taken in a dose of 1.0 ml were applied into each test tube with a two-fold dilution of antiseptic solution and into one test tube containing 1.0 ml of nutrient broth ("negative" control). All test tubes with the exception of "negative" control were incubated in the standard atmosphere at 35 ° C for a day. The "negative" control test tube was kept at a temperature 4 ° C until the results were recorded.

Biofilm-forming properties of clinical strains *C. albicans* and *C. glabrata* were assessed by using the Christensen's spectrophotometric method (MtP-test "microtiter plate test"). Biofilms were modelled in Sabouraud liquid nutrient medium in wells of a sterile, flat-bottom 96-well polystyrol tablet (Corning, USA) and stained with 1% solution of crystalline violet. The properties of microorganisms to form a biofilm were measured by absorbance of the dye in units of optical density (ODU) using a spectrophotometer with a wavelength of 570 nm. The ability of microorganisms to form biofilms was evaluated as low (ODU <0.120), average (ODU = 0.121-0.239) and high (ODU > 0.240) [10].

The influence of the antiseptics on *C. albicans* and *C. glabrata* film forms was assessed by the reproduction of the biofilms according to the above-described procedure with adding antiseptics in sub-bacteriostatic concentrations and the subsequent spectrophotometric ODU assessment.

In the study we used antiseptics based on cationic surfactants, chlorhexidine digluconate 0.05 (Chlorhexidine-KR, manufactured by PJSC "Khimfarmzavod "Chervona zirka"", Kharkiv, Ukraine (CHH)) and decamethoxin 0.2 (Decasan, produced by Yuria-Farm LLC ", Kyiv, Ukraine (DCM)).

The statistical analysis of the obtained findings was performed by using the standard SPSS Statistics 23 and Microsoft Excel 2010 software packages. We calculated the arithmetic mean (M), the mean error of the arithmetic mean (± m), and the criterion of the reliability of the differences (p). The differences between the indicators studied were evaluated according to Student's t-test.

Results and discussion

The obtained findings demonstrated that CHH and DCM were found to produce varying antifungal effect on investigated clinical strains of yeast fungi (Table 1). The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFcC) of CHH for *C. glabrata* significantly exceeded the MIC and MFcC showed by DCM for *C. albicans* in 1.6 times ($p < 0.05$). This indicated a lower sensitivity of *C. glabrata* strains to CHH when compared with the *C. albicans* strain sensitivity. In addition, the activity of DCM against the investigated representatives of *Candida spp.* did not differ significantly ($p < 0.05$). However, it should be noted that MFcC demonstrated by DCM against clinical strains of *C. glabrata* was 1.7 times lower than MFcC of CHH against the relevant strain ($p < 0.05$). This pointed out a significantly higher sensitivity of *C. glabrata* to DCM than to CHH.

Table 1
Susceptibility of Candida spp clinical strains to antiseptics, 10^{-3} mg/ml, M±m

Microorganism	N	Chlorhexidine digluconate		Decamethoxin	
		MIC	MFcC	MIC	MFcC
C. albicans	20	3,02±1,48	4,39±2,18	3,51±1,75	5,55±3,38
C. glabrata	15	4,93±2,20*	7,29±3,87**	3,13±1,77	4,38±1,89§

Note: ** - the reliability of the difference between the chlorhexidine digluconate MIC values relative to C. glabrata and the chlorhexidine digluconate MIC values relative to C. albicans, p <0,05;*
*** - the reliability of the difference between the chlorhexidine MFcC digluconate values relative to C. glabrata to the MFTSC values of chlorhexidine digluconate relative to C. albicans, p <0,05; §- the reliability of the difference between the decamethoxin MFcC values relative to C. glabrata and the chlorhexidine digluconate MFcC values relative to C. glabrata, p <0.05.*

The study showed the representatives of the genus *Candida* differed in their ability to biofilm formation (Fig. 1). The ability to absorb the dye by *C. glabrata* (0,251 ± 0,04 ODU) exceeded the *C. albicans* values (0,203 ± 0,05 ODU) in 1,2 times (p = 0,004), i.e. the clinical strains of *C. albicans* demonstrated average film-forming properties, while those of *C. glabrata* strains were higher.

It should be noted that the cationic surfactants antiseptics studied had a strong activity against the film formation by *Candida spp*. clinical strains. CHH contributed to a decrease in the optical density of the biofilms of the investigated fungi species in 1,1 times (p = 0,001), compared with their indices without the presence of the antiseptics. Similarly, there was a decrease in the film-forming properties by *C. albicans* and *C. glabrata* in the presence of DCM in a 1.1 time and 1.3 times respectively, compared with baseline (p = 0.001).

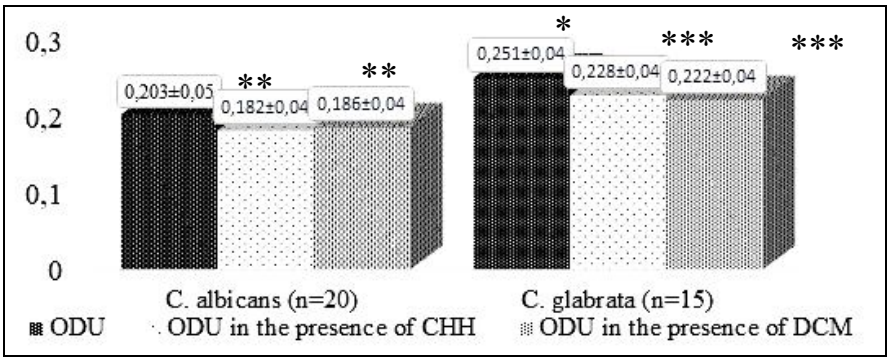

Fig. 1. Characteristics of biofilm-forming properties of Candida spp. clinical strains in the presence of antiseptics

(- the reliability of the differences between the ODU value of C. glabrata biofilms and the ODU value of C. albicans biofilms, p <0.001;*
*** - the reliability of the differences between the ODU value of C. albicans biofilms in the incidence of CHH and DCM and the ODU values demonstrated by biofilms without antiseptics, p <0.001;*
**** - the reliability of the difference of the ODU value of the C. glabrata biofilms in the presence of CHH and DCM to the index of ODU values demonstrated by biofilms without antiseptics , p <0.001).*

The biofilm formation by *C. albicans* is known to start by the microorganism adhesion to the artificial surface with the subsequent production of the extracellular matrix. Cells embedded into this matrix multiply, forming a biofilm. In turn, the ability of *C. glabrata* to form a similar extracellular matrix has not been experimentally proven. Accordingly, the powerful film-forming properties of the latter are genetically determined and associated with the presence of a number of surface adhesins involved in the process of biofilm formation. Recently, the genome of representatives of the *Candida* species is being actively studied, establishing a connection between their strains' properties of biofilm formation and the presence of surperficial Epa6, Epa7 and other proteins [11].

Cationic surfactant antiseptics used in the study have been found out to reliably suppress the biofilm-forming properties of both *C. albicans* clinical strains and *C. glabrata* strains, which are different from *C. albicans* by their biological properties. The mechanism of action of the drugs of this group consists in the ability to change the surface tension of the cell of the microorganism. It impacts the surface structure of the cell and stimulates the disruption of its osmotic balance, resulting in "osmotic shock" and the death of the microorganism [12].

The obtained results point out the high efficacy of cationic surfactant antiseptics on planktonic and film forms of *Candida spp*. clinical strains. The equally potent anti-fungal action of CHH and DCM against both *C. albicans* and *C. glabrata* strains makes the medicines studied appropriate in the treatment of candidiasis in view of the significant differences in their antimycotic effects to microbes within the genus.

Conclusions

Cationic surfactants antiseptics (CHH and DCM) possess antifungal activity against clinical strains of *Candida spp*. Clinical strains of *C. albicans* demonstrate higher susceptibility to CHH, compared with the clinical strains of *C. glabrata*. In turn, DCM has a significantly higher anti-fungal effect against *C. glabrata*, compared with CHH. *C. albicans* clinical strains show average biofilm properties, while *C. glabrata* strains have high biofilm-forming properties. CHH and DCM significantly inhibit the ability to form biofilms by clinical strains of *Candida spp*.

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