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ANTIBIOFILM EFFECT OF ADAMANTANE DERIVATIVE AGAINST *STAPHYLOCOCCUS AUREUS*

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Currently, one of the most urgent problems in clinical practice is the antibiotic therapy ineffectiveness at chronic diseases treatment caused by biofilms-forming microorganisms. One of the ways to its solution is the search for new compounds with antibiofilm activity which can prevent the adhesion of microorganisms, disrupt the structure of the biofilm matrix and affect the Quorum sensing system. ***The aim*** *of the study was to investigate adamantane derivative 1-[4-(1-adamantyl) phenoxy]-3-(N-benzyl,N-dimethylamino)-2-propanol chloride (KVM-97) antimicrobial activity mechanism against Staphylococcus aureus biofilms.* ***Methods.*** *The ability of the adamantane derivative KVM-97 to prevent S. aureus biofilm formation and to destroy previously formed biofilms has been tested on polystyrene plates by gentian violet sorption on these structures, followed by desorption with organic solvent and use of resazurin (redox indicator). The S. aureus cells viability in mature biofilms was evaluated with specific dyes for living (acridine orange) and dead (propidium iodide) cells. Lowry method was used to assess the effect of KVM-97 on the matrix components for the total protein contents determination, the polysaccharides were detected spectrophotometrically (using phenol and sulfuric acid), Bap-protein – by test with Congo red. Persisters' subpopulation was detected by activation of the SOS response in bacteria when exposed to high concentrations of antimicrobial substances.* ***Results.*** *It was found that KVM-97 (the compound with the adamantyl radical) showed an antibiofilm effect against S. aureus, decreasing biofilm biomass: at the biofilm formation stage – by 22.5 % and 75.0 %, while in case of 2-day biofilms treatment – by 34.5 % and 32.4 % at 0.5 MIC and 5.0 MIC respectively. Compound KVM-97 was able to reduce the number of metabolically active S. aureus cells only at the stage of biofilm formation (reduction by 92.7 and 95.8 % at 2.0 and 5.0 MIC). Obtained results indicated that this adamantane-containing compound did not affect the protein and polysaccharides contents of S. aureus biofilms matrix. The changes of Bap-protein level caused by KVM-97 were not statistically significant ($p > 0.05$). It was shown that KVM-97 did not prevent the formation of metabolically inactive persister cells; their share was 0.71 % of the control.* ***Conclusions.*** *Thus, adamantane-containing compound KVM-97 is able to prevent S. aureus biofilm formation, causing significant biofilms' mass reduction, as well as lowering the viable cells number in them and destroying already formed biofilms. Its antibiofilm effects are not associated with matrix protein and polysaccharides synthesis impairments. Further thorough investigations are needed to establish the effect of this compound on eDNA synthesis, the Quorum sensing system, and the ica and arg genes expression of S. aureus responsible for biofilm formation.*

Keywords: biofilms, Staphylococcus aureus, adamantane derivative, matrix, persisters.

In recent years, the attention of scientists to biofilms has increased significantly. Pubmed data shows, that the number of publications dedicated to this problem in 2020 was about 60,000. The attention of researchers is focused on the processes of biofilm formation, biofilm components determination, their sensitivity to antibiotics, biofilms-associated diseases and also on the search for substances with antibiofilm antibiotic activity. Many gram-negative and gram-positive microorganisms, including bacteria of the genus *Staphylococcus*, can form biofilms.

Staphylococcal biofilms are formed on any abiotic and biotic surfaces, creating problems in various fields of economic activity and in medical practice. It is known that the formation

of *Staphylococcus aureus* biofilms can play the main roles in the pathogenesis of diseases such as osteomyelitis, endocarditis, rhinosinusitis, otitis, pneumonia and be one of the main causes of nosocomial infections and purulent-inflammatory processes associated with implants [1, 2]. Biofilms are formed on the surface of endotracheal tubes, implants, nets, stents, venous and urinary catheters, heart valves, contact lenses and other devices [3, 4]. Bacterial biofilms are considered especially dangerous due to their increased resistance to immune system factors, antibacterial agents and disinfectants. In experiments it was found that biofilms' microorganisms can survive when exposed to antibiotics at concentrations that are 500–1000 times higher than those for plankton microorganisms [5–7].

Biofilm bacteria antibiotic resistance is realized via formation of persisters (i.e. bacterial cells, which are characterized by reduced metabolic activity and resistance to antimicrobial agents action) and low permeability of the intercellular matrix. In the biofilms *Staphylococcus* cells synthetize such extracellular components as polysaccharide intercellular adhesin (PIA), extracellular DNA, proteins and myeloid fibrils [8]. The main protein components of staphylococcal biofilms matrix are surface proteins C and G (SasC, SasG), ClfB (clumping factor B), Bap-protein (biofilm-associated protein, which in the biofilm exists in the form of amyloid (protein-polysaccharide) aggregates), the fibronectin/fibrinogen-binding proteins (FnBPA and FnBPB) [8–10]. Polysaccharide and protein components of the matrix are involved into cell adhesion processes, determining biofilms architecture and structural organization.

Despite considerable achievements in studying of microbial communities patterns of development and vital activity, at present there are no antibiotics with profound antibiofilm activities and with appropriate effectiveness and safety for clinical use. The duration and severity of the inflammatory processes require further intense search for new solutions of this problem. Now a search is underway for substances that can disrupt the structure of the biofilm matrix to increase antimicrobial drug concentration in the biofilm. Some devices with coatings using substances with anti-adhesive and antimicrobial properties are being developed. Also new compounds are being searched for modification the *Quorum sensing* (QS) system functioning, etc. Taking into account all abovementioned, adamantane derivatives, which exhibit a wide range of biological activity, including antimicrobial and antibiofilm effect against bacteria and fungi, deserve special attention [11–13].

The **aim** of this work is to establish the mechanism of the first synthesized adamantane derivative KVM-97 action on biofilms formed by *Staphylococcus aureus*.

Materials and methods

Test compound. A derivative of adamantane, 1-[4-(1-adamantyl)phenoxy]-3-(N-benzyl,N-dimethylamino)-2-propanol chloride (KVM-97), used in the experiments, was synthesized at the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine. The macrolide antibiotic azithromycin (*Azithromycin*, substance, series 1502000319, obtained from ZAO NPC Borshchagovsk Chemical and Pharmaceutical Plant, Ukraine) was used as a reference item.

Bacterial strain and growth conditions. Studies were performed using a clinical strain of *S. aureus* 222 isolated from a patient in a surgical hospital. *S. aureus* 222 showed resistance to the action of oxacillin and ciprofloxacin, as soon as sensitivity to clindamycin and azithromycin. The strain was grown on Muller-Hinton agar medium or in tryptone soy broth (TSB) for 24 hours at 37 °C.

Quantitative biofilm test. KVM-97 effects (concentrations of 0.5 MIC and 5.0 MIC) on *S. aureus* biofilms and their formation processes was estimated by microtiter dish biofilm formation assay described by O'Toole [14]. When evaluating the compound's effect on the biofilm formation, KVM-97 solution and the inoculum applications were performed simultaneously. At compounds effects on mature biofilms studies, the solution was applied on the 2nd day of the experiment after biofilms' pre-growth for 48 h at 37 °C. To prepare the inoculum, the overnight culture was 100-fold (1:100) diluted in liquid medium (TSB). The incubation period with the compound was 24 h at 37 °C. To determine the biomass of the biofilm, the contents of the plates were removed, the wells were washed three times with distilled water, 0.1 % solution of gentian violet was added and kept for (10–15) minutes. To detect the formed biofilm, the dye was extracted with ethanol (15 min). Optical density measurements were performed on "Adsorbance Microplate Reader ELx × 800" (VioTek, USA) at a wavelength of 630 nm. Intact cultures of microorganisms grown under the same conditions without the addition of the compound were used as a control. The experiments were performed in at least 3 replicates of 6 replicates in each study.

Determination of metabolically active cells in the biofilm (Resazurin test). The metabolic activity of *S. aureus* cells was determined at the stage of biofilm formation and in the mature 2-day biofilms using the redox indicator resazurin according to [15, 16]. Compound KVM-97 (0.5, 2.0 and 5.0 MIC) was added to the incubation medium simultaneously with the inoculum and at the 2nd day of incubation, respectively. To prepare the inoculum, the overnight culture was diluted in a nutrient medium 100 times (1:100). To determine the metabolic activity of the bacteria, contents of the plate wells were removed, 200 µl of TSB and 10 µl of resazurin solution (Sigma, USA) (0.5 µg / well) were added. The metabolic activity of *S. aureus* cells was evaluated by fluorescence intensity after 30 min of incubation in the dark at room temperature on a fluorescence spectrophotometer "HITACHI, MPF-3" (Japan) (λex 550 nm – λem 590 nm). The experiments were accompanied by controls of culture, culture medium and appropriate solutions of compounds / preparations and were performed at least 3 times.

Determination of total protein and polysaccharides. To evaluate the effect of KVM-97 on the content of proteins and polysaccharides in the matrix of biofilms formed by gram-positive microorganisms, the culture was grown in TSB containing test or reference compounds at concentrations 0.5 and 2.0 MIC for 48 h at 37 ºC. Then cells were precipitated by centrifugation at 3000 rpm for 15 min, followed by obtained pellets washing with 0.9 % NaCl solution. Proteins and polysaccharides extractions were performed by 1.5 M NaCl solution, cells were precipitated by centrifugation at 8000 g for 10 min [17].

Protein content determination in biofilm matrix was carried out by the method of Lowry et al. [18] using reagent A (2.0 % solution of Na_2CO_3 in 0.1 N NaOH), reagent B (0.5 % solution of $CuSO_4 \times \times H_2O$ in 1.0 % sodium citrate solution), reagent C – alkaline copper solution (mixture of 50 ml of reagent A with 1 ml of reagent B), reagent E – 2 times diluted Folin's reagent ("Merck", USA). To 0.5 ml of the obtained supernatant 2.5 ml of reagent C was added and left for 10 min at a temperature of (20–25) ºC. 0.5 ml of reagent E was added and mixture was incubated for 30 min under the same conditions and then analyzed on a SF-46 spectrophotometer at a wavelength of 750 nm. Protein concentration was determined by a standard calibration curve for bovine serum albumin ("Fluka", USA).

Determination of polysaccharides total content in bacterial biofilms matrix was carried out according to Dubois et al. [19]. To 0.2 ml of the supernatant was added 0.2 ml of 5 % phenol solution and 1.0 ml of concentrated sulfuric acid. The mixture was kept at a temperature of (20–25) ºC for 40 minutes. The carbohydrate content was determined at microplates spectrophotometer "Absorbance Microplate Reader ELx800" (VioTek, USA) at 490 nm using a standard curve for glucose.

Determination of Bap-protein. Gram-positive bacteria ability to produce Bap-protein was estimated by determination of the level of matrix amyloid component binding with Congo red dye [20, 21]. A night culture of *S. aureus* was used in the research. Test or reference compounds solutions were added at concentrations of 0.5 and 2.0 MIC and incubated for 90 min at 37 °C. Bacterial cells were precipitated by centrifugation at 3000 rpm for 15 minutes and washed with phosphate buffer. To the cell suspension (OD_{600} – 0.3) 50 µg/ml of Congo red dye solution was added and then mixture was kept for 10 min at 37 °C. Bacterial cells with bound Congo red were precipitated by centrifugation at 8000 rpm for 10 minutes. The supernatant was transferred into the wells of the plate and the amount of unbound dye was determined at 490 nm. Optical density measurements were performed on "Adsorbance Microplate Reader ELx800" (VioTek, USA). Congo red concentration was calculated using a standard calibration curve.

Persisters formation. The presence of persisters in *S. aureus* population with adamantane-containing compound action was determined according to [22]. For cells-persisters isolation, the culture (OD_{600} – 0.8) was incubated with KVM-97 or azithromycin (5.0 MIC) for 5 h at 37 ^{0}C, which corresponded to the stationary phase of culture growth. At the end of the incubation period, a series of 10-fold dilutions was prepared and plated on Mueller-Hinton agar containing 1 % ($MgCl_2 \times 7H_2O$) according to the "6x6 drops" method [23]. After the incubation period (24 h at 37 °C), colonies were counted. The results are presented as a decimal logarithm of total number of colony-forming units (CFU).

Fluorescent microscopy. Test or reference compounds effect on bacterial cells viability in mature biofilms was investigated at a concentration of 5.0 MIC by fluorescent staining using acridine orange (5 µg/ml) and propidium iodide (3 µg/ml) according to [24, 25]. The formed biofilms were incubated with solutions of the compound/preparation for 24 h at 37 °C. The

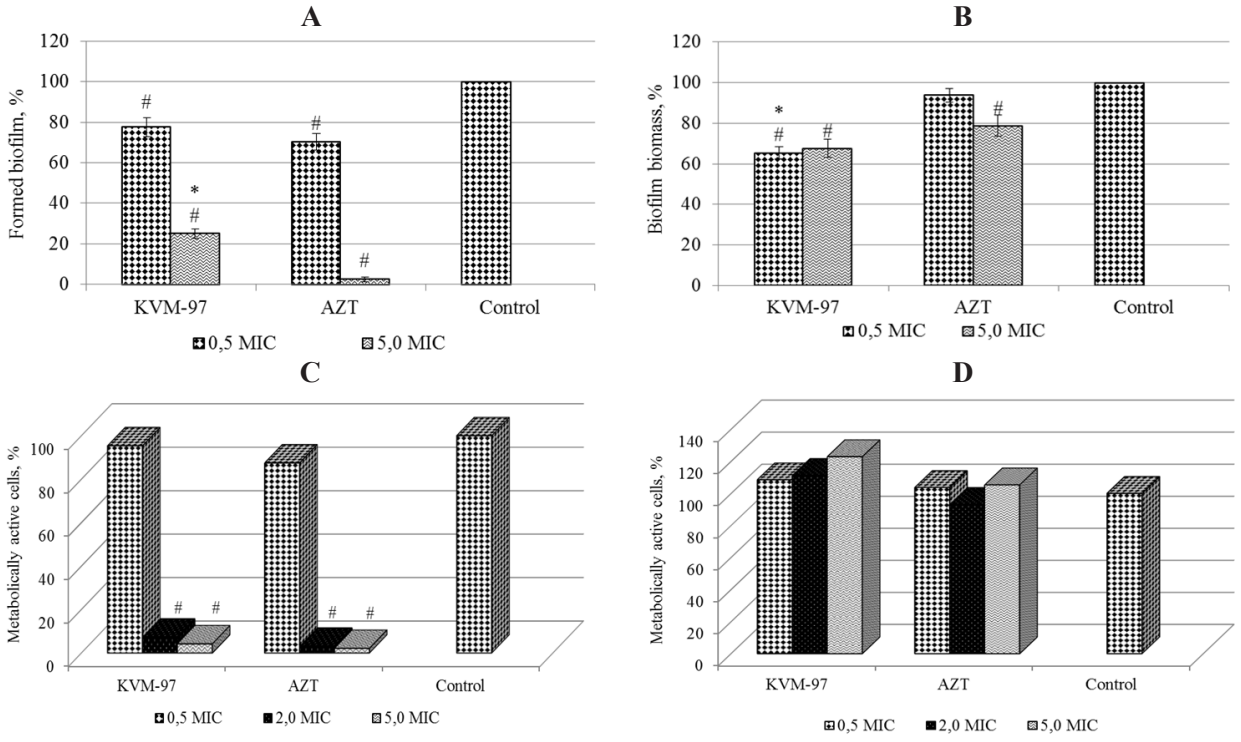
contents of the dishes were removed, washed twice with phosphate buffer (pH 7.2 ± 0.2) and then dye was added. Biofilms with dye were kept for 10 min in the dark at room temperature, washed twice with phosphate buffer (pH 7.2 ± 0.2) and evaluated on a fluorescent microscope "Olympus BX-41" (Japan). The number of viable cells was counted using the computer program BioFilmAnalyzer v.1.0 [26].

Statistical Analysis. The ANOVA method, Newman-Keils and Tukey criteria were used to evaluate the results of the studies and to identify differences between the action of test and reference compounds. Statistical processing was performed using the computer program "Statistica 6.0" (StatSoft. Inc., USA) [27, 28]. Research data are presented as M ± m, where M is the mean value, m is the standard error of the mean.

Results. Our previous studies have found that the MIC of KVM-97 against *S. aureus* 222 was 2.0 μg/ml. The obtained data (Fig. 1, A) show that the compound KVM-97 inhibits *S. aureus* biofilms formation: when exposed to a concentration of 5.0 MIC, the biomass decreases by 75.0 %, at a concentration of 0.5 MIC – by 22.5 % (compared to intact control). The *S. aureus* biofilm with azithromycin treatment at a concentration of 5.0 MIC is practically not formed (inhibition of 97.6 %), with 0.5 MIC – reduction of biomass was by 29.8 %.

The activity of KVM-97 was also registered at a 2-day biofilm formed by *S. aureus*: with concentration of 5.0 MIC the inhibition was 32.4 %, with 0.5 MIC – 34.5 % (Fig. 1, B). Azithromycin treatment at a concentration of 5.0 MIC decreased the biofilm biomass by 21.3 %, at 0.5 MIC – by 6.2 %. KVM-97 in the subinhibitory concentration has higher antibiotic action compared to azithromycin ($p < 0.05$).

The viability of intact *S. aureus* cells at the stage of biofilm formation was the same that in the mature biofilm: 48.0 ± 1.4 conditional unit (CU) and 47.0 ± ± 6.9 CU respectively. With KVM-97 treatment at the stage of *S. aureus* biofilm formation, the number of metabolically active cells decreased at 2.0 MIC and 5.0 MIC: by 92.7 – 95.8 % (compared to control), at 0.5 MIC differences compared with the control were not detected (Fig. 1, C). Metabolically active *S. aureus* cells were practically not detected in case of azithromycin treatment, the inhibition at

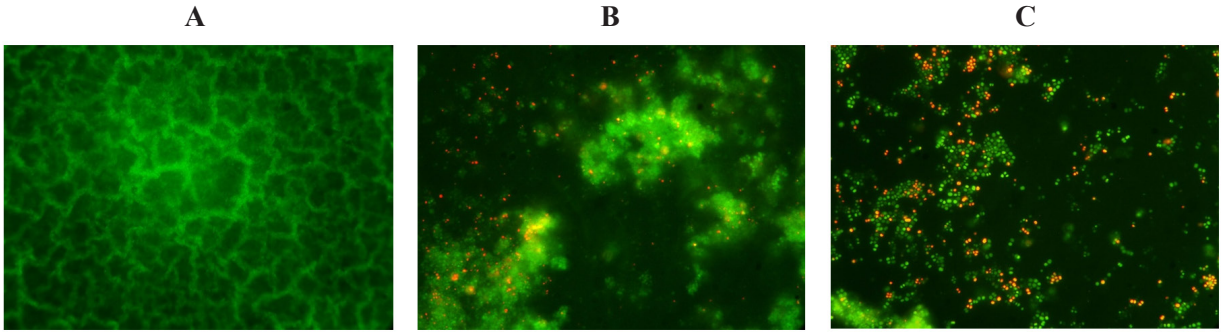


F i g. 1. Anti-biofilm activity of KVM-97: A – the effect on *S. aureus* biofilm formation process (formed biofilm, %); B – the effect on a 2-day mature biofilm (biomass, %); C – effect on the viability of *S. aureus* cells at the stage of biofilm formation (metabolically active cells, %); D – the effect on the viability of *S. aureus* cells in a 2-day mature biofilm (metabolically active cells, %).
***Notes*: AZT — azithromycin; * — $p < 0.05$ in comparison with the corresponding concentration of azithromycin (AZT); # — $p < 0.05$ in comparison with control.**

5.0 MIC and 2.0 MIC was 96.9–97.9 %.

No significant changes in the viable *S. aureus* cells numbers compared to the intact control were registered (p> 0.05) in case of 2-day-biofilms treatment by KVM-97 (Fig. 1, D). Reference item azithromycin showed the same effect.

Microscopy results (Fig. 2, A) showed that the control cells formed a dense layer, stained green, i.e. they were metabolically active. When KVM-97 and azithromycin were added, separate bacterial clusters were observed and the number of unviable cells visually increased (from yellow-orange to red).



F i g. 2. Cell viability of mature *S. aureus* biofilm with KVM-97 and azithromycin treatment at a concentration of 5.0 MIC. Fluorescence microscopy, magnification: × 1000: A – culture control; B – KVM-97; C – azithromycin; green color – living cells; red color – dead cells

Study results (Table 1) demonstrated that both KVM-97 and azithromycin decreased the number of *S. aureus* cells in comparison with control, but these changes are not statistically significant (p> 0.05).

When exposed to adamantane-containing compound and azithromycin, a significant decrease in *S. aureus* cells viability in the biofilm was by 11.4 % and 24.7 %, respectively (compared with the intact control, p <0.05). The antibiofilm effect of KVM-97 could be associated with its action on matrix components of staphylococcal biofilms.

The obtained results show (Fig. 3, A) that KVM-97 in both studied concentrations does not decrease polysaccharide contents in *S. aureus* biofilms matrix in comparison to the control (their content in the control was 45.3 μg). Azithromycin inhibitory effect on this parameter is registered at a concentration of 2.0 MIC (21.7 %), at 0.5 MIC – such effect is absent.

When determining the effect of KVM-97 on the protein components of the matrix of *S. aureus* 222, it was found (Fig. 3, B) that the compound at a concentration of 2.0 MIC reduces the protein content in the matrix by 7.5 %, with 0.5 MIC – does not cause changes.

The obtained data are not statistically significant compared to the intact control (content is 27.9 μg) (p> 0.05). The comparison drug azithromycin does not show inhibitory activity on the production of protein components of the matrix of *S. aureus*.

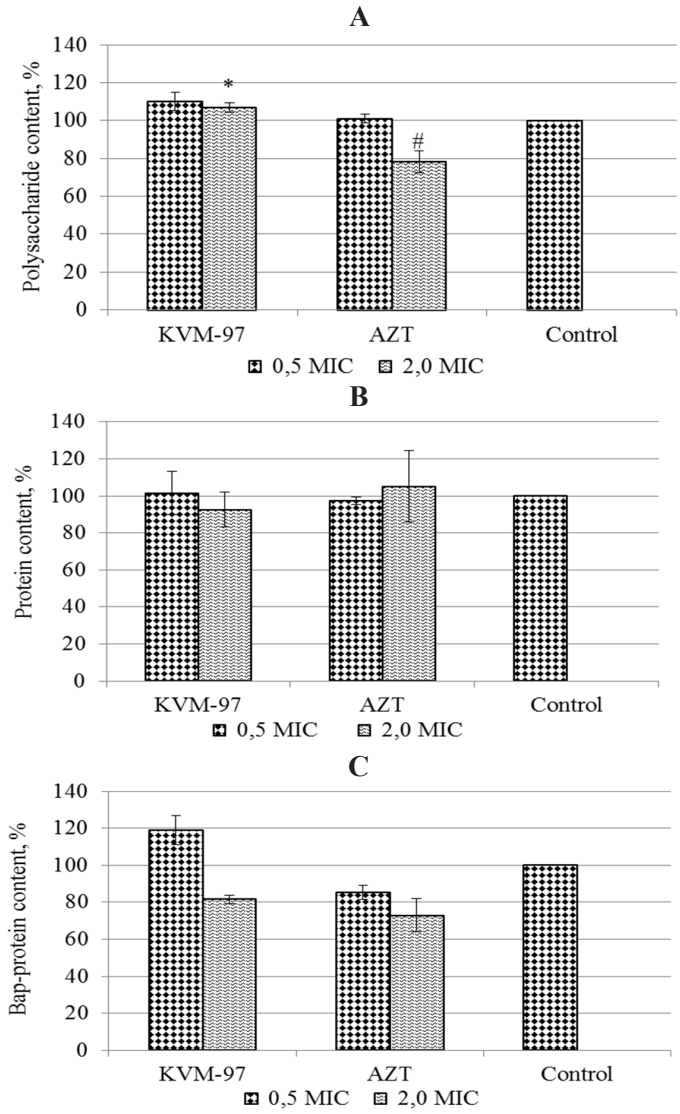
When studying the effect of KVM-97 on the matrix component of Bap-protein (biofilm associated protein), it was found (Fig. 3, C) that when exposed to a concentration of 2.0 MIC, a decrease in its content by 18.5 % compared to intact control was registered. At the subinhibitory concentration (0.5 MIC), the level of Bap-protein increases by 19.0 %. The comparison drug azithromycin dose-dependently reduces the content of Bap-protein by 14.6 % and 27.1 % when used at a concentration of 0.5 MIC and 2.0 MIC, respectively. However, the detected changes were not statistically significant (p> 0.05).

Table 1

The effect of KVM-97 on the viability of *S. aureus* cells in the 2-day mature biofilms

Experiment conditions	Cells number in biofilm, per visual field	
	Total number	Viable cells, %
Culture control	19302 ± 4581	99.4 ± 0.6
KVM-97	11461 ± 1844	88.6 ± 3.0*,#
Azithromycin	12799 ± 525	75.3 ± 2.3#

Notes: * differences are statistically significant in comparison with azithromycin, (p <0.05); # differences are statistically significant in comparison with control (p <0.05).



F i g. 3. The contents of *S. aureus* 222 biofilms matrix components (%) with KVM-97: A – polysaccharides; B – total protein; C – Bap-protein.
***Notes*: AZT – azithromycin; * – p <0.05 in comparison with the corresponding concentration of azithromycin (AZT); # – p <0.05 in comparison with control.**

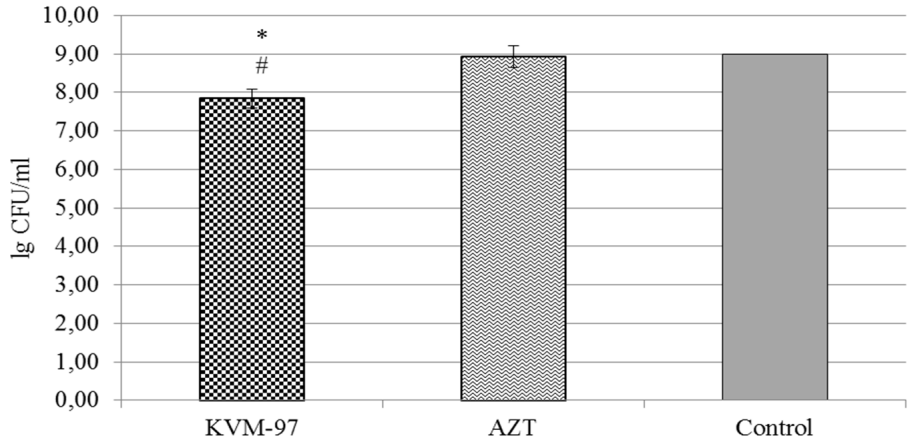
Decreased cell viability at different stages of biofilm formation under the action of compounds and drugs may occur due to the formation of persistent. The results obtained (Fig. 4) show that the number of *S. aureus* cells in the culture control was 9.7×10^8 CFU/ml (or 8.99 ± 0.22 lg CFU/ml).

It is established (Fig. 4) that with KVM-97 cells-persisters formation was present, their share reached 0.71 % from control.

Azithromycin did not change the number of cells-persisters compared to the control ($p > 0.05$). Cells remained metabolically active, which was confirmed by the reaction with resazurin. It could be assumed that in the absence of nutrients in the incubation medium, azithromycin retained a specific effect on *S. aureus*.

Discussion. Recently, scientific interest to biofilms contents, structure, functioning and search for active antibiofilm agents are rapidly increasing. Intensive search for perspective compounds and preparations able to act directly and indirectly on biofilm formation processes, bacterial cells viability in the biofilm, main features of mature biofilms, extracellular matrix, content of its components, etc. is currently underway. Such an integrated approach to this specific antimicrobial activity studies will make it possible to predict the possible mechanism of such compounds action and to evaluate the prospects for their use in clinical practice.

Thus, it was found that while the bactericidal compounds effect is accompanied by a decrease in biofilms' biomass and disruption of micro-



F i g. 4. The number of *S. aureus* persister cells (lg CFU/ml) at 5.0 MIC KVM-97 concentration. *Notes*: AZT — azithromycin; * – $p < 0.05$ in comparison with the corresponding concentration of azithromycin (AZT); # – $p < 0.05$ in comparison with control.

organisms' energy processes, substances with bacteriostatic effect only reduce biofilms' biomass without any changes in their metabolic processes [29].

Experimental data showed that KVM-97 caused dose-dependent inhibitory effect on *S. aureus* biofilms at the stage of biofilm formation decreasing biomass and viable bacterial cells number. A similar inhibiting effect had CD437 (the retinoid compound with adamantyl radical) in experiments with *Enterococcus faecalis, S. epidermidis, S. aureus* biofilms biomass and bacteria metabolic activity [30]. According to scientific publications [31], the antibiotic action of various substances may be due to their ability to cause impaired adhesion of microorganisms, violations in biofilms' matrix components synthesis, changes in the physicochemical characteristics of the bacterial cell surface, etc. Data of other authors [29] indicate that the antibiotics telithromycin and daptomycin in bactericidal (8.0 MIC) and subinhibitory (0.25 MIC) concentrations show activity against biofilms formed by methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) *S. aureus*. The antibiofilm effect of antibiotics is associated with their influence on the expression of *agrA, agrC, clfA, icaA, sigB* genes, responsible for the *QS* system regulation, intercellular adhesion, production of proteins and polysaccharides involved into *S. aureus* biofilm formation.

Our experiments have shown that a compound with an adamantyl radical, in addition to disrupting biofilm formation, can cause destruction of the mature *S. aureus* biofilm, which was confirmed by a decrease in its biomass both with bactericidal and subinhibitory concentrations. Such antibiofilm action at a concentration of 0.5 MIC was detected for vancomycin in experiments with *S. epidermidis* [31]. Its effect was accompanied by an increase in the amount of eDNA in the biofilm matrix and by stimulation of chemical interaction between them, which led to a limitation of the antibiotic permeability into the biofilm and its resistance to the glycopeptide [31]. Whereas, according to our results, some bacteria in the mature biofilm remain viable after KVM-97 treatment, it could be supposed that just the insufficient concentration of this compound in the mature biofilm was the reason of biofilm's incomplete destruction.

According to the obtained data, KVM-97 did not significantly change the contents of proteins and polysaccharides in the matrix of *S. aureus* biofilms. According to other authors' data [30] in the presence of the retinoid adamantane-containing compound CD437 in the incubation medium, a decrease in the content of polysaccharide components of the matrix in the biofilms of gram-negative bacteria *Pseudomonas aeruginosa* was registered. Inhibition of the exopolysaccharides synthesis by *S. aureus* cells was found with the combined use of azithromycin and Vitexin (subinhibitory concentrations) [32]. Simultaneously with such their action was registered also eDNA content decreasing in *S. aureus* biofilms matrix [32].

Persister bacterial cells formation is one of the factors that cause biofilms resistance. Such cells have reduced metabolic activity and are resistant to antimicrobial drugs. Most of persister cells are formed under the influence of antibiotics affecting the metabolic processes in bacteria, including fluoroquinolones (ciprofloxacin, ofloxacin),

aminoglycosides (tobramycin, gentamicin) etc. It has been experimentally proven that the compound KVM-97 does not prevent totally the formation of persister cells, but their number does not exceed that under the action of other antimicrobial agents. According to other authors' data [33], the frequency of persistence in the population of *S. aureus* under the influence of levofloxacin is 0.5 %.

Thus, the compound with the adamantyl radical KVM-97 has an inhibitory effect on *S. aureus* biofilms – it is able to prevent biofilm formation and destroy the formed biofilms. The antibiotic action of KVM-97 is accompanied by both a decrease in biomass and in the number of viable *S. aureus* cells in the biofilm. Our experiments have shown that the activity of KVM-97 against *S. aureus* biofilms may be associated with its effect on matrix protein component, but such changes were not statistically significant. It was found that this adamantane radical-containing compound does not affect the synthesis of exopolysaccharides in staphylococcus cells. It has been shown that KVM-97 does not prevent the formation of *S. aureus* persister cells, but the frequency of their formation does not exceed that parameter of other modern antimicrobials (≤ 1.0%). Our experiments have shown that KVM-97 demonstrates a pronounced activity against planktonic cells of *S. aureus*, similar to this parameter of reference item azithromycin (p> 0.05). As for biofilm-disseminated *S. aureus* cells, the activity of KVM-97 is less profound.

Conclusions. Obtained results allowed supposing that in addition to abovementioned changes, the antibiotic action of adamantane radical-containing compound KVM-97 could be realized via influencing eDNA synthesis, *Quorum sensing* system state, expression of *ica* and *arg* genes responsible for *S. aureus* biofilm formation, which requires further in-depth studies.

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Conflict of interests. The authors declare no conflict of interest.

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АНТИБІОПЛІВКОВА ДІЯ ПОХІДНОГО АДАМАНТАНА ЩОДО *STAPHYLOCOCCUS AUREUS*

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Резюме

Нині актуальною проблемою в клінічній практиці є неефективність антибіотикотерапії при лікуванні хронічних захворювань, спричинених біоплівкоутворюючими мікроорганізмами. Одним із напрямків її вирішення є пошук нових сполук з антибіоплівковою активністю, здатних запобігати адгезії мікроорганізмів, порушувати структуру матриксу біоплівки та впливати на систему *Quorum sensing*. **Мета** роботи – встановити механізм антимікробної активності похідного адамантану 1-[4-(1-адамантил)-фенокси]-3-(N-бензил, N-диметиламіно)-2-пропанол хлориду (КВМ-97) щодо біоплівок, сформованих *Staphylococcus aureus*. **Методи.** Здатність адамантанвмісної сполуки КВМ-97 запобігати плівкоутворенню та руйнувати сформовані біоплівки *S. aureus* досліджували на полістиролових планшетах методом сорбції генціанвіолету на її структурах з наступною десорбцією в органічний розчинник та з використанням окисно-відновного індикатора резазурину. Життєздатність клітин *S. aureus* у складі зрілих біоплівок оцінювали з використанням специфічних барвників для живих (акридиновий, оранжевий) та неживих (пропідій йодид) клітин. Для оцінки впливу КВМ-97 на компоненти матриксу для визначення загального білка використовували метод Lowry, полісахаридів – спектрофотометричний метод з використанням фенолу та сульфатної кислоти, Вар-білка – метод з використанням Конго червоного. Виділення субпопуляції персистерів проводили згідно методу, заснованому на активації SOS-відповіді у бактерій за дії високих концентрацій антибіотиків. **Результати.** Встановлено, що сполука з адамантильним радикалом КВМ-97 виявляє антибіоплівковий ефект щодо *S. aureus,* про що свідчить зменшення біомаси біоплівки: на етапі плівкоутворення – на 22,5 % та 75,0 %, за дії на 2-добо-

ві біоплівки – на 34,5 % та 32,4 %, за 0,5 МІК та 5,0 МІК відповідно. Сполука КВМ-97 здатна зменшувати кількість метаболічно активних клітин *S. aureus* лише на етапі плівкоутворення (зменшення на 92,7 % та 95,8 % при 2,0 та 5,0 МІК відповідно). Згідно з отриманими результатами адамантанвмісна сполука не впливає на вміст білкових та полісахаридних компонентів матриксу біоплівок золотистого стафілокока. Виявлені зміни рівня Bap-білка за дії КВМ-97 не були статистично достовірними ($p > 0,05$). Експериментально встановлено, що сполука КВМ-97 не запобігає формуванню персистерів, їх частка становить 0,71 % від контролю. **Висновки.** Таким чином, адамантанвмісна сполука КВМ-97 здатна запобігати плівкоутворенню *S. aureus*, що підтверджується достовірним зниженням біомаси біоплівок, зменшенням кількості життєздатних клітин у їх складі, а також руйнувати сформовані біоплівки. Антибіоплівковий ефект сполуки не пов'язаний із порушенням синтезу білкових та полісахаридних компонентів матриксу. Для встановлення впливу сполуки на синтез еДНК, систему *Quorum sensing*, експресію генів *ica* та *arg*, відповідальних за плівкоутворення *S. aureus,* необхідні подальші поглиблені дослідження.

Ключові слова: біоплівки, *Staphylococcus aureus,* похідне адамантану, матрикс, персистери.

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