https://doi.org/10.15407/microbiolj87.05.025

**S. MALANCHUK[1]*, M. MISHYNA[2], Yu. KOT[1],**
**S. DZHORAIEVA[3], Yu. MOZGOVA[2], B. MALANCHUK[4], Yu. MISHYN[2]**

[1] Department of Infectious Diseases and Clinical Immunology,
Department of Biochemistry, V.N. Karazin Kharkiv National University,
4 Svobody Sq., Kharkiv, 61022, Ukraine
[2] Department of Microbiology, Virology and Immunology named after D.P. Grynyov,
Department of General and Clinical Pathological Physiology
named after D.O. Alpern Kharkiv National Medical University,
4 Nauky aven., Kharkiv, 61022, Ukraine
[3] Laboratory and experimental department,
State Institution «Institute of Dermatology and Venereology
of the National Academy of Medical Sciences of Ukraine»,
7/9 Chernyshevska Str., Kharkiv, 61057, Ukraine
[4] Municipal Non-Profit Enterprise Uzhhorod City Multidisciplinary Clinilal Hospital,
71 Mynaiska Str., Uzhhorod, 88000, Ukraine
* Author for correspondence; e-mail: s.malanchuk@karazin.ua

# EFFECT OF 3,3'-DIINDOLYLMETHANE IN DIFFERENT SOLVENTS ON *PSEUDOMONAS AERUGINOSA* BIOFILM

*Today, the most common pathogens causing nosocomial infections are Gram-negative bacteria, especially Pseudomonas aeruginosa. One of the leading factors in determining the resistance of bacteria is their ability to form biofilms. One promising class of agents capable of affecting biofilms is indoles (diindolylmethane). The* ***aim*** *of this work was to determine the effect of diindolylmethane in different solvents on the formation of P. aeruginosa biofilm.* ***Methods.*** *For microbiologic examination, biological material was collected from purulent-inflammatory complicated gunshot and shrapnel wounds. The antimicrobial activity of samples containing 0.5% solution of 3,3'-diindolylmethane derivatives (Ts-D5-1 — sample 1, Ts-D8-1 — 2, VE-D67-1 —3, VE-D68-1—4, VE-D71-1—5, W-014-1—6, W-015-1—7, and W-016-1—8 in dimethylsulfoxide solvent and Ts-D5-2 —9, Ts-D8-2 — 10, VE-D67-2 — 11, VE-D68-2 —12, VE-D71-2 — 13, W-014-2 — 14, W-015-2 —15,and W-016-2 — 16 in N-methylpyrrolidone solvent) was determined. The study was carried out by the method of diffusion in agar. The biofilm formation study was performed according to the method of O'Toole.* ***Results****. Samples 14, 15, and 16 showed a high antimicrobial activity, among which sample 14 was the most efficient: the diameter of the lysis zone was 10 mm after 24 hours, 11 mm after 48 hours, and 12 mm after 72 hours. It was demonstrated that all other samples had less marked antibacterial activity, which was slightly potentiated over time.*

Citation: Malanchuk S., Mishyna M., Kot Yu., Dzhoraieva S., Mozgova Yu., Malanchuk B., Mishyn Yu. Effect of 3,3'-Diindolylmethane in Different Solvents on *Pseudomonas aeruginosa* Biofilm. *Microbiological journal.* 2025 (5). P. 25—35. https://doi.org/10.15407/microbiolj87.05.025

***Conclusions****. Sample 14 showed the highest antimicrobial activity. Moreover, the effect of potentiation of the antimicrobial activity of the solution was observed. The test solution prevented the formation of a biofilm when it was applied to the well surface, and also led to the destruction of the already formed daily biofilm of P. aeruginosa.*

***Keywords:*** *Pseudomonas aeruginosa, antibiotic resistance, biofilms, diindolylmethane.*

The steady growth of resistance of microorganisms to antibiotics predicts a disappointing picture for humanity in the future. Doctors around the world admit that the COVID-19 pandemic was a factor that led to an increase in the range of antibiotics to which bacteria have become resistant. Also, the level of nosocomial infections has recently increased, especially in Ukraine, which is extremely dangerous in wartime conditions. Despite the experience and efforts of Ukrainian doctors, perfectly performed surgical interventions for gunshot, fragment and burn wounds, quite often patients die as a result of infection with multi-resistant hospital strains. Among the pathogens that cause this kind of infection and are resistant to antibiotics, gram-negative bacteria, in particular, *Pseudomonas aeruginosa (P. aeruginosa)*, are the leaders today.

Recently, scientists around the world identified a separate of microorganisms called ESKAPE (*Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*.); these bacteria based on enhanced and increased resistance to antibacterial drugs lead to high mortality (Rabin et al., 2015a, 2015b; Lin et al., 2015; Penesyan et al., 2019).

The World Health Organization classifies *P. aeruginosa* as one of the most dangerous pathogens due to multidrug resistance (MDR), which requires an urgent search for new drugs (World Health Organization, 2024). The genome of *P. aeruginosa* is large (5.5—7 million base pairs) and has remarkable properties (Stover et al., 2000). Pathogenesis of *P. aeruginosa* is caused by some factors such as adhesion (flagella and type IV pili) and pigments, which induce adhesion, regulate or interrupt host cell pathways, and interact with the external matrix; the ability to produce toxins, proteases, and effector proteins (such as ExoS, ExoT, ExoU, and ExoY produced by the type III secretion system). Moreover, it can exist in the form of biofilms, which prompts quorum sensing plasticity (Reynolds et al., 2021).

R. Freddy Langendonck and his colleagues at the Institute of Infectious, Veterinary and Environmental Sciences at the University of Liverpool divide the mechanisms of antibiotic resistance into the following groups: intrinsic, acquired, and adaptive. Intrinsic resistance mechanisms are mechanisms genetically encoded in the main genome of an organism, while adaptive resistance mechanisms are mechanisms induced by environmental stimuli, and acquired resistance occurs due to receiving resistance genes from other organisms or selecting beneficial mutations (Langendonk et al., 2021). One of the leading roles that determine the resistance of bacteria to therapeutic agents is played by their ability to form biofilms, which poses a challenge to modern medicine antibiotics (Wolska et al., 2015). The existence of pathogens in biofilm associations significantly increases their chances of multiresistance due to both the mechanical protection of bacteria by a glycocalyx layer and the possibility of horizontal exchange of resistance genes (Donlan, 2002). As part of a biofilm, bacteria are able to switch their mode of existence from sessile to planktonic (free-floating) depending on their needs; these changes are regulated by individual genes (Berlanga & Guerrero, 2016).

Bacterial biofilms stimulate the development of resistance of microorganisms to drugs and also lead to the development of persistent inflammation of the macroorganism (Chen et al., 2011; Ciszek-Lenda et al., 2019; Lebeaux et al., 2014). Thus, combating biofilms requires a combined strategy targeting the biofilm phenotype

as well as the signaling molecules involved in the regulatory processes of biofilm existence.

Quite promising agents capable of affecting the biofilm are photochemical substances, in particular indole (Hu et al., 2011; Kim et al., 2015; Lee et al., 2009; Pandey et al., 2013; Van den Bergh et al., 2017). In addition to the fact that indole (an aromatic hydrocarbon) is produced by both gram-positive and gram-negative bacteria, it is also present in some plants, mostly cruciferous (Chimerel et al., 2012).

Studies show that indole has anti-inflammatory, antimicrobial, and anti-cancer properties; in addition, it controls the process of biofilm formation, transition from the exponential to the stationary phase, responds to stress, and affects virulence (El-Sawy et al., 2010; Fiester & Actis, 2013).

There is an opinion that indole, as an intercellular signaling molecule, affects the physiological processes of bacteria, including virulence, spore and biofilm formation, plasmid stability, and drug resistance (Kim et al., 2015; Gaimster et al., 2014; Wang et al., 2001).

Thus, the study of the influence of indole derivatives, namely 3,3'-diindolylmethane (DIM) on the biofilm of pathogenic microorganisms is a very relevant direction. The **purpose** of our experiment was to determine the effect of DIM in different solvents on the *P. aeruginosa* biofilm formation.

**Materials and Methods.** Biological material from purulent-inflammatory complicated gunshot and shrapnel wounds was taken for microbiological research. Isolation and identification were performed using Micro-la-test kits (Czech Republic).

The antimicrobial activity of composites was studied on clinical strains of *P. aeruginosa* (n=10) isolated from venflons, drainage devices, catheters, and clinical material from patients (n=42) of the Military Medical Clinical Center of the Northern Region (Kharkiv) with purulent-inflammatory processes (strains isolated in the bacteriological laboratory of the State Institution «Institute of Dermatology and Venereology of the National Academy of Medical Sciences of Ukraine»), the Kharkiv Regional Council Municipal Non-Profit Enterprise «Regional Clinical Hospital», and on reference strains of *P. aeruginosa* (n=2) obtained from the L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine (Table 1).

*Table 1.* **Origin of Clinical (Multidrug-Resistant) and Reference Strains**

| No. | Clinical Strains (Isolates) | Diagnosis |
|---|---|---|
| 1 | *P. aeruginosa* 10A | Shrapnel abdominal injury — peritonitis, sepsis |
| 2 | *P. aeruginosa* 51**T** | Gunshot thoracic injury — community-acquired bilateral subtotal pneumonia |
| 3 | *P. aeruginosa* 37T | Gunshot thoracic injury — postoperative pneumonia, sepsis |
| 4 | *P. aeruginosa* 10T | Shrapnel thoracic injury — pulmonary abscess |
| 5 | *P. aeruginosa* 45A | Gunshot abdominal injury — postoperative complications, sepsis |
| 6 | *P. aeruginosa* 3 | Polytrauma, postoperative complications, sepsis |
| 7 | *P. aeruginosa* S:Cl 10 | Gunshot wound — wound-related bilateral pneumonia |
| 8 | *P. aeruginosa* 10 (multidrug-resistant, thanatological) | Blast injury, purulent postoperative complications, peritonitis, bilateral subtotal pneumonia, sepsis |
| 9 | *P. aeruginosa* 49A | Polytrauma, peritonitis, sepsis |
| 10 | *P. aeruginosa* 38T | Polytrauma, bilateral subtotal pneumonia, sepsis |
| | Reference strains | |
| 1 | *P. aeruginosa* 27853 = NCDCF-51 (7419) | Obtained from the L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases, NAMS of Ukraine |
| 2 | *P. aeruginosa* 043102 = NCIB 862 | |

The sensitivity of clinical strains of microorganisms to antimicrobial drugs was studied using a microtest system with semiquantitative registration of data «SENSILAtest G-I, G-II» and Kirby-Bauer Disk Diffusion Susceptibility Test. Biofilms were grown in glass-bottomed Petri dishes.

The research was carried out by the well method (the method of diffusion of the experimental drug in agar). This method is based on the ability of the substance and its active ingredient to diffuse into agar (Muller Hinton's agar), on which the test culture is sown (Volyanskyi et al., 2004).

Research samples were presented in the form of multi-component compositions: 1 — Ts-D5-1 — 0.5% solution of 3,3'-diindolylmethane derivative Ts-D5 in dimethylsulfoxide, 2 — Ts-D8-1 — 0.5% solution of 3,3'-diindolylmethane derivative Ts-D8 in dimethylsulfoxide, 3 — VE-D67-1 — 0.5% solution of 3,3'-diindolylmethane derivative VE-D67 in dimethylsulfoxide, 4 — VE-D68-1 — 0.5% solution of 3,3'-diindolylmethane derivative VE-D68 in dimethylsulfoxide, 5 — VE-D71-1 — 0.5% solution of 3,3'-diindolylmethane derivative VE-D71 in dimethylsulfoxide, 6 — W- 014-1 — 0.5% solution of 3,3'-diindolylmethane derivative W-014 in dimethyl sulfoxide, 7 — W-015-1 — 0.5% solution of 3,3'-diindolylmethane derivative W-015 in dimethyl sulfoxide, 8 — W-016-1 — 0.5% solution of 3,3'-diindolylmethane derivative W-016 in dimethyl sulfoxide, 9 — Ts-D5-2 — 0.5% solution of 3,3'-diindolylmethane derivative Ts-D5 in N-methylpyrrolidone, 10 — Ts-D8-2 — 0.5% solution of derivative 3,3'-diindolylmethane Ts-D8 in N-methylpyrrolidone, 11 — VE-D67-2 — 0.5% solution of derivative 3,3'-diindolylmethane VE-D67 in N-methylpyrrolidone, 12 — VE-D68-2 — 0.5% solution of 3,3'-diindolylmethane derivative VE-D68 in N-methylpyrrolidone, 13 — VE-D71-2 — 0.5% solution of 3,3'-diindolylmethane derivative VE-D71 in N-methylpyrrolidone, 14 — W-014-2 — 0.5% solution of 3,3'-diindolylmethane derivative W-014 in N-methylpyrrolidone, 15 — W-015-2 — 0.5% solution of 3,3'-diindolylmethane derivative W-015 in N-methylpyrrolidone, 16 — W-016-2 — 0.5% solution of 3,3'-diindolylmethane derivative W-016 in N-methylpyrrolidone. As a control (K) — samples of solvents of 3,3'-diindolylmethane derivatives (Ts-D5, Ts-D8, VE-D67, VE-D68, VE-D71, W-014, W-015, and W-016): 1K — dimethyl sulfoxide, and 2K — N-methylpyrrolidone.

The study focused on biofilm formation using the O'Toole method (O'Toole et al., 2000), which involved testing the ability of bacterial strains to adhere to polystyrene plates. Bacterial cultures were grown following standard microbiology practices, with specific cultivation and suspension media recommended for each bacterial family. After obtaining the cultures, they were washed off with individualized suspension media. The initial bacterial suspension was measured for its optical density using a Densi-La-Meter and adjusted to the McFarland plateau using the suspension medium. A negative control was included by adding nutrient broth and suspension medium. The number of inoculated planktonic cells was counted on a Multiskan EX 355 photometer at 540 nm and expressed in conventional units of optical density. After obtaining a bacterial suspension with the required concentration of microorganisms, 200 μL of this suspension was inoculated into the plate cells with the appropriate nutrient medium, followed by incubation according to the conditions for each bacterial family in a humid container under a closed lid of the plate.

Intravital cells' viability was assessed by laser scanning confocal microscopy after fluorescence staining nucleoid DNA with DAPI/PI-based «Bacstain Bacterial Viability Detection Kit» (Dojindo, Cat. No. BS08). DAPI dye (λEx — 405 nm, λEm — 461 nm) is a minor groove binder specific to the AT sequence of

DNA, which permeates into bacteria to stain nucleic acids regardless of membrane damage. PI (λEx — 493 nm, λEm = 636 nm) is a parallel intercalator into the DNA double helix that stains nucleic acid; it passes only through damaged bacterial membranes. The samples were stained for 15 min in the dark. Bacteria-associated and extracellular polysaccharide matrix was label-free visualized by green autofluorescence (λEx — 488 nm, λEm — 532 nm). Live-cell imaging was conducted using an Olympus FV10i-LIV laser scanning a confocal microscope equipped with a 60/1.2 NA water immersion objective. Confocal images were acquired with a scanning mode format of 1024×1024 pixels. The pinhole aperture was 1 Airy unit. Z-reconstruction of serial single optical sections was performed with a scanning mode of 1024×1024 pixels with an electronic zoom at 2.0 and a Z stack of 0.2 μm/slice. The confocal images shown are representative images of ten fields of view in different regions of the coverslip. Post-rendering of the obtained images of optical sections was performed using Olympus cell Sence software (Olympus licensed). The imaging was carried out in triplicate with three independent repeats.

The statistical method of analyzing the research results was performed using Statistica 7. The obtained data were statistically processed by calculating the arithmetic average, the error of the arithmetic average, and the significance of the difference. Student's t-test was used to determine the statistical significance of the results. For all types of analysis, differences were significant at $p<0.05$ between experimental samples and $p<0.001$ between experimental samples and control (Xiuhua & Fuzhong, 2024).

**Results.** Bacteriological examination of biological material taken from infected gunshot, shrapnel, and burn wounds revealed that Gram-negative microorganisms predominated among the identified pathogens, and *P. aeruginosa* was 23.8% of the total (Fig. 1).

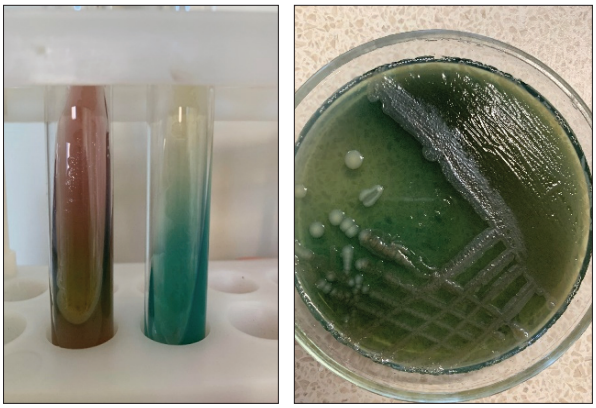

***Fig. 1.*** Isolates of *P. aeruginosa*

Antimicrobial activity of samples 1 and 2, containing components Ts-D5 and Ts-D8 against *P. aeruginosa,* on the next day after obtaining the test samples was absent. The lysis zones under the action of the samples containing Ts-D5 and Ts-D8 components increased slightly 72 hours after the drug had been introduced into the wells, which indicates the effect of increasing the antimicrobial effect. However, this increase was only 1 mm, that is, these samples have a rather low antimicrobial effect.

When determining the antimicrobial activity of samples . 3, 4, and . 5, containing components VE-D67, VE-D68, and VE-D71, on the next day after obtaining the test samples, it was established that the samples did not show activity toward *P. aeruginosa*. But 72 hours after the introduction of the drug into the wells, under the influence of all experimental samples, an increase in the lysis zone around the strains by 1 mm was observed like under the influence of samples 1 and 2 ($p<0.05$; $p<0.001$).

Another regularity was observed when determining the antimicrobial activity of samples 6, 7, and 8, containing components W-014, W-015, and W-016. It was found that samples 7 and 8 did not show activity against *P. aeruginosa* on the next day, but the diameter of the lysis zone under the influence of sample 7 increased by 2 mm after 48 and 72 hours, and by 2 mm

and 1 mm after 48 and 72 hours, respectively, under the influence of sample 8. As for the antimicrobial activity of sample 6, on the first day after obtaining the tested sample, the diameter of the lysis zones of microorganisms was 4 mm and increased by 1 mm after 48 and by another 1 mm after 72 hours. This characterizes the sample as «moderately active» with the effect of potentiating antimicrobial action ($p<0.05$; $p<0.001$).

When determining the antimicrobial activity of samples 6, 7, and 8, containing components W-014, W-015, and W-016, two months after obtaining them, it was established that samples 6 and 7 showed high antimicrobial activity with the effect of potentiation during the entire period (Figs. 2 and 3).

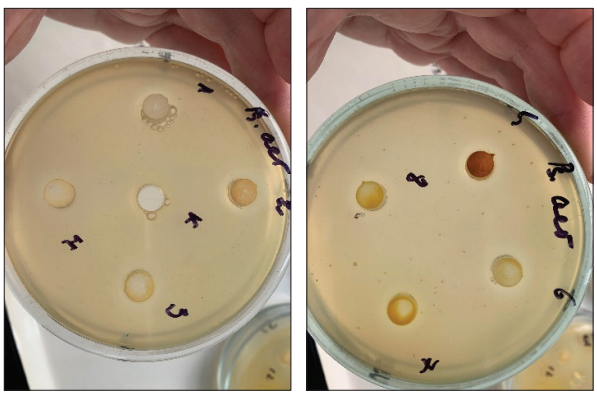

***Fig. 2***. Effect of samples 1—8 on *P. aeruginosa* strains after 24 hours

An experimental study of the antimicrobial activity of samples 9 and 10, which contained components Ts-D5 and Ts-D8, showed a high level of effectiveness against *P. aeruginosa*: the lysis zone increased by 10 mm after 72 hours ($p<0.05$; $p<0.001$). Even 2 months after application, the test samples retained a fairly high antimicrobial activity.

Samples 11, 12, and 13, which contained components VE-D67, VE-D68, and VE-D71, also showed a high lytic activity: the diameter of the lysis zones increased to 5—7 mm after 24 hours, and 6—8 mm after 72 hours ($p<0.05$; $p<0.001$).

Determination of the antimicrobial activity of samples 14, 15, and 16, which contained components W-014, W-015, and W-016, on the next day after obtaining the test samples, showed that all of them possessed a high antimicrobial activity toward the strains (Figs. 4 and 5). Sample 14 was the most active against *P. aeruginosa*: the diameter of the lysis zone was 10 mm after 24 hours, 11 mm after 48 hours, and 12 mm after 72 hours upon introducing the solution into the wells ($p<0.05$; $p<0.001$).

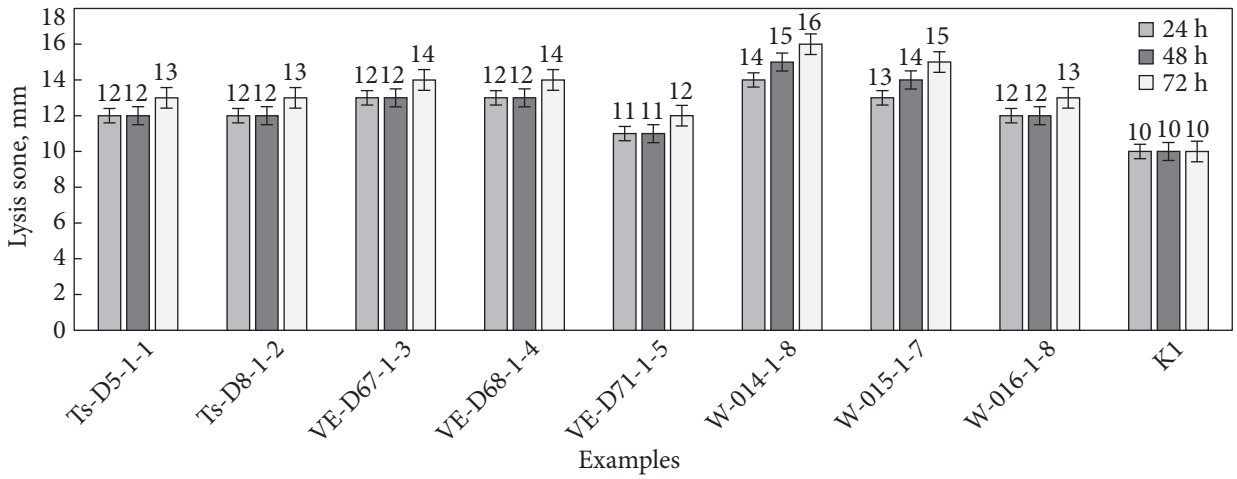


***Fig. 3***. Dynamics of changes in *P. aeruginosa* lysis zone under the action of experimental samples 1—8

Moreover, the high antimicrobial activity against strains of *P. aeruginosa* of samples 14, 15, and 16 was maintained even after two months.

Taking into account the obtained results, sample 14 was chosen to study its effect on the biofilm of *P. aeruginosa*.

The effect of experimental sample 14 — W-014-2 — 0.5% solution of 3,3'-diindolylmethane derivative W-014 in N-methylpyrrolidone — on *P. aeruginosa* was determined in two variants: 1 — first sample 14 was introduced into a glass-bottom Petry dish, then the culture

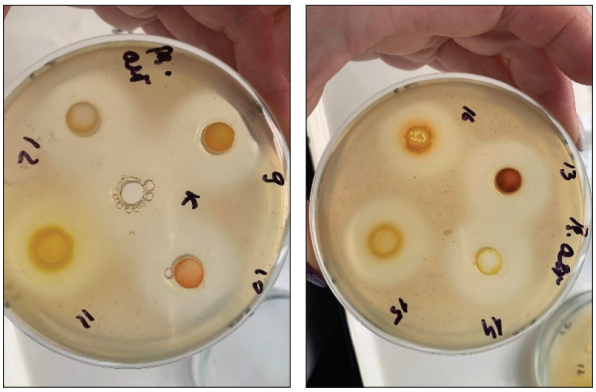

***Fig. 4***. Effect of samples 9—16 on *P. aeruginosa* strains after 24 hours

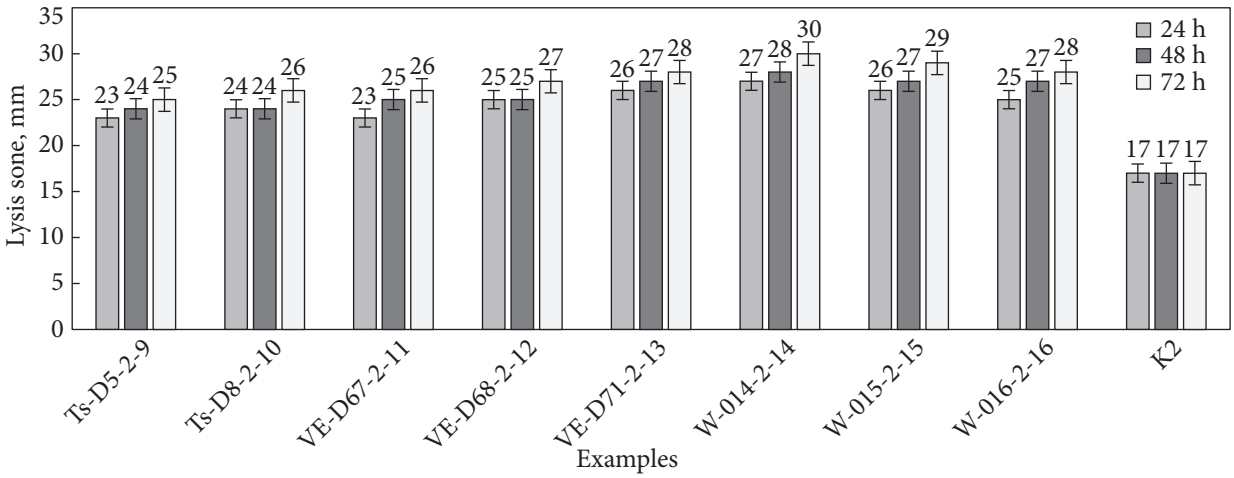


***Fig. 5***. Dynamics of changes in the *P. aeruginosa* lysis zone under the action of experimental samples 9—16

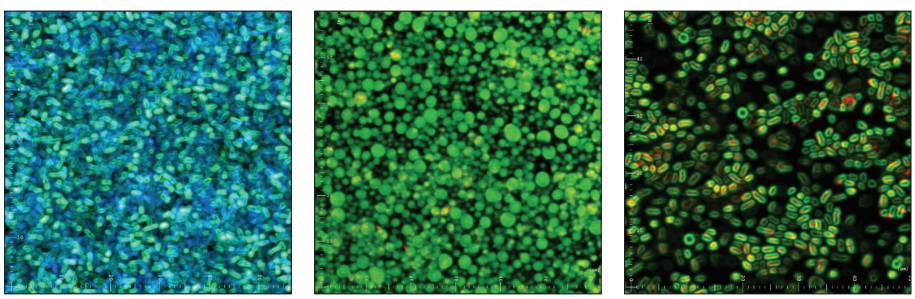

***Fig. 6***. *P. aeruginosa* biofilm: *a* — native daily biofilm; *b*— biofilm formed in the well with sample 14; *c*— daily formed biofilm after exposure to sample 14. Single slice (thickness 0.2 μm) of a confocal stack. Composite image of green, blue, and red emission bands: green fluorescence (polysaccharide matrix autofluorescence) was excited with 488 nm; blue fluorescence (nucleoid DNA+DAPI) was excited with 405 nm, and red fluorescence (damaged cells of nucleoid DNA+PI) was excited with 493 nm

of *P. aeruginosa,* and the ability to form a biofilm was investigated next day; 2 — sample 14 was applied to the already formed daily biofilm of *P. aeruginosa* to investigate the ability of the drug to penetrate and destroy the biofilm.

The native biofilm of *P. aeruginosa* is a dense three-dimensional structure covered with glycocalyx; the bacteria are in different phases of division, the membranes are intact, undamaged, and free DNA is not observed (Fig. 6, *a*).

When *P. aeruginosa* inoculum is introduced into a well containing test solution 14, the bacteria do not form a biofilm; individual cells transform into L-forms, division processes are not observed, and bacteria with damaged membranes and a small amount of free DNA are visible (Fig. 6, *b*).

When test sample 14 was introduced into a well with a formed *P. aeruginosa* biofilm, the effect of its destruction was observed. The biofilm looks significantly damaged, not dense: a well-stained nucleoid due to the defects in the bacterial cell wall, free DNA is also present, and some cells are transformed into L-forms (Fig. 6, *c*).

**Discussion**. As a result of the research, it was established that the test samples had different antimicrobial effects on strains of *P. aeruginosa*. Drug sample 14 showed the highest antimicrobial activity, so the diameter of the lysis zones increased by 12 mm (after 72 hours) compared to the control. Moreover, the effect of potentiation of the antimicrobial activity of the solution is observed. Two months after using the sample (9 passages) against *P. aeruginosa*, it retained a high lytic effect. The test solution prevented the formation of a biofilm when it was applied to the well surface, and also led to significant destruction of the already formed daily biofilm of *P. aeruginosa*.

It is known that biofilms cause numerous chronic infections and slow down the wound healing process, which explains the long-term persistence of the pathogen. Due to their compatibility and biostability with the macroorganism cells, plant materials have found biomedical applications (Mishyna et al., 2024). Although plant metabolites have long been used in traditional medicine, they are currently being studied for their antibacterial properties in connection with the resistance of pathogens to antimicrobial drugs. Our study highlights the potential of DIM derivatives for use as new antibacterial agents.

The research data is consistent with studies conducted by foreign experts (Golberg et al., 2022), who investigated the antimicrobial activity of DIM in relation to wound healing and found that the failure of chronic wounds to heal is associated with the presence of *P. aeruginosa* biofilms. They created two-day *P. aeruginosa* biofilms on an experimental wound model in laboratory animals and applied a polyurethane film with DIM and combined treatment with DIM and gentamicin, showing a significant reduction in wound size. In our in vitro study, the effect of DIM derivatives in various solvents on the formed daily biofilms of multidrug-resistant strains of *P. aeruginosa* was revealed.

**Conclusions**. Based on the obtained results, the following conclusions can be made:

- among all experimental composites based on dimethylsulfoxide, sample 6 (W-014-1 — 0.5% solution of 3,3'-diindolylmethane derivative W-014) had the highest antimicrobial effect with potentiation of action over time;
- among all experimental composites based on N-methylpyrrolidone, sample 14 (W-014-2 — 0.5% solution of 3,3'-diindolylmethane derivative W-014) had the highest antimicrobial effect with potentiation of action over time;
- tested solution prevented the formation of a biofilm when it was applied to the well surface, and also led to a significant destruction of the already formed daily biofilm of *P. aeruginosa*.

The research results confirm the antibacterial properties of diindolylmethane derivatives and require the finding of the best solvents that do

not reduce the effectiveness of 3,3'-diindolylmethane and do not exert any harmful effect on the macroorganism.

**Information about financing.** The study was supported by grant of the National Research Foundation of Ukraine 2022.01/0087 «Development of components of antimicrobial drugs to increase the effectiveness of their action and prevent resistance of microorganisms» (State Registration Number 0123U103756).

**Acknowledgments.** The authors would like to thank the scientists of the Institute of Single Crystals for supplying experimental pharmaceutical compositions.

**Conflict of interest.** The authors declare that there is no conflict of interest.

REFERENCES

Berlanga, M., & Guerrero, R. (2016). Living together in biofilms: the microbial cell factory and its biotechnological implications. *Microbial cell factories*, *15*(1), 165.

Chen, L., & Wen, Y. M. (2011). The role of bacterial biofilm in persistent infections and control strategies. *International journal of oral science*, *3*(2), 66—73.

Chimerel, C., Field, C. M., Piñero-Fernandez, S., Keyser, U. F., & Summers, D. K. (2012). Indole prevents Escherichia coli cell division by modulating membrane potential. *Biochimica et biophysica acta*, *1818*(7), 1590—1594.

Ciszek-Lenda, M., Strus, M., Walczewska, M., Majka, G., Machul-Żwirbla, A., Mikołajczyk, D., Górska, S., Gamian, A., Chain, B., & Marcinkiewicz, J. (2019). Pseudomonas aeruginosa biofilm is a potent inducer of phagocyte hyperinflammation. *Inflammation research: official journal of the European Histamine Research Society ... [et al.]*, *68*(5), 397—413.

Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerging infectious diseases*, *8*(9), 881—890.

El-Sawy, E. R., Bassyouni, F. A., Abu-Bakr, S. H., Rady, H. M., & Abdlla, M. M. (2010). Synthesis and biological activity of some new 1-benzyl and 1-benzoyl-3-heterocyclic indole derivatives. *Acta pharmaceutica (Zagreb, Croatia)*, *60*(1), 55—71.

Fiester, S. E., & Actis, L. A. (2013). Stress responses in the opportunistic pathogen Acinetobacter baumannii. *Future microbiology*, *8*(3), 353—365.

Gaimster, H., Cama, J., Hernández-Ainsa, S., Keyser, U. F., & Summers, D. K. (2014). The indole pulse: a new perspective on indole signalling in *Escherichia coli*. *PloS One*, *9*(4), e93168.

Golberg, K., Markus, V., Kagan, B. E., Barzanizan, S., Yaniv, K., Teralı, K., Kramarsky-Winter, E., Marks, R. S., & Kushmaro, A. (2022). Anti-Virulence Activity of 3,3'-Diindolylmethane (DIM): A Bioactive Cruciferous Phytochemical with Accelerated Wound Healing Benefits. *Pharmaceutics*, *14*(5), 967.

Hu, M., Zhang, C., Mu, Y., Shen, Q., & Feng, Y. (2010). Indole affects biofilm formation in bacteria. *Indian journal of microbiology*, *50*(4), 362—368.

Kim, J., & Park, W. (2015). Indole: a signaling molecule or a mere metabolic byproduct that alters bacterial physiology at a high concentration? *Journal of microbiology (Seoul, Korea)*, *53*(7), 421—428.

Kim, S. K., Park, H. Y., & Lee, J. H. (2015). Anthranilate deteriorates the structure of Pseudomonas aeruginosa biofilms and antagonizes the biofilm-enhancing indole effect. *Applied and environmental microbiology*, *81*(7), 2328—2338.

Langendonk, R. F., Neill, D. R., & Fothergill, J. L. (2021). The Building Blocks of Antimicrobial Resistance in *Pseudomonas aeruginosa*: Implications for Current Resistance-Breaking Therapies. *Frontiers in cellular and infection microbiology*, *11*, 665759.

Lebeaux, D., Ghigo, J. M., & Beloin, C. (2014). Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiology and molecular biology reviews: MMBR*, *78*(3), 510—543.

Lee, J. H., & Lee, J. (2010). Indole as an intercellular signal in microbial communities. *FEMS microbiology reviews*, *34*(4), 426—444.

Lin, Q., Deslouches, B., Montelaro, R. C., & Di, Y. P. (2018). Prevention of ESKAPE pathogen biofilm formation by antimicrobial peptides WLBU2 and LL37. *International journal of antimicrobial agents*, *52*(5), 667—672.

Mishyna, M., Mozgova, Yu, Malanchuk, S., Marchenko, I., Kot, Yu., & Mishyn, Yu. (2024). Detection of antimicrobial effect of new compositions with diindolilmethane on formed microbial biofilms. *Modern Medicine, Pharmacy and Psychological Health, 4* (18), 60—68.

O'Toole, G., Kaplan, H. B., & Kolter, R. (2000). Biofilm formation as microbial development. *Annual review of microbiology*, *54*, 49—79.

Pandey, P., Mehta, R., & Upadhyay, R. (2013). Physico-chemical and preliminary phytochemical screening of Psoralea corylifolia. *Archieves of Applied Sciences Research*, *5*, 261—265.

Penesyan, A., Nagy, S. S., Kjelleberg, S., Gillings, M. R., & Paulsen, I. T. (2019). Rapid microevolution of biofilm cells in response to antibiotics. *NPJ biofilms and microbiomes*, *5*(1), 34.

Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y., Bonsu, E., & Sintim, H. O. (2015). Biofilm Formation Mechanisms and Targets for Developing Antibiofilm Agents. *Future Medicinal Chemistry*, *7*(4), 493—512.

Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y., Bonsu, E., & Sintim, H. O. (2015). Agents that Inhibit Bacterial Biofilm Formation. *Future Medicinal Chemistry*, *7*(5), 647—671.

Reynolds, D., & Kollef, M. (2021). The Epidemiology and Pathogenesis and Treatment of *Pseudomonas aeruginosa* Infections: An Update. *Drugs*, *81*(18), 2117—2131.

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., Brinkman, F. S., Hufnagle, W. O., Kowalik, D. J., Lagrou, M., Garber, R. L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L. L., Coulter, S. N., Folger, K. R., Kas, A., Larbig, K., … Olson, M. V. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959—964.

Van den Bergh, B., Fauvart, M., & Michiels, J. (2017). Formation, physiology, ecology, evolution and clinical importance of bacterial persisters. *FEMS microbiology reviews*, *41*(3), 219—251.

Volyanskyi, Yu. L., Hrytsenko, I. S., & Shirobokov, V. P. (2004). Study of the specific activity of antimicrobial drugs. Methodological recommendations of the Ministry of Health of Ukraine. Kyiv, 38. www.umj.com.ua/uk/publikatsia-30960- chutlivist-klinichnix-shtamiv-staphylococcus-aureus-do-antibakterialnix preparativ # list. [In Ukrainian].

Wang, D., Ding, X., & Rather, P. N. (2001). Indole can act as an extracellular signal in Escherichia coli. *Journal of bacteriology*, *183*(14), 4210—4216.

Wolska, K. I., Grudniak, A. M., Rudnicka, Z., & Markowska, K. (2016). Genetic control of bacterial biofilms. *Journal of applied genetics*, *57*(2), 225—238.

World Health Organization. (2024). *WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance*. World Health Organization.

Xiuhua, Guo, & Fuzhong, Xue (2024). Textbook of Medical Statistics. Zhengzhou University Press, XII, 220 p.

Received 24.03.2025

*Світлана Маланчук[1*], Марина Мішина[2], Юрій Кот[1],*
*Світлана Джораєва[3], Юлія Мозгова[2], Богдан Маланчук[4], Юрій Мішин[2]*

[1] Кафедра інфекційних хвороб та клінічної імунології, кафедра біохімії,
Харківський національний університет імені В. Н. Каразіна,
майдан Свободи, 4, Харків, 61022, Україна
[2] Кафедра мікробіології, вірусології та імунології імені Д. П. Гриньова,
Харківський національний медичний університет,
проспект Науки, 4, Харків, 61022, Україна
[3] Лабораторно-експериментальний відділ,
Державна установа «Інститут дерматології та венерології НАМН України»,
вул. Чернишевська, 7/9, Харків, 61057, Україна
[4] Комунальне некомерційне підприємство «Ужгородська міська багатопрофільна клінічна лікарня»,
вул. Минайська, 71, Ужгород, 88000, Україна

ЕФЕКТ 3,3'-ДІІНДОЛІЛМЕТАНУ В РІЗНИХ РОЗЧИННИКАХ
НА БІОПЛІВКИ *PSEUDOMONAS AERUGINOSA*

На сьогодні найпоширенішими збудниками нозокоміальних інфекцій є грамнегативні бактерії, особливо *Pseudomonas aeruginosa*. Одним із провідних факторів, що визначають резистентність бактерій, є їх здатність до утворення біоплівок. Перспективним класом речовин, здатних впливати на біоплівки, є індоли (дііндолілметан). **Метою** даного експерименту було визначення впливу дііндолілметану в різних розчинниках на формування біоплівки *P. aeruginosa*. **Методи**. Для мікробіологічного дослідження матеріал був зібраний із гнійно-запальних ускладнених вогнепальних та осколкових ран. Визначали антимікробну активність зразків, що містили 0,5% розчин похідних 3,3'-дііндолілметану (Ts-D5-1 — зразок 1, Ts-D8-1 — 2, VE-D67-1 — 3, VE-D68-1 — 4, VE-D71-1 — 5, W-014-1 — 6, W-015-1 — 7, W-016-1 — 8 у розчиннику диметилсульфоксид) та (Ts-D5-2 — зразок 9, Ts-D8-2 — 10, VE-D67-2 — 11, VE-D68-2 — 12, VE-D71-2 — 13, W-014-2 — 14, W-015-2 — 15, W-016-2 — 16 у розчиннику N-метилпіролідон). Дослідження проводили методом дифузії в агарі. Утворення біоплівки проходило за методом O'Toole. **Результати**. Зразки 14, 15 та 16 продемонстрували високу антимікробну активність, серед яких зразок 14 був найефективнішим: діаметр зони лізису становив 10 мм через 24 години, 11 мм через 48 годин і 12 мм через 72 години. Інші зразки мали менш виражену антибактеріальну активність, яка з часом дещо посилювалася. **Висновки**. Зразок 14 продемонстрував найвищу антимікробну активність. Крім того, спостерігався ефект посилення антимікробної активності. Тестований розчин запобігає утворенню біоплівки при нанесенні на поверхню лунки, а також приводить до руйнування вже сформованої добової біоплівки *P. aeruginosa*.

**Ключові слова***: Pseudomonas aeruginosa,* антибіотикорезистентність, біоплівки, дііндолілметан.