One of the serious problems of medicine is the emergence and spread of antibiotic-resistant strains of microorganisms. Multidrug-resistant pathogens reduce the effectiveness of therapy, increase the length of hospital stay and material costs for patients treatment. On May 2015, at the 68th World Health Assembly it was endorsed a global action plan to tackle antimicrobial resistance [1], and in 2017, WHO presented a list of 12 types of bacteria that pose a threat to human health. On urgency of new drugs need, these pathogens are divided into three groups: critical, high and medium priority. The most important group is Acinetobacter, Pseudomonas and various Enterobacteriaceae (including Klebsiella, E. coli, Serratia, and Proteus) [2]. E. coli causes both acute inflammatory diseases and chronic recurrent diseases. The recurrent nature of the course of the inflammatory process is caused by biofilms. Biofilm is a complex matrix of microbial communities made up of polysaccharides, proteins and other organic components, in which cells bind together to form strong attachments to biotic or abiotic surfaces. Biofilm microorganisms are insusceptible to the action of therapeutic concentrations of antimicrobial drugs, which is due to both the structure of biofilms and physiological characteristics. Insufficient efficacy of antimicrobial chemotherapy in acute inflammatory diseases, the practically absence of drugs with antibiofilm activity motivates the search for promising compounds and the development of effective and safe drugs on their basis. Adamantane derivatives are promising antibiofilm agents.

Amantadine, Rimantadine and Tromantadine are used for the treatment of influenza (amantadine is also used as an antiparkinsonian drug). Memantine is known as a drug for symptomatic treatment of Alzheimer’s disease. A naphthoic acid derivative Adapalene is used for the topical treatment of acne. Vildagliptin and Saxagliptin are dipeptidyl peptidase IV (DPP-IV) inhibitors for the treatment of type 2 diabetes mellitus [3]. Adamantane derivatives have shown antimycobacterial [4–6] and antiviral [7, 8] activities. The interest of medicinal chemists in adamantane compounds is due to their unique spatial structure, high lipophility, and carbon cage rigidity. As a result, these molecules can easily penetrate biological lipid membranes and often have unique target-specific...
activity profile. For illustration, amantadine acts via modulation of a number of targets belonging to different families such as membrane receptors, enzymes, ion channels, transporters, transcription factors and others. For example, ethylenediamine derivative SQ-109 is one of the promising drug candidates for the treatment of multidrug-resistant tuberculosis, which is currently at stage 3 of clinical trials [9]. Adamantane derivatives, which exhibit a wide range of biological activity, including antimicrobial and antibiofilm effect against bacteria and fungi, deserve special investigation [10–12].

The aim of this work was to study the effect of 4-(adamantyl-1)-1-(1-aminobutyl) benzole on the adhesive properties of E. coli and the expression of genes that regulate adhesion.

Materials and methods. The bacterial strain used in the present study was E. coli isolated from pus. The test strain showed resistance to amikacine, norfloxacine, cefoperazone, susceptibility to ciprofloxacin, and gentamicin. The strain was subcultured at 37 °C on Tryptone Soya Agar plates.

The 4-(adamantyl-1)-1-(1-aminobutyl) benzole (AM-166) used in the present study was synthesized within the PJSC SIC «Borshchahivskiy CPP». AM-166 was dissolved in 10.0 % dimethyl sulfoxide; the stock solution concentration was 1 mg/mL. All other chemicals were obtained from commercial sources. Meropenem, ciprofloxacin, gentamicin and ceftazidime were purchased in the pharmacy under the trade name Meronem (MEM, powder for solution for injection, manufactured by AstraZeneca UK Limited, United Kingdom), Ciprinol (CIP, solution for infusion, manufactured by KRKA, Slovenia), Gentamicin (GEN, substance, manufactured by PJSC SIC «Borshchahivskiy CPP) and Ceftum (CAZ, powder for solution for injection, manufactured by LLC «ARTERIUM LTD», Ukraine) were used as reference preparations. The following media were used in the present study: Luria-Bertani broth (Conda, Spain), Luria-Bertani agar and Tryptone Soya Broth (TSB, HiMedia, India).

Biofilm assay. AM-166 effects (concentrations of 0.5 minimal inhibitory concentration (MIC) and 5.0 MIC) on E. coli biofilms and their formation processes was estimated by microtiter dish biofilm formation assay described by O’Toole [13]. When evaluating the compound’s effect on the biofilm formation, AM-166 solution and the inoculum applications were performed simultaneously. To prepare the inoculum, the overnight culture was diluted 100-fold (1:100) 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) min. To detect the formed biofilm, the dye was extracted with ethanol (15 min). Optical density (OD) were measured using «Adsorbance Microplate ReaderELx × 800» (VioTek, USA) at a wave length of 630 nm. Intact cultures of microorganisms grown under the same conditions without the addition of the compound were used as a control.

Bacterial surface hydrophobicity assay. E. coli surface hydrophobicity was measured using previously described microbial adhesion to solvents (MATS) method with modifications [14]. The affinity to ethyl acetate that is a monopolar and basic solvent was studied. An overnight culture in TSB medium was 10-fold diluted with fresh TSB medium. The hydrophobicity properties were estimated by growing strain in TSB (optical density OD_{600} 0.3)
with or without AM-166 (12.5 μg • mL⁻¹), meropenem (0.005 μg • mL⁻¹ or 0.02 μg • mL⁻¹), ciprofloxacin (0.0075 μg • mL⁻¹ or 0.03 μg • mL⁻¹), gentamicin (0.125 μg mL⁻¹ or 0.5 μg • mL⁻¹) or ceftazidime (0.075 μg • mL⁻¹ or 0.3 μg • mL⁻¹), at 37 °С for 90 min. After incubation, bacteria were washed twice in 0.9 % NaCl solution by centrifugation for 15 min at 3000 rpm and were resuspended in same solution to OD₆₀₀ 0.18–0.22 (A₀). Afterwards, ethyl acetate (0.5 mL) was added to the bacterial suspensions (3.0 mL), which were then kept at room temperature (RT) for 10 min to saturate. Each sample was then mixed by vortexing (model V-3, ELMI, Latvia) for 2 min and then allowing the mixture to stand for 15 min at room temperature for phase separation. The aqueous phase was collected and the OD₆₀₀ was measured (A). The results were expressed as the percentage decreased in the OD of the aqueous phase (A) compared with the OD of the initial cell suspension (A₀): 100 • [1 – (A/A₀)]. Each assay was repeated three times in duplicate.

**Motility assay.** The swarming, swimming, and twitching motilities of E. coli were investigated using the following media: (I) swim plates [1 % tryptone, 0.5 % yeast extract, 0.5 % NaCl, 0.3 % agar], (II) swarm plates [1 % tryptone, 0.5 % yeast extract, 0.5 % NaCl, 0.5 % agar, 1M MgSO₄, 0.5 % glucose], and (III) twitch plates [1 % tryptone, 0.5 % yeast extract, 0.5 % NaCl, 1.0 % agar]. An overnight cell culture in TSB medium was incubated 30–45 min with 0.5 MIC or 2.0 MIC AM-166 (12.5 μg • mL⁻¹ and 50 μg • mL⁻¹ respectively), meropenem (0.005 μg • mL⁻¹ and 0.02 μg • mL⁻¹ respectively), ciprofloxacin (0.0075 μg • mL⁻¹ and 0.05 μg • mL⁻¹ respectively), gentamicin (0.125 μg • mL⁻¹ or 0.5 μg • mL⁻¹) or ceftazidime (0.075 μg • mL⁻¹ or 0.3 μg • mL⁻¹). Control cultures contained no antimicrobials. Each assay was repeated three times in duplicate. For the swimming motility assay, the plates were inoculated in the centre with a sterile toothpick and incubated for 16–20 h at RT [15]. Motility was assessed by observation of the circular turbid zone formed by bacteria migrating away from the inoculation point. For the swarming motility assay, the bacterial cells were gently inoculated by micropipette (2 μL) into the top of semisolid agar, and the plates were incubated at 37 °C for 16–24 h [16]. For the twitching motility assay, the cells were stab inoculated with a sterile toothpick through an agar layer to the bottom of the Petri dish. After incubation for 24–48 h at 37 °C, a hazy zone of growth at the interface between the agar medium and the glass surface was observed. The ability of bacteria to twitch strongly on the glass surface was examined by removing the agar, washing out the untouched cells and staining the attached cells by a crystal violet solution [15].

**Adhesion assay.** The adhesion of E. coli was estimated by the method by Christensen [17]. An overnight culture in TSB medium was grown at 37 °C in the presence or absence of 5.0 MIC AM-166 (125 μg • mL⁻¹), meropenem (0.05 μg • mL⁻¹), ciprofloxacin (0.075 μg • mL⁻¹), gentamicin (1.25 μg • mL⁻¹) or ceftazidime (0.75 μg • mL⁻¹). After 1 h, 3 h and 5 h the bacterial cells were diluted 100-fold with fresh TSB medium; cell suspension (100 μL) was transferred into individual wells of sterile, polystyrene, 96-well plate and incubated at 37 °C. After a 24-hour incubation, the TSB medium was discarded, and the wells were washed thrice with distilled water to remove non-adherent bacteria. Adherent cells were fixed in place for 15 min with
96 % ethanol, dried and then stained for 5 min with 0.1 % crystal violet. Excess stain was rinsed off. After drying, the OD of stained adherent bacterial films were measured using Absorbance Microplate Reader (model ELx800, BioTek, USA) at 630 nm. Adherence measurements were repeated at least three times in quadruplicate; the values were then averaged. The adherence capability of the test strain was classified into four categories: non-adherent, slightly adherent, moderately adherent, or strongly adherent, based upon the OD of bacterial films. The cut-off optical density (ODc) was determined as three standard deviations above the mean OD of the negative control. The strength of adhesion was calculated by the following formula: OD ≤ ODc – non-adherent; ODc < OD ≤ 2 · ODc – slightly adherent; 2 · ODc < OD ≤ 4 · ODc – moderately adherent; 4 · ODc < OD – strongly adherent.

Polymerase chain reaction (PCR)-analysis. DNA was extracted from a 24-hour E. coli cell culture using the boiling method [18]. The PCR-mix in a total volume of 25 μl contained 1 × PCR-buffer (Thermo Scientific), 2.5 mM MgCl₂ (Thermo Scientific), 2.5 mM dNTP mix (Thermo Scientific), 10 pmol of each primer (Table 1), 1 unit of Platinum™ Taq DNA Polymerase (Thermo Scientific) and 5 μl of DNA template. The temperature conditions were specific for each primer pair [19, 20]. For fliC, motB, gyrA they were: an initial denaturation at 95 °C for 5 min, followed by 45 cycles of 10 s at 95 °C, 30 s at 56 °C, and 20 s at 72 °C, with a final extension step at 72 °C for 5 min. For fimA PCR parameters was included an initial denaturation step at 95 °C for 4 min; 35 amplification cycles of 30 s at 95 °C, 30 s at 55 °C, 40 s at 72 °C and an extension step of 3 min at 72 °C; for papC – an initial denaturation step at 95 °C for 4 min; 35 amplification cycles of 60 s at 95 °C, 60 s at 65 °C, 60 s at 72 °C and an extension step of 2 min at 72 °C; for afa – an initial denaturation step at 95 °C for 5 min; 35 amplification cycles of 60 s at 95 °C, 30 s at 60 °C, 180 s at 72 °C and an extension step of 7 min at 72 °C. PCR-products were separated on 1.2 % agarose gel and visualized under UV-light (Bio-Rad, Germany).

Quantitative Real-Time PCR (qRT-PCR). Total RNA was isolated from a 24-hour E. coli cell suspension cultured with or without 0.5 MIC of 4-(adamantyl-1)-1-(1-aminobutyl) benzol using

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primers sequences (5′–3′)</th>
<th>Size, bp</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fliC (Fw)</td>
<td>ATTCCCGTTCCCTCCTCGGTG</td>
<td>131</td>
<td>[19]</td>
</tr>
<tr>
<td>fliC (Rv)</td>
<td>TGACACTTGGGCTCGCATAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>motB (Fw)</td>
<td>GAACGTAGTGGTGGAGGTTT</td>
<td>146</td>
<td>[19]</td>
</tr>
<tr>
<td>motB (Rv)</td>
<td>GCCTGTTCGGGTGGTGTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fimA (Fw)</td>
<td>GTTGTGCTGGCTGGCTGTC</td>
<td>400</td>
<td>[20]</td>
</tr>
<tr>
<td>fimA (Rv)</td>
<td>ATGTTGCTGGTGGTGTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>papC (Fw)</td>
<td>GACGGCGGTACGTGAGGCGGTGCG</td>
<td>328</td>
<td>[20]</td>
</tr>
<tr>
<td>papC (Rv)</td>
<td>ATATCTTCTGGCAAGGATGCAATA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>afa (Fw)</td>
<td>GCTTGGCGCAGCAAATGATAACTCT</td>
<td>750</td>
<td>[20]</td>
</tr>
<tr>
<td>afa (Rv)</td>
<td>CATCAAGCTGTTTGTTCGTCCGCCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gyrA (Fw)</td>
<td>GTCTGGGCGGAAAGGTG AAA</td>
<td>106</td>
<td>[19]</td>
</tr>
<tr>
<td>gyrA (Rv)</td>
<td>CGGCTGGAAAGCCACAGAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Direct-zol™ RNA Miniprep Plus (Zymo Research). The reaction mix for qRT-PCR contained 10 μl of Luna Universal One-Step Reaction Mix (2X), 1 μl of Luna_WarmStart® RT Enzyme Mix, 10 pmol of each primer (forward and reverse) (Table 1) and 5 μl of RNA bacteria. Amplification was carried out using CFX96 Real-Time System (Bio-Rad, Germany). The expression of gyrA gene was considered as an internal control. Amplification for each sample and for each gene was performed in duplicate. The relative gene expression level was calculated with $2^{-\Delta\Delta Ct}$ method [21] and verified for statistical significance with t-test. The specificity of primers and dimer formation were confirmed by melting curve analysis.

**Statistical Analysis.** The nonparametric Kruskal-Wallis H-test was used to compare the continuous variables for the biofilm and adhesion assays. The expression levels of the genes before and after treatment were compared using the paired t-test. A p-value of $< 0.05$ was considered as significant. STATISTICA, version 10 (StatSoft, USA) was used for the data analysis. The data obtained were expressed as $M \pm m$, where $M$ is the mean value and $m$ is the standard error of the mean.

**Results.** *Effect of AM-166 on adhesion of E. coli.* Our previous studies have found that the MIC of AM-166 against *E. coli* 311 was 25.0 μg/mL. *E. coli* 311 was a highly adherent strain. The influence of time of pre-treatment with AM-166, meropenem, ciprofloxacin, gentamicin and ceftazidime on adhesion ability of *E. coli* was compared with the intact control sample (100 %) (Fig. 1).

The experiments showed that AM-166 decreased adhesion of *E. coli* cells by 46.0 – 76.4 %, meropenem – 33.1 – 45.0 % after 1, 3 and 5 h of incubation; ceftazidime – 22.3 % after 3 h of incubation, ciprofloxacin – 25.8 % after 5 h of incubation ($p < 0.05$).

**Biofilm formation.** The data obtained (Fig. 2) show that the compound AM-166 inhibits *E. coli* 311 biofilms formation: when exposed to a concentration of 5.0 MIC, the biomass decreases by 69.2 %, at a concentration of 0.5 MIC – by 36.9 % (compared to intact control).

The biomass of *E. coli* biofilm with ciprofloxacin treatment decreases by 79.4 % at a concentration of 5.0 MIC, with 0.5 MIC a reduction of biomass was by 88.9 %. Meropenem produces the biomass reduction by 91.0 % and 77.0 %, gentamicin – 79.8 % and 85.8 %, ceftazidim – 91.7 % and 82.2 %, respectively.

**Cell surface hydrophobicity.** Bacterial cell surface hydrophobicity (CSH) is one of the most important factors that govern bacterial adhesion to various
surfaces. Depending on the type of surface, hydrophobicity of cells can increase the propensity of microorganisms to adhesion [22, 23]. The present study investigated the influence of AM-166, meropenem, ciprofloxacin, gentamicin, and ceftazidim on the hydrophobic properties of 

\[ E. \text{coli} \]. The data reported in the Table 2 showed that AM-166 at 0.5 MIC reduced bacterial hydrophobic properties by 4.0 % compared to the control.

High turbidity of medium was observed in the presence of AM-166 at 2.0 MIC, which caused erroneous results (data not shown).

Compound AM-166 and reference drugs in the studied concentrations did not significantly change the hydrophobic properties of 

\[ E. \text{coli} \] cells, for the action of ciprofloxacin hydrophobicity decreases by (3.6–11.0) %, meropenem – by (4.7–5.1) %, gentamicin – by (4.2–3.0) %. The reference drug ceftazidime increased the hydrophobicity of cells (by 13.8–15.7 %) compared to the control.

**Effect of AM-166 on E. coli motility.** Flagellum- and fimbriae-mediated motility enables bacteria to migrate toward nutrients or away from toxic substances [24, 25] and plays key roles in bacterial biofilm formation and host-pathogen interactions [26, 27]. In the study of the effect of adamantane derivatives on the mobility of the

![Fig. 2. Biofilm-forming of Escherichia coli using 0.5 and 5.0 MIC AM-166 (formed biofilm, %)](image)

**Note.** CIP – ciprofloxacin; MEM – meropenem; GEN – gentamicin; CAZ – ceftazidim; *p < 0.05 in comparison with the corresponding concentration of ciprofloxacin (CIP); **p < 0.05 in comparison with the corresponding concentration of meropenem (MEM); ***p < 0.05 in comparison with the corresponding concentration of gentamicin (GEN); **p < 0.05 in comparison with the corresponding concentration of ceftazidime (CAZ), *p < 0.05 in comparison with control.

**Table 2**

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Solvent affinity to ethyl acetate (%)</th>
<th>0.5 MIC</th>
<th>2.0 MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM-166</td>
<td>35.1 ± 6.7</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>34.5 ± 5.1</td>
<td>34.1 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>37.8 ± 3.7</td>
<td>34.9 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>35.0 ± 1.8</td>
<td>36.2 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>53.0 ± 0.2*</td>
<td>54.9 ± 3.2*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39.2 ± 1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note.** *p < 0.05 in comparison with control (bacterial growth without antimicrobials).
«swimming» type of *E. coli* bacteria, the growth of culture throughout the agar thickness in Petri dishes was recorded. The results of studying the effect of the AM-166 compound on swarming and twitching migration of *E. coli* are shown in the Table 3.

AM-166 (0.5 MIC and 2.0 MIC) affected swarming. The diameter of motility zone was increase 1.5-fold and 1.2-fold compared to the control. Ciprofloxacin and ceftazidime at 0.5 MIC increased swarming zone 2.4-fold and 1.9-fold, at 2.0 MIC – 1.2-fold and 1.8-fold, respectively. Meropenem (0.5 MIC) reduced motility zone of the culture 1.2-fold, gentamicin (0.5 MIC and 2.0 MIC) – 2-fold compared to the control (p < 0.05).

Compound AM-166 inhibited the twitching migration of *E. coli*. The motility zones diameter reduction by 1.3-fold and 1.8-fold under the influence of AM-166 at 0.5 MIC and 2.0 MIC respectively was registered. Ciprofloxacin, meropenem and gentamicin also inhibited the motility of *E. coli* 1.5-fold, 1.8-fold and 1.4-fold at a concentration of 0.5 MIC, 1.4-fold, 2.3-fold and 1.2-fold at 2.0 MIC respectively. Pretreatment with cef-

The evaluation of the relative expression level of genes responsible for the motility of *E. coli* 311 was performed using real-time PCR (*fliC*, *motB*, *fimA*, *papC*) (Fig. 3). The effects of AM-166 on the transcriptional activity of genes were studied at a subinhibitory concentration (0.5 MIC).

It was found that after AM-166 treatment the transcriptional activity of *fliC* and *motB* genes was 2-fold less as compared to control (p < 0.05). At the same time, statistically significant increase of *fimA* gene expression was registered (Fig. 4). The data obtained suggest that adamantane derivative AM-166 led to the slight decrease expression (not statistically significant) of *papC* gene in *E. coli* 311 at sub-inhibitory concentration (p > 0.05).

The effect of AM-166 on motility of *E. coli* depending on concentration

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Motility, mm (mean ± m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swarming</td>
</tr>
<tr>
<td></td>
<td>0.5 MIC</td>
</tr>
<tr>
<td><strong>AM-166</strong></td>
<td></td>
</tr>
<tr>
<td>0.5 MIC</td>
<td>45.2 ± 2.2*</td>
</tr>
<tr>
<td>2.0 MIC</td>
<td>36.5 ± 2.2*</td>
</tr>
<tr>
<td><strong>Ciprofloxacin</strong></td>
<td></td>
</tr>
<tr>
<td>0.5 MIC</td>
<td>73.0 ± 1.0*,##</td>
</tr>
<tr>
<td>2.0 MIC</td>
<td>35.0 ± 1.0##</td>
</tr>
<tr>
<td><strong>Meropenem</strong></td>
<td></td>
</tr>
<tr>
<td>0.5 MIC</td>
<td>32.0 ± 9.0*,##</td>
</tr>
<tr>
<td>2.0 MIC</td>
<td>18.5 ± 1.6##</td>
</tr>
<tr>
<td><strong>Gentamicin</strong></td>
<td></td>
</tr>
<tr>
<td>0.5 MIC</td>
<td>15.7 ± 2.0*,##</td>
</tr>
<tr>
<td>2.0 MIC</td>
<td>14.0 ± 1.0##</td>
</tr>
<tr>
<td><strong>Ceftazidime</strong></td>
<td></td>
</tr>
<tr>
<td>0.5 MIC</td>
<td>57.5 ± 6.9*,##</td>
</tr>
<tr>
<td>2.0 MIC</td>
<td>53.7 ± 10.4*,##</td>
</tr>
<tr>
<td><strong>Control (without antimicrobials)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29.8 ± 1.7</td>
</tr>
</tbody>
</table>

Note. *p < 0.05 in comparison with control (bacterial growth without antimicrobials); *p < 0.05 in comparison with the same concentration of ciprofloxacin; **p < 0.05 in comparison with the same concentration of gentamicin; *p < 0.05 in comparison with the same concentration of ceftazidime.
Discussion. *E. coli* is a type of gram-negative rod-shaped bacteria, facultative anaerobes, which is a part of the normal microflora of the human gastrointestinal tract, and is capable of causing both intestinal and extraintestinal infections. *E. coli* can attach to surfaces and assemble into multicellular communities enclosed in extracellular polymeric substances called biofilms [26, 27]. Biofilm is frequently found on implanted medical devices, prostheses and implants. Short-term contacts of *E. coli* with the surfaces of tissue cells or medical devices lead to the bacterial attachment and biofilm production [28]. The compound AM-166 inhibits the biofilm formation of *E. coli*. The most pronounced effect was registered at a concentration of 5.0 MIC (69.2 %).

The attachment of microbial cells to surfaces depends on a number of factors including, e. g., Brownian movement, van der Waals attraction, gravitational forces and surface electrostatic charges [22]. One of the important factors is the hydrophobicity of the cells. Depending on the type of surface, hydrophobicity of cells can increase the propensity of microorganisms to adhesion. The more hydrophobic cells adhere more strongly to hydrophobic surfaces, while hydrophilic cells strongly adhere to hydrophilic surfaces [23].

However, one should take into consideration also the heterogeneity of microbial population. In the culture with planktonic, freely living microorganisms, it is possible to observe the presence of both hydrophilic and hydrophobic cells, hence only a part of them participate in the adhesion. Another important tenet is that microorganisms can switch between hydrophobic and hydrophilic phenotypes in response to changes in the environmental conditions (temperature, composition of nutrients, etc.) and growth phases [22, 23].
Our studies have found that the adamantane derivative AM-166 and reference drugs in the studied concentrations (0.5 MIC, 2.0 MIC) did not significantly change the hydrophobic properties of *E. coli* cells by 3.6–11.0 %, the reference drug ceftazidime increased the hydrophobicity cells (by 13.77–15.72 %) in comparison with control.

To survive in changing conditions, bacteria have the ability to quickly adapt their structure and physiology. The mechanisms of this process are based on the existence of numerous regulatory networks that enable the coordinated regulation of gene expression in response to environmental signals. An example of such a complex regulatory system is the coordination of the processes of bacterial motility and chemotaxis. Many bacterial strains are motile by means of flagella.

An *E. coli* cell has 5–10 flagella located randomly on the cell surface. Each of them consists of a basal body, a long outer filament of flagellin protein, and a «hook» connecting the two parts. The outer filament of the bacterial flagellum consists of the FliC flagellin, which regulates the protein synthesis of the *fliC* gene [27]. The mobility of microorganisms is associated with pathogenicity and antibiotic resistance.

The rotation of the flagellum is carried out by the interaction between the rotor and the stator. The rotor is a C-shaped ring that is located under the basal body and consists of FliG, FliM and FliN.

There are two types of flagellar motors, depending on the bond ion. One is the proton engine with a stator made up of MotA and MotB, and is found in bacteria such as *E. coli* or *Salmonella*. The MotA and MotB proteins are associated with the cytoplasmic membrane. Most of the MotB protein is located in the periplasmic space. The production of the MotB protein is regulated by the *motB* gene [29, 30].

Our studies have shown that the adamantane derivative AM-166 does not interfere with the swimming of *E. coli*; we recorded the growth of the culture across the entire thickness of the agar in Petri dishes. Compound AM-166 inhibits the expression of *fliC* and *motB* genes in *E. coli*.

Despite the inhibition of the expression of *fliC* and *motB* genes, the swimming-type motility of bacteria was not impaired, swimming was increased [(45.2 ± 2.2) mm and (36.5 ± 11.0) mm at 0.5 MIC and 2.0 MIC, in control (29.8 ± 1.7) mm]. Perhaps this effect depends on the cell level of a secondary messenger cyclic di-GMP (c-di-GMP), which is involved in the formation of biofilms and is of the key importance in modulating the transition between a mobile and a sedentary lifestyle, which are important for acute and chronic infections respectively [25]. The mobility of bacteria, in particular, swimming motility is also regulated by Quorum Sensing systems [27, 31]. It should be noted that increased motility with the participation of *E. coli* flagella is important for the colonization of the upper urinary tract and mucous membranes of the digestive tract by microorganisms [27].

Twitching motility is a flagella-independent form of bacterial translocation over the moist surfaces. It occurs by the extension, tethering, and then retraction of polar type IV pili, which operate in a manner similar to a grappling hook. Twitching motility is equivalent to social gliding motility in *Myxococcus xanthus* and is important in host colonization by a wide range of plant and animal pathogens, as well as in the formation of biofilms. The biogenesis and function of type IV pili is controlled by a large number of genes,
almost 40 of which have been identified in *Pseudomonas aeruginosa*. A number of genes required for pili assembly are homologous to genes involved in type II protein secretion and competence for DNA uptake, suggesting that these systems share a common architecture. Twitching motility is also controlled by a range of signal transduction systems, including two-component sensor-regulators and a complex chemosensory system [32, 33]. The data obtained suggested that AM-166 largely affected the motility of *E. coli* due to type IV pili (than flagella). Both AM-166 concentrations caused considerable changes in twitching motility. In further studies, it is necessary to establish whether this effect depends on the inhibition of the expression of genes that regulate twitching motility or is associated with a dysfunction of the fimbriae.

*E. coli* adhesion to the substrate is provided by type 1 fimbriae, P-fimbriae, Afa/Dr-adhesins, and Curli invasion [33, 34]. Type 1 fimbriae (pili) carrying the adhesin FimH protein are able to recognize the structure of mannose receptors in epithelial tissue and interact with them.

The structural gene fimbria 1 type *fimA* encodes a polypeptide of 158–159 amino acids with an approximate molecular weight of 17 kDa [35–37]. Type 1 fimbriae adhere to mammalian buccal cells, intestinal epithelial cells, lung, proximal tubular cells of kidney [38–40]. Type 1 pili make bacteria hydrophobic and reduce their electrophoretic mobility. In experiments *in vivo* on a model of urinary tract infection in mice, it was shown that increased transcription of *fimA* and *fimB* is recorded at an early stage of infection; by the 5\(^{th}\) day of the disease, transcription decreases [38]. *E. coli* strains the type 1 fimbriae are combined with other virulence determinants.

The results obtained indicate an increase in the expression of the *fimA* gene under the action of a subinhibiting concentration of AM-166, which indicates the possibility of an adamantane-containing compound to stimulate the formation of fimbriae and enhance the adhesion of microorganisms to epithelial cells. An increase in *fimA* expression under the action of AM-166 may also be a protective reaction of *E. coli* to the action of an antimicrobial substance and an increase in biofilm formation processes, including the formation of type 1 fimbriae, since the biofilm acts as a natural barrier protecting bacteria from the treatment with antimicrobial drugs [41–42].

P-fimbriae also provide adhesion of *E. coli* to the substrate. P-fimbriae are composed of heteropolymeric fibers containing various protein subunits encoded by the *papA-K* operon. The adhesive part of P-pili consists of several protein subunits PapA, PapH, PapK, PapE, PapF, PapG, and PapC, each of which is controlled by a corresponding gene [43]. It was found that the degree of PapC synthesis affects the number of pili expressed on the cell surface. The PapC protein is localized on the outer membrane of *E. coli*, where it can form a transmembrane channel, due to which the pilin subunits are localized on the surface. In *E. coli*, the *papC* gene has been identified as a determinant of antibiotic resistance [44]. A correlation was established between the *papC* gene and the carriage of beta-lactamase genes and commensal *E. coli* strains [44–46]. On the contrary, the carriage of the pap gene is inversely proportional to resistance to fluoroquinolones in *E. coli*, which may be due to the partial loss of islets of genomic pathogenicity as a result of resistance mutations in genes encoding topoisomerases II and IV [47].
It was found by experiments that under the action of an adamantane derivative at a concentration of 0.5 MIC, the expression of the *papC* gene does not change, and statistically significant differences are absent.

Afa/Dr adhesins is a heterogeneous group of homopolymeric adhesive organelles identified in uropathogenic *E. coli* (UPEC) and promoting UPEC invasion of uroepithelial cells [48]. The *afa/dr* gene is not detected in the clinical strain *E. coli* 311.

**Conclusions**

1. The present study showed that 4-(adamantyl-1)-1-(1-aminobutyl)benzole inhibits the biofilm formation of *E. coli*, affecting adhesion factors.

2. The AM-166 at subinhibitory concentration (0.5 MIC) decreased the twitching migration of *E. coli*, which led to reduction of *E. coli* attachment to polystyrene. Swimming motility at a concentrations of 0.5 MIC and 2.0 MIC increased.

3. Gene expression under the action of AM-166 (0.5 MIC) changes: *fliC* and *motB* decreased 2-fold, *fimA* increased (p < 0.05). A decrease in *papC* gene expression (p > 0.05) was revealed, however there was no statistically significant difference.


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**Anti-adhesion properties of 4-(adamantyl-1)-1-(1-aminobutyl) benzole against *Escherichia coli***

One of the serious problems of medicine is the formation and spread of antibiotic-resistant *E. coli* strains capable of causing acute and chronic persistent infections due to biofilms. Insufficient effectiveness of antimicrobial chemotherapy in acute inflammatory diseases and the practical absence of drugs with an antibiofilm activity actualize the search for promising compounds and the development of effective and safe drugs on their basis.

The aim of this work is to study the effect of 4-(adamantyl-1)-1-(1-aminobutyl) benzole (AM-166) on the adhesive properties of *E. coli* and the expression of genes that regulate adhesion.

The antibiofilm activity of the adamantane-containing compound AM-166 was studied by the sorption of gentian violet on biofilm structures, the hydrophobicity of the *E. coli* cell surface was assessed by adhesion to a solvent (MATS test), swimming, swarming, and twitching migration – by standard methods. Cell adhesion to the abiotic surface was assessed by the Christensen method, the effect of AM-166 on the expression of the *fimA*, *papC*, *fliC*, and *motB* genes was investigated using real-time PCR.

It was shown that the AM-166 compound disrupts the formation of *E. coli* biofilm: the biomass decreases at 5.0 MIC by 69.2 %, at 0.5 MIC – by 36.9 %. It was found that AM-166 reduces the hydrophobicity of *E. coli* cells (by 4.0 % at 0.5 MIC) and adhesion to an abiotic substrate (by 46.0–76.4 %, depending on the incubation time), inhibits twitching migration, and stimulates swarming migration. The diameters of the *E. coli* mobility zones increased by 1.5 times (0.5 MIC) and 1.2 times (2.0 MIC). Under the action of AM-166, an increase in the expression of the *fimA* gene, as well as a decrease in the expression of the *fliC* and *motB* genes (by 2 times) were recorded. The decrease in *papC* gene expression was statistically insignificant.

Thus, the antibiofilm activity of AM-166 is due to the impaired mobility of microorganisms and altered expression of the *fimA*, *fliC*, and *motB* genes. In the future, it is necessary to study the effect of the adamantane derivative on the Quorum Sensing systems in *E. coli*.

**Key words:** aminoadamantane derivatives, bacteria, *Escherichia coli*, biofilm, hydrophobicity, motility, adhesion, gene expression
Однією з серйозних проблем медицини є поява та розповсюдження антибіотикорезистентних штамів *E. coli*, здатних сприяти гострі та хронічні персистуючі інфекції, зумовлени біоплівками. Недостатня ефективність противимикробної хіміотерапії за гостріх запальних захворювань, практична відсутність препаратів з антибіоплівковою активністю спонукають до пошуку перспективних сполук і розробки на їхній основі ефективних і безпечних препаратів.

Мета дослідження — вивчити вплив 4-(адамантил-1)-1-(1-амінобутил)бензолу (АМ-166) на адгезивні властивості *E. coli* та експресію генів, що регулюють адгезію. Антибіоплівкову активність адамантанамісної сполуки АМ-166 досліджували методом сербції генціанвіолету на структурах біоплівки. Гідрофобність клітинної поверхні оцінювали за адгезією до розчинника (тест MATS), swimming-, swarming- та twitching-міграцію *E. coli* досліджували стандартними методами. Адгезію клітин до абіотичної поверхні оцінювали за методом Christensen, вплив АМ-166 на експресію генів *fimA*, *papC*, *fliC* та *motB* — за допомогою ПЛР у реальному часі.

Показано, що сполука АМ-166 порушує формування біоплівки *E. coli*, біомаса зменшується у разі 5,0 МІК на 69,2 %, 0,5 МІК — на 36,9 %. Встановлено, що АМ-166 знижує гідрофобність клітин *E. coli* (на 4,0 % у разі 0,5 МІК), адгезію до абіотичного субстрату (на 46,0–76,4 %, залежно від часу інкубації), пригнічує twitching-міграцію та стимулює swarming-міграцію, діаметри зон рухливості збільшились у 1,5 (0,5 МІК) та 1,2 (2,0 МІК) рази. За дії АМ-166 реєструється збільшення експресії гена *fimA*, зменшення експресії генів *flic* та *motB* (у 2 рази). Зніження експресії гена *papC* статистично недостовірне.

Таким чином, антибіоплівкова активність АМ-166 обумовлена порушенням рухливості мікроорганізмів та зміною експресії генів *fimA*, *flic* та *motB*. У подальших дослідженнях необхідно вивчити вплив похідного адамантану на систему Quorum Sensing у *E. coli*.

**Ключові слова:** похідні аміноадамантану, бактерії, *Escherichia coli*, біоплівка, гідрофобність, рухливість, адгезія, експресія генів

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**Вступ**

Однією з серйозних проблем медицини є поява та розповсюдження антибіотикорезистентних штамів *E. coli*, здатних сприяти гострі та хронічні персистуючі інфекції, зумовлени біоплівками. Недостатня ефективність противимикробної хіміотерапії зумовлена біоплівками. Антибіотикорезистентні штами *E. coli*, здатних сприяти гострі та хронічні персистуючі інфекції, зумовлени біоплівками. Антибіотикорезистентні штами *E. coli*, здатних сприяти гострі та хронічні персистуючі інфекції, зумовлени біоплівками.