N. Hrynchuk[1], T. Bukhtiarova[1], N. Vrynchanu[1], L. Ishchenko[2], E. Vazhnichaya[3]

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

[1]State Institution «Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine», Kyiv

[2]National University of Life and Environmental Sciences of Ukraine, Kyiv

[3]Poltava state medical university

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 lipophilicity, and carbon cage rigidity. As a result, these molecules can easily penetrate biological lipid membranes and often have unique target-specific

380

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

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)

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. *Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393*

381

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 °C 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 OD$_{600}$ 0.18–0.22 (A$_0$). 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 OD$_{600}$ 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 (A$_0$): $100 \cdot [1 - (A/A_0)]$. 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 MgSO$_4$, 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 stabinoculated 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 distillate water to remove non-adherent bacteria. Adherent cells were fixed in place for 15 min with

382

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

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: $OD \leq ODc$ − non-adherent; $ODc < OD \leq 2 \cdot ODc$ − slightly adherent; $2 \cdot ODc < OD \leq 4 \cdot ODc$ − moderately adherent; $4 \cdot ODc < OD$ − 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 $1 \times$ PCR-buffer (Thermo Scientific), 2.5 mM $MgCl_2$ (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 ℃ for 5 min, followed by 45 cycles of 10 s at 95 ℃, 30 s at 56 ℃, and 20 s at 72 ℃, with a final extension step at 72 ℃ for 5 min. For *fimA* PCR parameters was included an initial denaturation step at 95 ℃ for 4 min; 35 amplification cycles of 30 s at 95 ℃, 30 s at 55 ℃, 40 s at 72 ℃ and an extension step of 3 min at 72 ℃; for *papC* − an initial denaturation step at 95 ℃ for 4 min; 35 amplification cycles of 60 s at 95 ℃, 60 s at 65 ℃, 60 s at 72 ℃ and an extension step of 2 min at 72 ℃; for *afa* − an initial denaturation step at 95 ℃ for 5 min; 35 amplification cycles of 60 s at 95 ℃, 30 s at 60 ℃, 180 s at 72 ℃ and an extension step of 7 min at 72 ℃. 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 1

Primers used in the study

Primers	Primers sequences (5′–3′)	Size, bp	Reference
fliC (Fw) *fliC* (Rv)	ATTCCGTTCTTCCCTCGGTG TGGACACTTCGGTCGCATAG	131	[19]
motB (Fw) *motB* (Rv)	GAACTGATGGTCGGAGGGTT GCCTGTTCGGCTTGTTTGTTC	146	[19]
fimA (Fw) *fimA* (Rv)	GTTGTTCTGTCGGCTCTGTC ATGGTGTTGGTTCCGTTATTC	400	[20]
papC (Fw) *papC* (Rv)	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	[20]
afa (Fw) *afa* (Rv)	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTITTGTTCGTCCGCCG	750	[20]
gyrA (Fw) *gyrA* (Rv)	GTCGTGGCGGGAAAGGTAAA CGGCTGGAGAAGCACAGAA	106	[19]

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

383

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 ± m, where M is the mean value and m is the standard error of the mean.

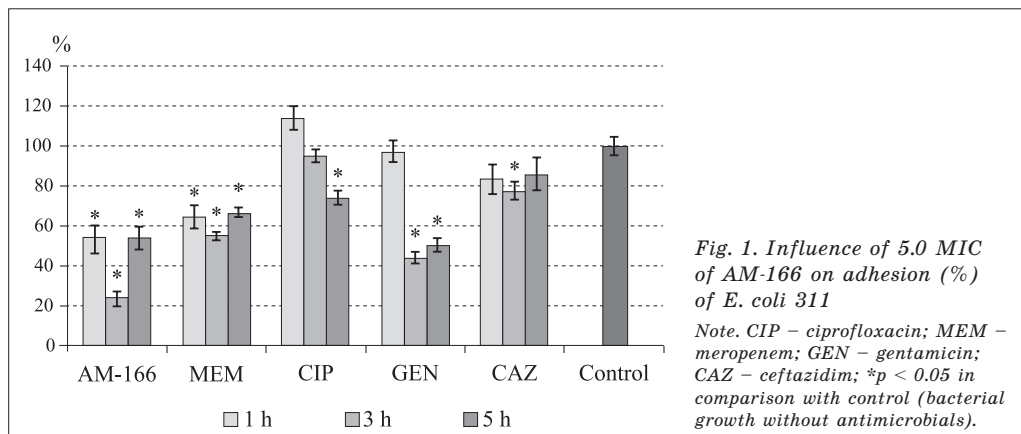**Results.** *Effect of AM-166 on adhesion of E. coli.* Our previous studies have found that the MIC of AM-166 against *E. coli* 311 was 25.0 µg/mL. *E. coli* 311 was a highly adherent strain. The influence of time of pretreatment with AM-166, meropenem, ciprofloxacin, gentamicin and ceftazidime on adhesion ability of *E. coli* was compared with the intact control sample (100 %) (Fig. 1).
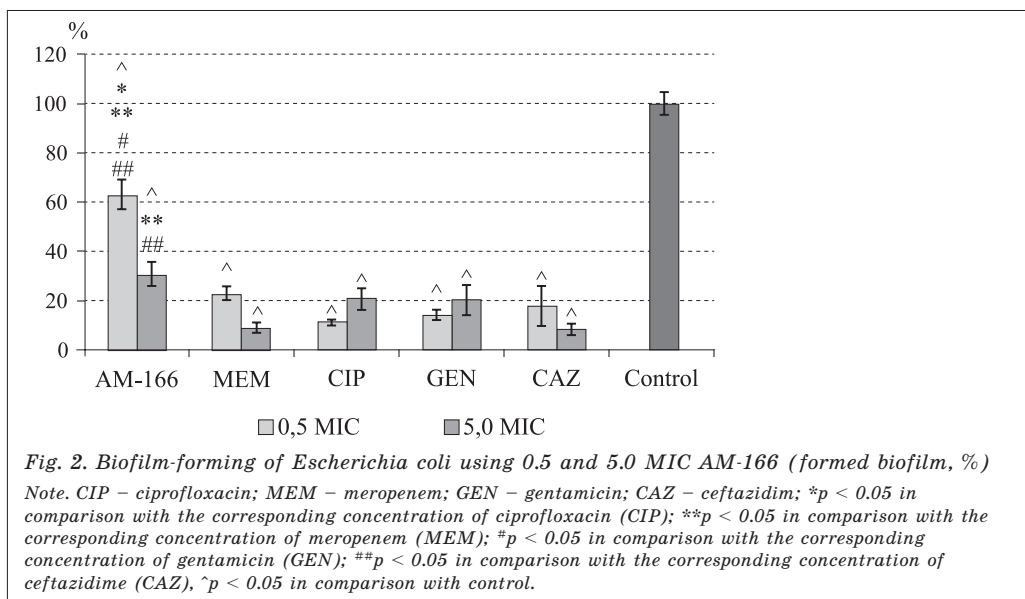
The experiments showed that AM-166 decreased adhesion of *E. coli* cells by 46.0 – 76.4 %, meropenem – 33.1 – 45.0 % after 1, 3 and 5 h of incubation; ceftazidime – 22.3 % after 3 h of incubation, ciprofloxacin – 25.8 % after 5 h of incubation (p < 0.05).

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

Fig. 1. Influence of 5.0 MIC of AM-166 on adhesion (%) of E. coli 311

*Note. CIP – ciprofloxacin; MEM – meropenem; GEN – gentamicin; CAZ – ceftazidim; *p < 0.05 in comparison with control (bacterial growth without antimicrobials).*

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

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.

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 ceftasidim on the hydrophobic properties of *E. 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. 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

Table 2

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

Antimicrobials	Solvent affinity to ethyl acetate (%, mean ± m)	
	0.5 MIC	**2.0 MIC**
AM-166	35.1 ± 6.7	N/A
Meropenem	34.5 ± 5.1	34.1 ± 2.6
Ciprofloxacin	37.8 ± 3.7	34.9 ± 1.7
Gentamicin	35.0 ± 1.8	36.2 ± 2.5
Ceftazidime	53.0 ± 0.2*	54.9 ± 3.2*
Control (without antimicrobials)	39.2 ± 1.2	

Note. *p < 0.05 in comparison with control (bacterial growth without antimicrobials).

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

385

«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 a 2.0 MIC respectively. Pretreatment with cef-

tazidime (0.5 MIC and 2.0 MIC) led to twitching zones induction 1.1-fold and 1.3-fold.

Gene detection and gene expression analysis. The results of PCR-analysis showed that *E. coli* 311 genome contained genes responsible for the motility *fliC*, *motB*, *fimA*, *papC* (Fig. 3).

According to the data obtained, *E. coli* 311 lacks the *afa* gene encode afimbrial adhesins.

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. 4). 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 subinhibitory concentration (p > 0.05).

Table 3

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

Antimicrobials	Motility, mm (mean ± m)			
	Swarming		Twitching	
	0.5 MIC	2.0 MIC	0.5 MIC	2.0 MIC
AM-166	45,2 ± 2,2*	36,5 ± 11,0##	27,7 ± 2,5##	21,0 ± 1,0^, ##
Ciprofloxacin	73,0 ± 1,0^, **, #, ##	35,0 ± 0,7	24,0 ± 2,5##	27,5 ± 4,2##
Meropenem	32,0 ± 9,0*, ##	18,5 ± 1,6##	21,0 ± 1,8^, ##	16,0 ± 0,8^, ##
Gentamicin	15,7 ± 2,0*, ##	14,0 ± 1,0##	27,0 ± 1,3##	30,5 ± 7,3##
Ceftazidime	57,5 ± 6,9^, *, **, #	53,7 ± 10,4^, **, #	42,3 ± 1,6*, **, #	46,0 ± 4,3*,**, #
Control (without antimicrobials)	29,8 ± 1,7		36,8 ± 3,3	

*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 meropenem; #p < 0.05 in comparison with the same concentration of gentamicin; ##p < 0.05 in comparison with the same concentration of ceftazidime.*
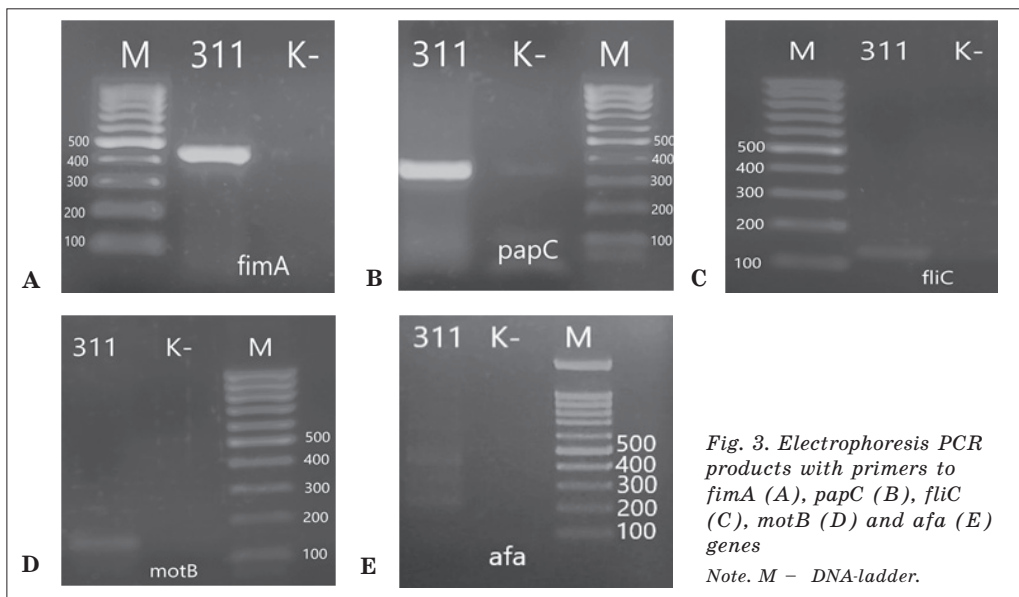
Fig. 3. Electrophoresis PCR products with primers to fimA (A), papC (B), fliC (C), motB (D) and afa (E) genes

Note. M — DNA-ladder.

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].
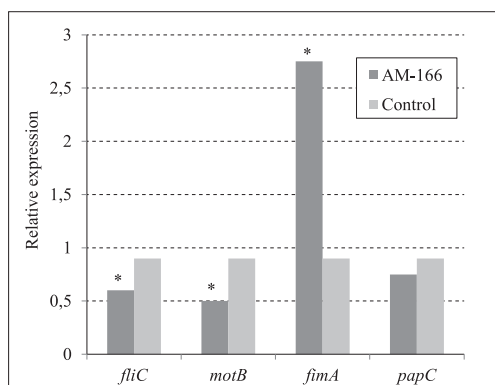

Fig. 4. Relative expression of the genes ($2^{-\Delta\Delta Ct}$) in E. coli exposed to AM-166 (0.5 MIC)
Notes. *$p < 0.05$ in comparison with control (bacterial growth without antimicrobials).

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380—393

387

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, swarming 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, swarming 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,

388

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

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 5th 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].

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

389

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 *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.

1. World Health Organization. Global Action Plan on Antimicrobial Resistance. URL: http://www.emro.who.int/health-topics/drug-resistance/global-action-plan.htmL.

2. World Health Organization. URL: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

3. *Wanka L., Iqbal K., Schreiner P. R.* The Lipophilic Bullet Hits the Targets: Medicinal Chemistry of Adamantane Derivatives. *Chemical Reviews*. 2013. V. 113 (5). P. 3516–3604. https://doi.org/10.1021/cr100264t.

4. Synthesis and Antimicrobial Activity of Adamantyl Substituted Pyridoxine Derivatives. R. Khaziev, N. Shtyrlin, R. Pavelyev et al. *Letters in Drug Design & Discovery*. 2019. V. 16 (12). P. 1360–1369. https://doi.org/10.2174/1570180816666190911150705.

5. Antibacterial activity of adamantyl substituted cyclohexane diamine derivatives against methicillin resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*. K. B. Beena, D. Kumar, W. Kumbukgolla et al. *RSC Advances*. 2014. V. 4 (23). P. 11962–11966. https://doi.org/10.1039/c4ra00224e.

6. Screening a library of 1600 adamantyl ureas for anti-*Mycobacterium tuberculosis* activity *in vitro* and for better physical chemical properties for bioavailability. M. S. Scherman, E. J. North, V. Jones et al. *Bioorg. Med. Chem*. 2012. V. 20 (10). P. 3255–3262. https://doi.org/10.1016/j.bmc.2012.03.058.

7. *Klimochkin Y. N., Shiryaev V. A., Russ M. V.* Antiviral properties of cage compounds. *New prospects. Chem. Bull*. 2015. V. 64 (7). P. 1473–1496. https://doi.org/10.1007/s11172-015-1035-y.

8. *Göktaş F., Vanderlinden E., Naesens L.* Microwave assisted synthesis and anti-influenza virus activity of 1-adamantyl substituted N-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide derivatives. *Bioorg. Med. Chem.* 2012. V. 20 (24). P. 7155–7159. https://doi.org/10.1016/j.bmc.2012.09.064.

9. Discovery and development of SQ109: A new anti-tubercular drug with a novel mechanism of action. K. A. Sacksteder, M. Protopopova, C. E. Barry et al. *Future Micro-biol*. 2012. V. 7 (7). P. 823–837. https://doi.org/10.2217/fmb.12.56.

10. *Вринчану Н. О.* Антибактеріальна дія 4-(адаманти-1)-1-(1-амінобутил) бензолу. *Мікробіологічний журнал.* 2014. Т. 69 (5). С. 42–47.

11. Антибактеріальна активність амінопропанолів з адамантильним і N-алкіларильним радикалом відносно біоплівок *E. coli*. Д. М. Дудікова, Н. О. Вринчану, Ю. В. Короткий, М. Л. Дронова, З. С. Суворова, А. О. Шарова, Н. І. Гринчук та ін. *Фармакологія та лікарська токсикологія*. 2017. № 6. С. 37–42.

12. Антибактеріальна активність 4-(1-адамантил)-(1-амінобутил)бензолу відносно біоплівок *S. aureus*. А. О. Шарова, Н. І. Гринчук, Д. М. Дудікова та ін. *Фармакологія та лікарська токсикологія*. 2018. № 2. С. 79–85.

13. *O'Toole G. A.* Microtiter dish biofilm formation assay. *J Vis Exp*. 2011. V. 47. P. e2437. https://doi.org/10.3791/2437.

14. *Bellon-Fontaine M. N., Rault J., Van Oss C. J.* Microbial adhesion to solvents: a novel method to determine the electron-donor/electron-acceptor or Lewis acid-base properties of microbial cells. *Colloids and surfaces B: Biointerfaces*. 1996. V. 7 (1–2). P. 47–53. https://doi.org/10.1016/0927-7765(96)01272-6.

15. *Fonseca A. P., Sousa J. C*. Effect of antibiotic-induced morphological changes on surface properties, motility and adhesion of nosocomial *Pseudomonas aeruginosa* strains under different physiological states. *Journal of applied microbiology*. 2007. V. 103 (5). P. 1828–1837. https://doi.org/10.1111/j.1365-2672.2007.03422.x.

16. *Kinscherf T. G., Willis D. K*. Swarming by *Pseudomonas* syringae B728a requires gacS (lemA) and gacA but not the acyl-homoserine lactone biosynthetic gene ahlI. *Journal of Bacteriology*. 1999. V. 181 (13). P. 4133–4136. https://doi.org/10.1128/JB.181.13.4133-4136.1999.

17. Adherence of coagulase-negative *Staphylococci* to plastic tissue culture plates: a quantitative model for the adherence of *staphylococci* to medical devices. G. D. Christensen, W. A. Simpson, J. J. Younger et al. *Journal of clinical microbiology*. 1985. V. 22 (6). P. 996–1006.

18. Ідентифікація генів антибіотикорезистентності у *Salmonella spp*. методом полімеразної ланцюгової реакції. Л. М. Іщенко, Л. М. Виговська, В. В. Данчук та ін. *Аграрний вісник Причорномор'я. Ветеринарні науки.* 2019. Вип. 93. С. 284–289.

19. Global gene expression analysis of *Escherichia coli* K-12 DH5α after exposure to 2.4 GHz wireless fidelity radiation. I. H. Said-Salman, F. A. Jebaii, H. H. Yusef et al. *Sci Rep*. 2019. V. 9 (1). P. 14425. https://doi.org/10.1038/s41598-019-51046-7.

20. Detection of pap, sfa, afa, foc, and fim adhesin-encoding operons in uropathogenic *Escherichia coli* isolates collected from patients with urinary tract infection. M. Rahdar et al. *Jundishapur J. Microbiology.* 2015. V. 8 (8). P. e22647. https://doi.org/10.5812/jjm.22647.

21. *Livak K. J., Schmittgen T. D*. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) *Method. Methods.* 2001. V. 25 (4). P. 402–428. https://doi.org/10.1006/meth.2001.1262.

22. *Krasowska A., Sigler K*. How microorganisms use hydrophobicity and what does this mean for human needs? *Front Cell Infect Microbiol*. 2014. V. 4 (112). https://doi.org/10.3389/fcimb.2014.00112.

23. The role of hydrophobicity in bacterial adhesion. R. Oliveira, J. Azeredo, P. Teixeira, A. P. Fonseca. *BioLine*. 2001. P. 11–22.

24. From swimming to swarming: *Escherichia coli* cell motility in two-dimensions. D. F. Blair, J. M. Swiecicki, O. Sliusarenko, D. B. Weibel. *Integr Biol (Camb)*. 2013. V. 5 (12). P. 1490–1494. https://doi.org/10.1039/c3ib40130h.

25. AmrZ Regulates Swarming Motility Through Cyclic di-GMP-Dependent Motility Inhibition and Controlling Pel Polysaccharide Production in *Pseudomonas aeruginosa* PA14. L. Hou, A. Debru, Q. Chen et al. *Front Microbiol*. 2019. V. 10. P. 1847. https://doi.org/10.3389/fmicb.2019.01847.

26. *Beloin C., Roux A., Ghigo J-M. Escherichia coli* biofilms. *Curr Top Microbiol Immunol.* 2008. V. 322. P. 249–289.

27. *Rütschlin S., Böttcher T*. Inhibitors of Bacterial Swarming Behavior. *Chemistry*. 2020. V. 26 (5). P. 964–979. https://doi.org/10.1002/chem.201901961.

28. What are the advantages of living in a community? A microbial biofilm perspective! A. L. S. D. Santos, A. C. M. Galdino, T. P. Mello et al. *Mem Inst Oswaldo Cruz*. 2018. V. 113 (9). P. e180212. https://doi.org/10.1590/0074-02760180212.

29. *Blair D. F*. Fine structure of a fine machine. *J. Bacteriol*. 2006. V. 188 (20). P. 7033–7035. https://doi.org/10.1128/JB.01016-06.

30. Регуляция экспрессии генов бактериальной флагеллы. И. А. Остерман, Ю. Ю. Дихтяр, А. А. Богданов и др. *Биохимия*. 2015. Т. 80, Вып. 11. С. 1662–1672.

31. AmrZ regulates swarming motility through cyclic di-GMP-dependent motility inhibition and controlling Pel polysaccharide production in *Pseudomonas aeruginosa* PA14. L. Hou, A. Debru, Q. Chen et al. *Frontiers in Microbiology*. 2019. V. 10. 1847. https://doi.org/10.3389/fmicb.2019.01847.

32. *Mattick J. S*. Type IV Pili and Twitching Motility. *Annual Review of Microbiology.* 2002. V. 56 (1). P. 289–314. https://doi.org/10.1146/annurev.micro.56.0123.

33. The type 4 pili of enterohemorrhagic *Escherichia coli* O157:H7 are multipurpose structures with pathogenic attributes. J. Xicohtencatl-Cortes, V. Monteiro-Neto, Z. Saldaña et al. *J Bacteriol*. 2009. V. 191 (1). P.411–421. https://doi.org/10.1128/JB.01306-08.

34. *Barnhart M. M., Chapman M. R*. Curli Biogenesis and Function. *Annual Review of Microbiology*. 2006. V. 60 (1). P. 131–147. https://doi.org/10.1146/annurev.micro.60.0808.

35. *Schwan W. R*. Regulation of fim genes in uropathogenic *Escherichia coli*. *World J Clin Infect Dis*. 2011. V. 1 (1). P. 17–25. https://doi.org/10.5495/wjcid.v1.i1.17.

36. *Orndorff P. E., Falkow S*. Nucleotide sequence of pilA, the gene encoding the structural component of type 1 pili in *Escherichia coli*. *J Bacteriol*. 1985. V. 162 (1). P. 454–457. https://doi.org/10.1128/jb.162.1.454-457.1985.

37. *Klemm P*. The fimA gene encoding the type-1 fimbrial subunit of *Escherichia coli*. Nucleotide sequence and primary structure of the protein. *Eur J Biochem*. 1984. V. 143 (2). P. 395–399. https://doi.org/10.1111/j.1432-1033.1984.tb08386.x.

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

391

38. *Schwan W. R., Ding H*. Temporal regulation of fim genes in uropathogenic *Escherichia coli* during infection of the murine urinary tract. *Open Access*. 2017. V. 2017. P. 1–3. https://doi.org/10.1155/2017/8694356.

39. Survival of FimH-expressing enterobacteria in macrophages relies on glycolipid traffic. D. M. Baorto, Z. Gao, R. Malaviya et al. *Nature*. 1997. V. 389 (6651). P. 636–639. https://doi.org/10.1038/39376.

40. *Mulvey M. A., Schilling J. D., Hultgren S. J*. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infection and Immunity*. 2001. V. 69 (7). P. 4572–4579. https://doi.org/10.1128/IAI.69.7.4572-4579.2001.

41. *Donlan R. M*. Biofilms and device-associated infections. *Emerg. Infect. Dis*. 2001. V. 7. P. 277–281. https://doi.org/10.3201/eid0702.010226.

42. *Valen H., Scheie A. A*. Biofilms and their properties. *Eur. J. Oral Sci*. 2018. V. 126. P. 13–18. https://doi.org/10.1111/eos.12425.

43. *Каюков И. Г*. Некоторые вопросы этиопатогенеза, диагностики и тактики ведения пациентов при инфекциях мочевых путей. *Почки и здоровье*. 2011. Т. 15 (1). С. 71–80.

44. *Karami N., Wold A. E., Adlerberth I*. Antibiotic resistance is linked to carriage of papC and iutA virulence genes and phylogenetic group D background in commensal and uropathogenic *Escherichia coli* from infants and young children. *Eur J Clin Microbiol Infect Dis*. 2017. V. 36 (4). P. 721–729. https://doi.org/10.1007/s10096-016-2854-y.

45. Colonization dynamics of ampicillin-resistant *Escherichia coli* in the infantile colonic microbiota. N. Karami, C. Hannoun, I. Adlerberth, A. E. Wold. *J Antimicrob Chemother.* 2008. V. 62 (4). P. 703–708. https://doi.org/10.1093/jac/dkn263.

46. Tetracycline resistance in *Escherichia coli* and persistence in the infantile colonic microbiota. N. Karami, F. Nowrouzian, I. Adlerberth, A. E. Wold. *Antimicrob Agents Chemother.* 2006. V. 50 (1). P. 156–161. https://doi.org/10.1128/AAC.50.1.156-161.2006.

47. *Soto S. M., Jimenez de Anta M. T., Vila J*. Quinolones induce partial or total loss of pathogenicity islands in uropathogenic *Escherichia coli* by SOS-dependent or –independent pathways, respectively. *Antimicrob Agents Chemother.* 2006. V. 50 (2). P. 649–653. https://doi.org/10.1128/AAC.50.2.649-653.

48. *Proft T., Bakerb E. N*. Pili in Gram-negative and Gram-positive bacteria – structure, assembly and their role in disease. *Cell. Mol. Life Sci.* 2009. V. 66 (4). P. 613–635. https://doi.org/10.1007/s00018-008-8477-4.

N. Hrynchuk, T. Bukhtiarova, N. Vrynchanu, L. Ishchenko, E. Vazhnichaya
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

Н. І. Гринчук, Т. А. Бухтіарова, Н. О. Вринчану, Л. М. Іщенко, О. М. Важнича
Антиадгезивні властивості 4-(адамантил-1)-1-(1-амінобутил) бензолу щодо
Escherichia coli

Однією з серйозних проблем медицини є поява та розповсюдження антибіотикорезистентних штамів *E. coli*, здатних спричинити гострі та хронічні персистуючі інфекції, зумовлені біоплівками. Недостатня ефективність протимікробної хіміотерапії за гострих 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-66) на адгезивні властивості *E. coli* та експресію генів, що регулюють адгезію.

Антибіоплівкову активність адамантанвмісної сполуки АМ-166 досліджували методом сорбції 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) разу. За дії АМ-166 реєструється збільшення експресії гена *fimA*, зменшення експресії генів *fliC* та *motB* (у 2 рази). Зниження експресії гена *papC* статистично недостовірне.

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

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

Н. И. Гринчук, Т. А. Бухтиарова, Н. А. Врынчану, Л. М. Ищенко, Е. М. Важничая
Антиадгезивные свойства 4-(адамантил-1)-1-(1-аминобутил) бензола в
отношении *Escherichia coli*

Одной из серьезных проблем медицины является формирование и распространение антибиотикорезистентных штаммов *E. coli*, способных вызвать острые и хронические персистирующие инфекции, обусловленные биопленками. Недостаточная эффективность противомикробной химиотерапии при острых воспалительных заболеваниях, практическое отсутствие препаратов с антибиопленочной активностью актуализирует поиск перспективных соединений и разработку на их основе эффективных и безопасных лекарственных средств.

Цель исследования – изучить влияние 4-(адамантил-1)-1-(1-аминобутил)бензола (АМ-166) на адгезивные свойства *E. coli* и экспрессию генов, регулирующих адгезию.

Антибиопленочную активность адамантансодержащего соединения АМ-166 исследовали методом сорбции генцианвиолета на структурах биопленки, гидрофобность клеточной поверхности *E. coli* оценивали по адгезии к растворителю (тест MATS), swimming-, swarming- и twitching-миграцию – стандартными методами. Адгезию клеток к абиотической поверхности оценивали методом Christensen, влияние АМ-166 на экспрессию генов *fimA*, *papC*, *fliC* и *motB* – с помощью ПЦР в реальном времени.

Показано, что соединение АМ-166 нарушает формирование биопленки *E. coli*, биомасса уменьшается при 5,0 МИК на 69,2 %, при 0,5 МИК на 36,9 %. Установлено, что АМ-166 снижает гидрофобность клеток *E. coli* (на 4,0 % при 0,5 МИК) и адгезию к абиотическому субстрату (на 46,0–76,4 %, в зависимости от времени инкубации), ингибирует twitching-миграцию, стимулирует swarming-миграцию, диаметры зон подвижности *E. coli* увеличились в 1,5 раза (0,5 МИК) и 1,2 раза (2,0 МИК). При действии АМ-166 регистрируется увеличение экспрессии гена *fimA*, уменьшение экспрессии генов *fliC* и *motB* (в 2 раза). Снижение экспрессии гена *papC* статистически недостоверное.

Таким образом, антибиопленочная активность АМ-166 обусловлена нарушением подвижности микроорганизмов и изменением экспрессии генов *fimA*, *fliC* и *motB*. В дальнейшем необходимо изучить влияние производного адамантана на системы Quorum Sensing у *E. coli*.

Ключевые слова: производные аминоадамантана, бактерии, Escherichia coli, биопленка, гидрофобность, подвижность, адгезия, экспрессия генов

Фармакологія та лікарська токсикологія, Том 15, № 6/2021
ISSN 2227-7943. Pharmacology and Drug Toxicology, 2021, 15 (6), 380–393

393