ORIGINAL ARTICLE

ARYLALIPHATIC AMINOALCOHOL DERIVATIVE KVM-194 AFFECTS *E. COLI* LIPOPOLYSACCHARIDE COMPOSITION

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Abstract

The newly synthesized compound KVM-194, a derivative of aryl-aliphatic amino alcohols, possesses inhibitory activity against a broad spectrum of pathogens. The aim of the present study was to investigate the effect of the novel compound KVM-194 on the content and composition of *Escherichia coli* lipopolysaccharide. The obtained data suggest that KVM-194, in the sub-inhibitory concentration, changes the fatty acid and monosaccharide composition of lipopolysaccharide. These alterations may lead to changes in the structure, properties of the outer membrane, cell viability and its susceptibility to antibiotics.

Rezumat

Compusul KVM-194, un derivat aminoalcoolic arilalifatic nou sintetizat, are proprietăți inhibitorii asupra unei game largi de agenți patogeni. Scopul prezentului studiu a fost să investigheze efectul acestui compus asupra conținutului de lipopolizaharide din *E. coli* și a compoziției acestuia. Datele obținute sugerează că KVM-194, administrat în concentrații inferioare celor inhibitorii, produce modificări la nivelul conținutului de acizi grași și monozaharide al lipopolizaharidului. Aceste modificări ar putea conduce la alterări ale structurii și proprietăților membranei, a viabilității celulare și a susceptibilității la antibiotice.

Keywords: *E. coli,* lipopolysaccharide, antimicrobial compounds

Introduction

The World Health Organization considers infectious diseases as one of the three leading causes of death [28].

A special role in the structure of morbidity belongs to gram-negative bacteria, causing severe human diseases. Species of *Salmonella, Shigella, Helicobacter* genera cause gastrointestinal diseases, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Neisseria gonorrhoeae* – predominantly, genitourinary disorders, *Moraxella catarrhalis, Legionella pneumophila, Hemophilus influenzae* – respiratory system diseases, *Neisseria meningitidis* – meningitis [19]. In addition, *Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Burkholderia cepacia, Acinetobacter baumanii* and species of the family *Enterobacteriaceae* are the main causative agents of nosocomial infections that are difficult to treat due to intrinsic and acquired resistance to antibiotics [18]. According to a large-scale monitoring study in Ukraine, most of *P. aeruginosa* isolates are insensitive to ceftriaxone and amoxicillin/clavulanate (84 % for each antibiotic). It was registered *Acinetobacter* spp. resistance to cefoperazone (71 %), ceftriaxone (79 %) and ciprofloxacin (79 %), and complete *Serratia* spp. insensitivity to ceftriaxone, ciprofloxacin and amoxicillin/ clavulanate (100 % of the tested strains) [23].

Among of the reasons of the relatively low susceptibility of gram-negative bacteria to antibiotics are the properties of the cell wall, which contains two lipid bilayers and lipopolysaccharides (LPS). LPS increase the negative charge of the membrane, stabilize it, and exhibit the endotoxin properties in the infected organism: cause the severe inflammation, possibly resulting in septic shock and death [29].

The chemical structure of LPS determines the permeability of the cell wall, and as a consequence – susceptibility of bacteria to antibiotics [3, 9, 12, 17]. Thus, increased susceptibility to beta-lactams in *P. aeruginosa* may be associated with changes in the content of monosaccharides (ribose, mannose, fucose, glucose, rhamnose) [9, 12] and/or fatty acids (dodecanoate, hexadecanoate, 2-hydroxydodecanoate) [12].

The significant role of lipopolysaccharide for bacterial cell integrity, its susceptibility to antibiotics and the development of pathological

process are the ground to study LPS as a potential target for antibacterial agents.

It is known [21] that lipid A, a component of LPS, is a target for antibacterial polycationic natural substances used in the treatment of bacterial infections. It can be explained by the fact, that some bacterial lipids A contain substituents, modifying the biological properties of LPS and whole bacterial cell. For example, substituents at the C4 ′-phosphate glucosamine II are responsible for the resistance of certain bacteria to the polycationic antibiotic, e.g., polymyxin B. If the OH-group at C4 ′-phosphate glucosamine II is not substituted, polymyxin binds to this site, and, thus, acts on bacterium. If the OH-group carries substituents such as 4-amino-4-deoxy-L-arabinose (L-Ara4N), bacterium becomes resistant because polymyxin B can't interact.

It was also determined, that the polymyxin B binding to LPS provides its anti-endotoxin properties [7], but it is not used for this purpose because the required doses are quite toxic. The pharmacophore, responsible for binding to LPS had been found, and a new substance with LPS-neutralizing properties, DS-96, was synthesized [20]. Similar effects were also found in some new antimicrobial peptides [2, 8, 10, 15, 16, 24].

One of the new strategies for searching of antibiotics is the screening for their feature to affect the synthesis of LPS. Thus, the new derivatives of sulphonamides, possessing pronounced antibacterial activity, have been investigated [11]. It is also known that inhibitors of fatty acid synthesis can affect the formation of the appropriate components of LPS [14], and aminoglycoside antibiotics are capable to reduce the LPS content in cells [4, 22] without changing its composition [13].

Our research is devoted to new potent antimicrobial compounds –derivatives of aryl-aliphatic amino-alcohols. The aim of the present study was to investigate the effect of the novel compound KVM-194 on the content and composition of *E. coli* LPS.

Materials and Methods

A strain *E. coli* was isolated from a patient with inflammation and was used for all experiments. The selected strain was susceptible to ceftriaxone, cefotaxime, gentamicin, amikacin, ciprofloxacin and meropenem. 24 h culture was used for all experiments. The minimum inhibitory concentration (MIC) was determined by serial macrodilution method in Mueller-Hinton broth. *Inoculum* density was 10^6 CFU/mL [5].

E. coli cells for analysis were grown for 24 h at 37°C in Mueller-Hinton broth, containing the compound KVM-194 at 0.5 MIC. Control cells were grown under the same conditions in the medium without additives. After the incubation, cells were centrifuged at 5000 g for 15 min, washed with normal saline and then dried by sequential treatment with acetone (twice) and ether (once).

LPS was isolated from dry bacterial mass by the method of Westphal and Jann [25, 27]. LPS purification from nucleic acids was carried by addition of 10 % trichloroacetic acid.

Determination of carbohydrate content was performed according to the method of Dubois et al. [6]. Absorbance was measured by SF-26 spectrophotometer at a 490 nm. Quantitative carbohydrate content was determined using a standard curve for glucose.

To determine the fatty acid composition, samples were mixed with 1.5 % solution of acetyl chloride in methanol and hydrolysed at a temperature of 100°C in sealed ampoule for 4 h [25, 27]. Fatty acid methyl esters were extracted with n-hexane. Analysis of fatty acid methyl esters was carried out by gas chromatography-mass spectrometry system Agilent 6890N/5973 inert (column HP 5MS, 30.0 m × 0.25 mm × 0.25 μm). Temperature range was 150-250 °C, temperature gradient – 4°C/min, carrier gas – helium, flow rate was 1.2 mL/min. Results were evaluated using a standard mixture of fatty acid methyl esters (Supelco, USA).

Identification of neutral monosaccharides was carried after acid hydrolysis of samples for 5 h at 100°C. Processing was performed by the method of Albersheim et al. [1]. The obtained samples contained the polyol acetates of neutral monosaccharides, that were separated by chromato-mass-spectrometry system Agilent 6890N/5973 inert (column DB-225 mS, 30.0 m × 0.25 m × 0.25 mm) with the following conditions: carrier gas – helium, flow rate – 1 mL/min, evaporation temperature – 250°C, interphase – 280°C, the thermostat – 220°C (isothermal mode). The sample was injected with a flow division ratio (1:100). Identification of monosaccharides was performed by comparing the data obtained with the retention time of the standard polyol acetates, and using a computer database ChemStation. The quantitative ratio of individual monosaccharides and fatty acids was represented as a percentage of their total peak areas [25].

Statistical analysis of the results was carried out using Student's t-test.

Results and Discussion

The data obtained suggest that aryl-aliphatic amino-alcohol derivative KVM-194 possesses inhibitory activity against *E. coli*, the MIC being 15 μg/mL. *E. coli* cultivation in the media, containing the sub-inhibitory concentration (0.5 MIC) of the tested compound resulted in 13.89 % decrease of biomass accumulation, 7 % decrease of carbohydrate and 2% decrease of protein content. The LPS output was not

changed significantly in comparison to the control (Table I).

Table I

The effect of KVM-194 on the biomass accumulation, LPS, protein and carbohydrate content in *E. coli* cells

Parameter	Control cells	KVM-194 treated cells
Biomass, g	3.6	3.1
LPS, %	2.1 ± 0.1	2.2 ± 0.1
Carbohydrate content, %	46.0 ± 2.3	39.0 ± 2.0
Protein content, %	10.0 ± 0.5	8.0 ± 0.4

Analysis of fatty acid-containing fractions of *E. coli* and LPS, isolated from cells, was performed. It was shown, that treatment with KVM-194 led to qualitative and quantitative changes in fatty acid composition (Table II, Figures 1-4).

Table II

The effect of KVM-194 on the fatty acid composition of *E. coli* cells and LPS (% of the total peak area)

Fatty acids	*E. coli*		LPS obtained from:	
	Intact cells	KVM-194 treated cells	Intact cells	KVM-194 treated cells
Dodecanoic	4.9 ± 0.24	0.5 ± 0.02	3.4 ± 0.16	1.2 ± 0.06
Tetradecanoic	7.6 ± 0.38	10.2 ± 0.51	20.7 ± 1.03	8.8 ± 0.44
3-Oxytetradecanoic	9.7 ± 0.48	14.9 ± 0.74	54.6 ± 2.73	67.6 ± 3.38
Hexadecanoic	44.6 ± 2.23	54.3 ± 2.71	15.1 ± 0.76	18.3 ± 0.91
Octadecanoic	2.6 ± 0.13	5.6 ± 0.38	-*	-*
Cis-octadecenic	1.9 ± 0.09	2.8 ± 0.14	-*	-*
Trans-octadecenic	12.6 ± 0.63	7.2 ± 0.36	0.5 ± 0.03	2.0 ± 0.1
Unidentified X_1	1.8 ± 0.09	1.4 ± 0.07	5.2 ± 0.26	2.1 ± 0.11
Unidentified X_2	1.1 ± 0.05	3.1 ± 0.13	-*	-*
Unidentified X_3	13.2 ± 0.66	-*	0.5 ± 0.02	-*

* "-" not determined

The data obtained (Figure 1) suggest that the intact *E. coli* cells contain dodecanoic (4.9 %), tetradecanoic (7.6 %), 3-oxytetradecanoic (9.7 %), hexadecanoic (44.6 %), octadecanoic (2.6 %), cis-octadecenoic (1.9 %), trans-octadecenoic (12.6 %) and three unidentified acids.

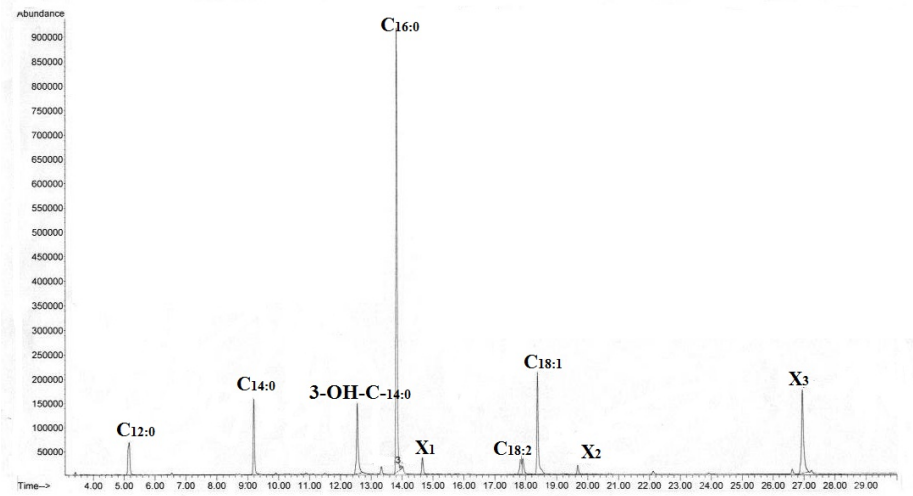

Figure 1.
Chromato-mass spectrogram of *E. coli* fatty acid composition (intact cells)

Incubation of *E. coli* cells in the presence of aryl-aliphatic amino-alcohol derivative KVM-194 (Figure 2) led to the alteration of overall fatty acid composition. It was found a marked decrease of dodecanoate (0.5 %) and trans-octadecenoate (7.2%) content, and increase of the amount of tetradecanoic (10.2 %), 3-oxytetradecanoic (14.9 %), hexadecanoic (54.3 %), octadecanoic (5.6 %) and cis-octadecenoic (2.8 %) acids.

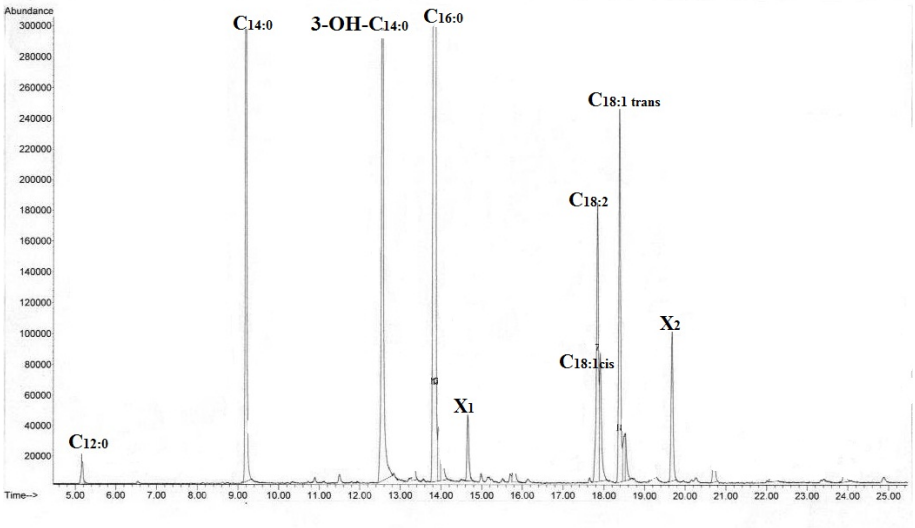

Figure 2.
Chromato-mass spectrogram of *E. coli* fatty acid composition (KVM-194 treated cells)

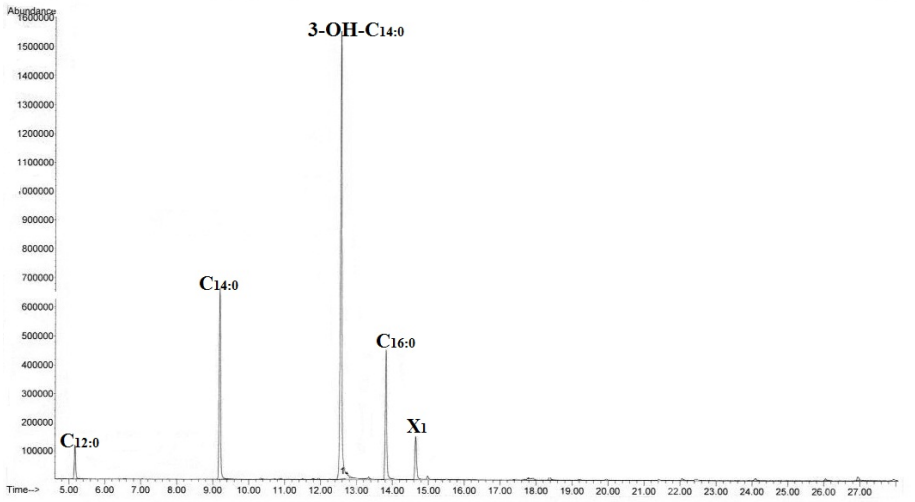

Figure 3.
Chromato-mass spectrogram of fatty acid composition of *E. coli* LPS (intact cells)

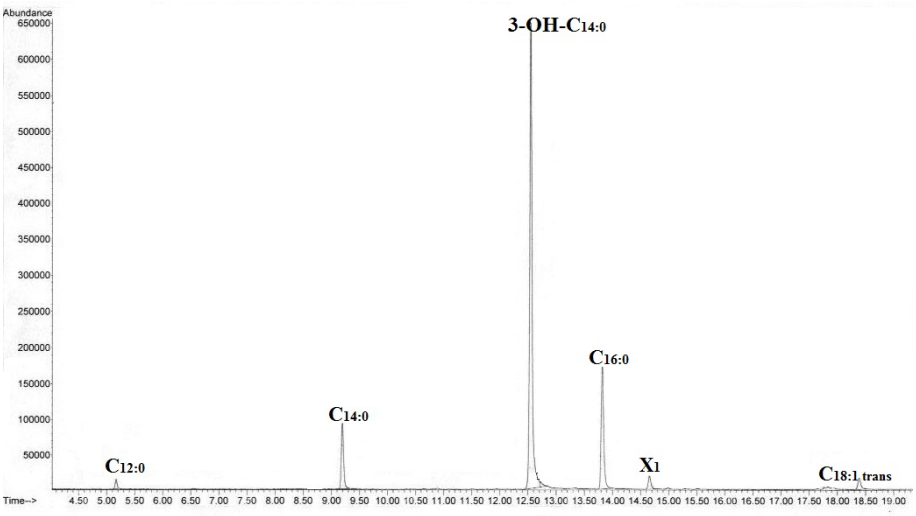

Figure 4.
Chromato-mass spectrogram of fatty acid composition of *E. coli* LPS (KVM-194 treated cells)

LPS isolated from the intact cells (Figure 3) contains dodecanoic (3.4 %), tetradecanoic (20.7 %), 3-oxytetradecanoic (54.6 %), hexadecanoic (15.1%), trans-octadecenoic (0.5 %) and unidentified acids. Analysis of the results indicates a significant impact of KVM-194 on the fatty acid composition of LPS, isolated from treated cells (Figure 4). Reduction of dodecanoic (1.2%) and tetradecanoic (8.8%) acids amount and an increase of the content of 3-oxytetradecanoic (67.6 %), hexadecanoic (18.3 %) and trans-octadecenoic (2 %) acids, as well as one of the unidentified compounds were noted.

For further investigations regarding the influence of aryl-aliphatic amino-alcohol derivative on the LPS composition, monosaccharide content was studied (Table 3, Figure 5, 6).

The predominant monosaccharides in the LPS obtained from intact *E. coli* cells (Figure 5) were glucose (44.7 %) and galactose (34.5 %). Ribose (3.9 %), mannose (3.1%), arabinose (1.1%), xylose (0.7 %), rhamnose (0.4 %), fucose (0.5 %), and heptose (11.1%) were also identified.

LPS, obtained from KVM-194 treated cells (Figure 6) contains reduced amount of mannose (0.9%), galactose (22.7%), glucose (34.7%), heptose (5.5 %) and an increased quantity of ribose (32.6 %), arabinose (2.1%), fucose (0.3 %) and rhamnose (0.5 %).

Table III

The effect of KVM-194on the monosaccharide composition of *E. coli* LPS (% of the total peak area)

Monosaccharide	LPS obtained from:	
	Intact cells	KVM-194 treated cells
Rhamnose	0.4 ± 0.02	0.5 ± 0.03
Fucose	0.5 ± 0.02	0.3 ± 0.01
Ribose	3.9 ± 0.19	32.6 ± 1.63
Arabinose	1.1 ± 0.06	2.1 ± 0.1
Xylose	0.7 ± 0.03	0.7 ± 0.04
Mannose	3.1 ± 0.15	0.9 ± 0.04
Galactose	34.5 ± 1.72	22.7 ± 1.13
Glucose	44.7 ± 2.23	34.7 ± 1.74
Heptose	11.1 ± 0.55	5.5 ± 0.27

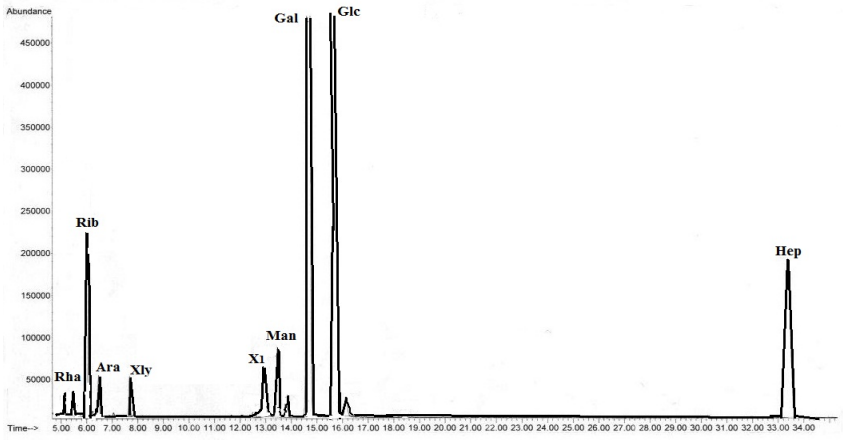

Figure 5.
Chromato-mass spectrogram of polyol acetates of *E. coli* LPS (intact cells)

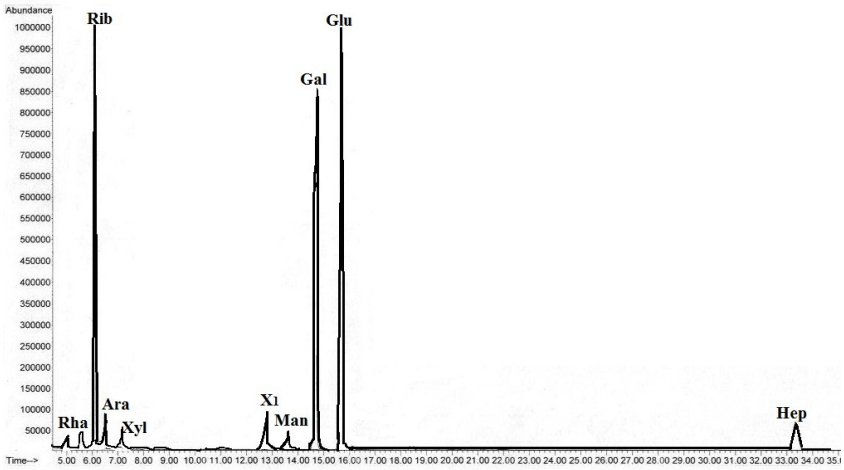

Figure 6.
Chromato-mass spectrogram of polyol acetates of *E. coli* LPS (KVM-194 treated cells)

Conclusions

The obtained data suggest that the aryl-aliphatic amino-alcohol derivative KVM-194, in the sub-inhibitory concentration changes the fatty acid and monosaccharide composition of LPS, which probably, indicate the influence of the studied compound on the processes of its synthesis. These alterations may lead to changes in the structure, properties of the outer membrane, its permeability, cell viability and its susceptibility to antibiotics.

References

1. Albersheim P., Nevins D.J., English P.D., Karr A., A method for analysis of sugars in plant cell wall polysaccharides by gas liquid chromatography. *Carbohydr. Res.*, 1967; 5(3): 340-345.
2. Bagheri M., Keller S., Dathe M., Interaction of W-substituted analogs of cyclo-RRRWFW with bacterial lipopolysaccharides: the role of the aromatic clusterin antimicrobial activity. *Antimicrob. Agents Chemother.*, 2011; 55(2): 788-797.
3. Bryan L.E., O'Hara K., Wong S., Lipopolysaccharide changes in impermeability-type aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, 1984; 26(2): 250-255.
4. Clements J.M., Coignard F., Johnson I., Chandler S., Palan S., Waller A., Wijkmans J., Hunter M.G., Antibacterial activities and characterization of novel inhibitors of LpxC. *Antimicrob. Agents Chemother.*, 2002; 46(6): 1793-1799.
5. Cucu A., Nica M., Ceauşu E., Cioran N., Antimicrobial resistance profile in infectious disease hospital intensive care unit. *Farmacia*, 2014; 62(4): 767-776.
6. Dubois M., Gilles K., Hamilton J., Rebers P.A., Smith F., Colorimetric method for determination of sugars and related substranses. *Anal. Chem.*, 1956; 28(2): 350-356.
7. Durando M.M., MacKay R.J., Linda S., Skelley L.A., Effects of polymyxin B and *Salmonella typhimurium* antiserum on horses given endotoxin intravenously. *Am. J. Vet. Res.*, 1994; 55(7): 921-927.
8. Giacometti A., Cirioni O., Ghiselli R., Mocchegiani F., Orlando F., Silvestri C., Bozzi A., Di Giulio A., Luzi C., Mangoni M.L., Barra D., Saba V., Scalise G., Rinaldi A.C., Interaction of antimicrobial peptide Temporin L with lipopolysaccharide *in vitro* and in experimental rat models of septic shock caused by gram-negative bacteria. *Antimicrob. Agents Chemother.*, 2006; 50(7): 2478-2486.
9. Godfrey A.J., Hatlelid L., Bryan L.E., Correlation between lipopolysaccharide structure and permeability resistance in beta-lactam-resistant *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, 1984; 26(2): 181-186.
10. Gough M., Hancock R.E.W., Kelly N.M., Antiendotoxin activity of cationic peptide antimicrobial agents. *Infect Immun*, 1996; 64(12): 4922-4927.
11. Karlowsky J.A., Saunders M.H., Harding G.A., Hoban D.J., Zhanel G.G., *In vitro* characterization of aminoglycoside adaptive resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, 1996; 40(6): 1387-1393.
12. Kropinski A.M., Kuzio J., Angus B. Hancock R.E., Chemical and chromatographic analysis of lipopolysaccharide from an antibiotic-supersusceptible mutant of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, 1982; 21(2): 310-319.
13. Kusser W.C., Ishiguro E.E., Effects of aminoglycosides and spectinomycin on the synthesis and release of lipopolysaccharide by *Escherichia coli*. *Antimicrob. Agents Chemother.*, 1988; 32(8): 1247-1250.
14. Martin N.L., Beveridge T.J. Gentamicin interaction with *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, 1986; 29(6): 1079-1087.
15. Okuda D., Yomogida S., Tamura H., Nagaoka I., Determination of the antibacterial and lipopolysaccharide-neutralizing regions of guinea pig neutrophil cathelicidin peptide CAP11. *Antimicrob. Agents Chemother.*, 2006; 50(8): 2602-2607.
16. Pulido D., Moussaoui M., Andreu D., Nogués M.V., Torrent M., Boix E., Antimicrobial action and cell agglutination by the eosinophil cationic protein are modulated by the cell wall lipopolysaccharide structure. *Antimicrob. Agents Chemother.*, 2012; 56(5): 2378-2385.
17. Rocque W.J., Fesik S.W., Haug A., McGroarty E.J., Polycation binding to isolated lipopolysaccharide from antibiotic-hypersusceptible mutant strains of *Escherichia coli*. *Antimicrob. Agents Chemother.*, 1988; 32(3): 308-313.
18. Shahinian I.A., Chernuha M.Y., Non-fermentative gram-negative bacteria in the etiology of nosocomial infections: clinical, microbiological and epidemiological features. *Clinical Microbiology and Antimicrobial Chemotherapy*, 2005; 7(3): 271-285.
19. Şerban C., Mitrea N., Ordeanu V., Research on bacterial resistance to antibiotics, in suspicion of nosocomial infections, in major hospitals in Bucharest. *Farmacia*, 2012; 60(3): 350-357.
20. Sil D., Shrestha A., Kimbrell M.R., Nguyen T.B., Adisechan A.K., Balakrishna R., Abbo B.G., Malladi S., Miller K.A., Short S., Cromer J.R., Arora S., Datta A., David S.A., Bound to shock: protection from lethal endotoxemic shock by a novel, nontoxic, alkylpolyamine lipopolysaccharide sequestrant. *Antimicrob. Agents Chemother.*, 2007; 51(8): 2811-2819.
21. Knirel, M.A., Valvano Y.A., Bacterial lipopolysaccharides. Structure, chemical synthesis, biogenesis and interactions with host cells. Springer-Verlag, Wien-N.Y., 2011; 433 p.
22. Six D.A., Yuan Y., Leeds J.A., Meredith T.C., Deletion of the β-acetoacetyl synthase FabY in *Pseudomonas aeruginosa* induces hypoacetylation of lipopolysaccharide and increases antimicrobial susceptibility. *Antimicrob. Agents Chemother.*, 2014; 58(1): 153-161.
23. Sprinsyan T., Rational use of antibiotics today – possibility to save them for future. *Health of Ukraine*, 2012; 8(2): 6-7.
24. Srivastava S., Ghosh J.K., Introduction of a lysine residue promotes aggregation of Temporin L in

lipopolysaccharides and augmentation of its antiendotoxin property. *Antimicrob. Agents Chemother.*, 2013; 57(6): 2457-2466.
25. Varbanets L.D., Zdorovenko G.M., Knirel Y.A., Methods of study of endotoxins. Naukova Dumka Publishing house, Kiev, 2006; 237 p.
26. Nicolae A.C., Mitrea N., Arsene A.L., Constantinescu M.Z., Vuţă V., Drăgoi C.M., *In vitro* P-glycoprotein inhibition assay on N2a murine cell line. *Farmacia*, 2013; 61(3): 481-491.
27. Westphal O., Jann K., Bacterial lipopolysaccharides. Methods of carbohydrates. Mir Publishing house, Moscow, 1975; p. 325-333.
28. World health statistics 2013. http://www.who.int/gho/publications / world_health_statistics/en.
29. Zdorovenko E.L., Pozur V.K., Kucherenko N.E., Biological activity of lipopolysaccharides of gram-negative bacteria. *Biopolymers and the cell*, 2000; 16(2): 5-15.