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Changes in lipid composition of *Escherichia coli* and *Staphylococcus areus* cells under the influence of disinfectants Barez®, Biochlor® and Geocide®

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The purpose of the work was to investigate the qualitative and quantitative composition of *Escherichia coli* and *Staphylococcus aureus* lipids after exposure to disinfectants. **Methods.** Quantitative and Qualitative composition of total lipids and phospholipids were identified using method of thin layer chromatography. Disinfectants Barez® (active ingredients: essential oils, benzalkonium chloride, nanoparticles of argentum), Biochlor® (sodium hypochlorite), Geocide® (benzalkonium chloride; polyhexamethylene guanide hydrochloride, deltametrin), were used according to manufacturer's recommendations. For this test, 0.5 ml of disinfectants (0.1 % Barez®, 0.1 % Biochlor®, 0.5 % Geocide®) were added into 3 ml of cell suspension (5×10^8 cell/ml). Controls were the cells nontreated with disinfectants. Cells suspensions were incubated in standard conditions for 1 hour. **Results.** The influence of disinfectants on the lipid composition of *E. coli* and *S. aureus* after the action of Barez®, Biochlor and Geocide was studied. Changes of quantitative and qualitative composition of the lipids containment were identified. Barez® had the most notable effect on quantitative and qualitative composition of total lipids and phospholipids. It was confirmed that reduction of total quantity of several phospholipids results to increased level of diglycerides. These data allow us to establish the sensitivity of microorganisms to the influence of disinfectants and to determine the optimal concentrations and exposure of disinfectants for high quality disinfection. **Conclusions.** It has been established that the disinfectants Barez®, Biochlor® and Geocide® cause significant changes in the lipid composition of *E. coli* and *S. aureus* cells, which leads to increased fluidity of cell membranes and loss of viability of bacteria.
Keywords: disinfectants; lipids; phospholipids; cholesterol; free fatty acids

Introduction

Development of new efficient disinfectants is an important direction in veterinary medicine. Identification of mechanisms of action of chemical compounds on microorganisms permits to improve and develop disinfectants. Lipids of bacterial membranes are important for this development. They are not only structural components of membranes (Lee,2004; Yeagle, 2005; Richards, 2016), but also have regulatory function in metabolic processes in bacterial cells. For instance, some isoenzymes of protein kinase C are activated by phosphatidyl serine, and phosphatidyl ethanolamine, that permits to open the active center of catalytic sub-unit responsible for completion of protein phosphorylation (DiRusso et al., 1992; Török et al., 2003). Phosphatidylinositol plays key role in cellular signaling pathways of calcium transport (Vaskovsky & Terekhova, 1979). Negatively charged phosphatidyl serine regulates electrostatic interaction between proteins and membranes, and some lipid domains of membranes of temperature sensors during cell reaction on heat stress permitting their survival (Török et al., 2003; Parker, 2004). Therefore, lipids can be a target for disinfectants (Lysytsya, 2015; Souza, 2015; Lysytsia & Rebriiev, 2014; Van Oosten, 2014).

The aim of this work was to identify quantitative and qualitative lipid composition of *E. coli* and *S. aureus* after treatment with disinfectants.

Material and methods

A set of reference test strains of microorganisms *Escherichia coli* ATCC 25922 (F-55), *Staphylococcus aureus* 209-P for bacteriological quality control of disinfection provided by the State Research and Control Institute of Biotechnology and strains of microorganisms.

Disinfectants Barez® (active ingredients: essential oils, benzalkonium chloride, nanoparticles of argentum), Biochlor® (sodium hypochlorite), Geocide® (benzalkonium chloride, polyhexamethylene guanide hydrochloride, and deltametrin), used according to manufacturer's recommendations. For this test 0.5 ml of disinfectants (0.1 % Barez®, 0.1 % Biochlor®, 0.5 % Geocide®) were added into 3 ml of cell suspension (5×10^8 cell/ml). Controls were cells nontreated with disinfectants. Cells suspensions were incubated in standard conditions for 1 hour.

Quantitative and Qualitative composition of total lipids and phospholipids were identified using method of thin layer chromatography using plates "Sorbil" PTSH-AF-A ("Imid Ltd", Krasnodar). Lipids were extracted from the sample using mix of chloroform/methanol (1:1 dilution). Thin layer chromatography of total lipids was conducted in one direction within a system of solvents diethyl hexane ether; acetic acid (85:15:1 dilution) (Kates, 1972). Thin layer chromatography of phospholipids was performed in two perpendicular directions (Vaskovsky & Terekhova, 1979). First system of solvents – chloroform-methanol-benzyl-ammonium (65:30:10:6; v/p). Second – chloroform-methanol-benzyl-acetone-acetic acid-water (70: 30: 10: 5: 4: 1; v/p). After evaporation of solvents, plates were treated with 10 % H_2SO_4 in methanol and heated for 5 min, 180 °C. Obtained chromatograms were scanned. Lipid content was calculated in percent. Statistical analysis was performed using Student t-test (Kovalenko et al., 2017).

Results and Discussion

The changes of the lipid composition of *E. coli* and *S. aureus* after one-hour incubation with disinfectants Barez®, Biochlor® and Geocide® were identified and presented in table 1. Control suspensions of *E. coli*, and *S. aureus* had low level of cholesterol. Cholesterol is the necessary structural content of cell membrane. It stabilizes membrane integrating via its hydroxyl groups with polarized components of phospholipids and sphingolipids. In these cells the main part of structural lipids was presented by phospholipids, diglycerides and triglycerides. Treatment with disinfectants Barez®, Biochlor®, Geocide® results in reduction of the total quantity of phospholipids, cholesterol and triglycerides. Diglycerides and triglycerides are generally main energy storages for prokaryotes and eukaryotes and phospholipids are the main components of membranes. Barez® reduced the total quantity of phospholipids, cholesterol and triglycerides in *E. coli* by 37 %, 53 % and 21 % respectively. Biochlor® reduced these components by 23 %, 27 % and 19 % respectively and Geocide® – by 22 %, 20 % and 6 % respectively. Increased level of free fatty acids and diglycerides was identified. Barez® increased level of free acids and diglycerides by 53 % and 58 % accordingly. Biochlor and Geocide® increased these parameters by 53 %, 37 % and by 113 % and 23 % respectively. *S. aureus* cells demonstrated the similar changes. After Barez® treatment total composition of phospholipids, cholesterol and triglycerides was reduced by 35 %, 21 % and 25 % respectively. While Biochlor® and Geocide reduced these parameters by 40 %, 16 %, 17 % and 26 %, 11 %, 16 % respectively (Table 1).

Table 1. Total lipids in *E. coli* and *S. aureus* after exposure for 1 hour with disinfectants, %, M±m (n=7)

Strain	Disinfectant	Total phospholipids	Mono-glycerides	Cholesterol	Free fatty acids	Diglycerides	Triglycerides
E. coli	Control	38.1±1.3	2.1±0.3	1.5±0.1	1.5±0.2	35.3±2.1	25.5±2.1
	Barez®	24.1±1.5*	2.7±0.2	0.7±0.1*	2.3±0.1*	55.7±1.7*	20.2±1.5
	Biochlor®	29.5±1.3*	2.5±0.1	1.1±0.2*	2.5±0.1*	48.5±1.3*	20.5±2.1
	Geocide®	29.6±1.5*	2.7±0.2	1.2±0.1	3.2±0.3*	43.3±2.1*	23.9±2.0
S. aureus	Control	42.7±2.1	3.7±0.3	1.9±0.2	2.4±0.1	27.5±1.8	21.3±1.7
	Barez®	27.5±2.1*	3.1±0.2	1.5±0.1	3.7±0.2*	43.4±1.5*	15.9±1.1*
	Biochlor®	25.7±1.1*	3.2±0.1	1.6±0.2	3.5±0.1*	43.2±1.2*	17.6±1.2
	Geocide®	31.5±1.3*	3.3±0.2	1.7±0.1	3.0±0.1*	38.9±2.1*	17.9±0.9

Note: * - $p<0.05$ comparing to control.

Fatty acids are included into complex fats such as phospholipids and triglycerides. Metabolites of fatty acids play key role in expression of transcription factors. Transcription factors with fatty acids regulated activity interact with specific promoters of genes due to direct connection with DNA or non-covalent protein-protein interaction (Kates, 1972). Fundamental achievement of recent decade was the discovery that the chemical composition of fatty acid is a key regulator of gene expression control (Gossett, 1996).

It was identified that *E. coli* has inducible systems for oxidation of fatty acids (Gossett, 1996). FaDr protein takes part in regulation of different genes that code enzymes of fatty acid synthesis and degradation. FadR belongs to the class of the receptors of gene transcription genes that participate in transport of fatty acids (DiRusso et al., 1992; DiRusso, 1998; Black, 1994; Jump, 2004).

In *Bacillus megaterium* suppression by fatty acids of DNA interaction of DNA repressor of transcription ion Bm3R1was identified. It is leading to activation of transcription operon coding for hydroxylase of fatty acids (CYP102) (Palmer, 1998). Changes of individual phospholipids are represented in Table 2.

Table 2. Content of individual phospholipids in *E. coli* and *S. aureus* after one-hour exposure to disinfectants, %, M±m (n=7)

Strain	Disinfectant	Phosphatidy-lethanolamine	Phosphati-dylcholine	Phosphati-dylserine	Phosphatidy-linositol	Sphingo-myelin
E. coli	Control	22.6±2.1	33.7±2.3	12.7±1.1	10.9±1.2	16.7±1.3
	Barez®	16.5±0.7*	44.5±2.3*	8.9±0.5*	12.5±1.3	11.3±1.2
	Biochlor®	17.3±1.1*	45.6±1.2*	10.5±0.5*	11.6±1.1	11.0±1.2*
	Geocide®	18.7±0.5	41.8±1.5*	10.3±0.3	12.3±0.5	11.6±0.6
S. aureus	Control	26.2±1.5	34.3±2.1	14.5±1.1	12.2±1.2	14.9±1.2
	Barez®	16.7±1.4*	49.5±1.8*	10.5±0.5*	11.3±0.2	11.9±0.5
	Biochlor®	19.9±0.9*	46.6±1.8*	10.1±0.2*	12.7±1.0	10.5±0.7
	Geocide®	21.1±0.2*	44.5±1.9*	11.1±0.3*	12.1±1.0	11.4±0.3

Note: * - $p<0.05$ comparing to control.

Tested disinfectants reduced quantity of phosphatidyl ethanolamine, phosphatidyl serine and sphingomyelin in *E. coli* and *S. aureus*. However, content of phosphatidylcholine was increased. Barez® decreased the levels of phosphatidylethanolamine, phosphatidylserine and sphingomyelin by 27 %, 30 % and 32 % respectively. Biochlor® and Geocide® reduced quantity of these lipids by 23 %, 17 % and 34 % as well as by 17 %, 19 % and 30 % respectively. But Barez®, Biochlor®, Geocide® increased level of phosphatidyl choline by 32 %, 35 % and 24 % respectively.

Bacterial DNA is connected to bacterial cell membrane. Therefore, the growth of bacterial cell (primary synthesis and DNA replication) is depending on the condition of membrane. Phospholipids are main components of cell membranes that define its physic-chemical properties, permeability for low molecular weight particles, activity of membrane linked enzymes as well as cell signaling (Parsons, 2013). Main phospholipids of membranes are phosphatidylcholine and phosphatidylethanolamine, Cholesterol and sphingomyelin are the components of the lipid raft residing in the external level of plasmatic membrane. These components participate in processes in membrane invagination, endocytosis and cell transduction (Parker, 2004).

Barez® apparently has the most noticeable effect on quantitative and qualitative composition of microorganism. Reduced quantity of phospholipids leads to increased content of diglycerides, possibly due to loss of connection of triglyceride. However, not all phospholipids are changed this way. Content of phosphatidylcholine is increased while content of phosphatidylethanolamine, phosphatidylserine, and sphingomyelin decreased. The level of cholesterol is also reduced. Such changes indicate the loss of integrity of *E. coli* and *S. aureus* (Black, 1994).

Conclusions

Therefore, disinfectants Barez®, Biochlor® and Geocide® induce significant changes of lipid composition in *E. coli* and *S. aureus* leading to increased instability if cell membranes and reduced viability of bacteria.

It was found that among sanitizers under investigation disinfectant Barez® influences qualitative and quantitative composition of general lipids and phospholipids. It causes a decrease in phosphatidylethanolamine, phosphatidylserine and sphingomyelin level by 27 %, 30 % and 32 % respectively. In *E. coli* and *S. aureus* the study medication provokes a decrease in general number of phospholipids, cholesterol and triglycerides by 37 %, 53 % and 21 % respectively. But Barez® caused an increase in the level of phosphatidylcholine by 32 %. As a result, decreased general number of phospholipids brings about an increased level of diglycerides that proves the disinfectant effectiveness.

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