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RESEARCH OF THE INFLUENCE OF DISINFECTANTS ON THE RATE OF ABSORPTION OF OXYGEN BY CELLS OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* BACTERIA

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Summary. The development and implementation of modern complex disinfectants involves the study of their direct effect on the test cultures of microorganisms, most often using *E. coli* and *S. aureus*. It is taken into account several indicators of the viability and resistance of bacterial cells, including the rate oxygen absorption. This is what led to the purpose of research — the study of the effect of disinfectants on the rate of oxygen absorption by the test cultures of microorganisms *E. coli* and *S. aureus*. In the experiments, the effect of disinfectants 'Orgasept', 'Biochlor', 'Asept', and 'Biolong' on the test cultures *E. coli* strain 1257, *S. aureus* strain 209-P from the strains collection of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms has been studied. The rate of oxygen absorption by bacteria cells *E. coli* and *S. aureus* under the influence of disinfectants on them was determined using the polarographic method (Frank et al., 1973). It has been established that solutions of preparations 0.1% 'Orgasept', 0.1% 'Biochlor', and 0.5% 'Asept' are effective disinfectants for *E. coli* and *S. aureus* bacteria. The use of the 'Biolong' drug is less effective than the use of disinfecting drugs 'Orgasept', 'Biochlor', and 'Aseptic'. The sensitivity and resistance of microorganisms to disinfectants have been determined and the optimal effective concentrations and exposures of these preparations have been investigated for their further application in production conditions.

Keywords: test cultures of microorganisms, *Escherichia coli*, *Staphylococcus aureus*, resistance and viability, cell respiration

Introduction. The effectiveness of disinfectants should be tested at the stage of their development and selection of constituent substances, because a significant amount of the proposed disinfectants is toxic, immunosuppressive and causes distant effects on the organism of animals.

In addition, recently more and more strains of microorganisms are resistant to traditional disinfectants. Therefore, when choosing a disinfectant, it is necessary to take into account that the mechanism of its action should be as close as possible to the natural mechanisms of protection of the organism of animals in the process of their growth and development, as well as to strengthen the existing natural mechanisms of protection.

Creation of the complex disinfectants, safe for humans and animals, is a prospect of their widespread use in veterinary medicine. When developing disinfectants it is necessary to conduct qualitative studies of their effects on the organism of animals in order to avoid the risks of suppressive action on the immune system and the occurrence of violations of physiological functions of the animal organism (Kotsiumbas et al., 2010).

For this purpose, additive ingredients are introduced into the composition of disinfectants which are not harmful to animals, but which can inhibit the reproduction of pathogens, exhibit surface-active action, have a prolonged effect of action, in order to avoid multiple disinfection (Kovalenko, 2008; Kovalenko and Nedosiekov, 2011).

A prerequisite for conducting research was the study of the specifics of the metabolism of bacteria cells, namely the active use by cells of certain substrates, as well as oxygen. The presence of oxygen and the rate of its absorption can be one of the important indicators of the viability of bacterial cells, especially for *E. coli* and *S. aureus* (Schnitzer and Grunberg, 1960; Rabotnova and Pozmogova, 1979).

The aim of the study. The purpose of the work was to investigate the effect of disinfectants on the rate of absorption of oxygen by test cultures of microorganisms *E. coli* and *S. aureus*, which may indicate their resistance and viability.

Materials and methods. The following disinfectants 'Orgasept' (active substances: lactic acid, benzalkonium chloride, argentum nanoparticles), 'Biochlor' (sodium hypochlorite), 'Asept' (benzalkonium chloride, silver nanoparticles, essential oils), and 'Biolong' (isopropyl alcohol, quaternary ammonium compounds) were tested. They had been used according to the stuffer.

In the course of research, strains of test cultures *E. coli* strain 1257, *S. aureus* strain 209-P from the collection of strains of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms were used. The cultivation of the cells of these strains was carried out according to the standard method.

The rate of absorption of oxygen by cells was determined using the polarographic method (Frank, 1973). For this purpose, the polarograph LP-7E (Czech Republic) and Clark closed platinum electrode were used. Into a polarographic well (1.5 ml per volume) containing PBS (pH 7.4), 0.6 ml of cell suspension was introduced, and after 3 minutes, 0.1 ml of disinfectant solution was added.

In the second series of experiments, the test cultures of *E. coli* and *S. aureus* cells were incubated at 37 °C for 30 and 60 minutes with the studied disinfectants, which were used according to the stuffer. At the same time, 0.5 ml of disinfectants: 0.1% 'Orgasept', 0.1% 'Biohlor', 0.5% 'Asept', 2,0% 'Biolong' were added to the 3 ml of the initial suspension of cells (5×10^8 cells/ml). As a control there were the test cultures of cells that were not exposed to disinfectants. Right after a certain period of incubation of the test culture of cells with disinfectant, 0.6 ml of suspension was added in a polar graph, and the rate of absorption of oxygen by microbial cells was determined. Statistical processing of the obtained results was carried out using the Student coefficient (Lakin, 1990).

Results. As a result of the studies, it has been found that the rate of absorption of oxygen by cells *E. coli* and *S. aureus* in the ordinary state is practically the same (Table 1).

Table 1 — The rate of absorption of oxygen by bacterial cells after 3 minutes of influence of disinfectants (oxygen nano atoms/min/10^8 cells; n = 5)

Disinfectants, %	*E. coli*	*S. aureus*
Control (without disinfectant)	0.95±0.05	0.98±0.06
'Orgasept', 0.1	0.18±0.09*	0.26±0.07*
'Biochlor', 0.1	0.24±0.08*	0.21±0.08*
'Asept', 0.5	0.19±0.07*	0.22±0.03*
'Biolong', 2.0	0.87±0.02	0.95±0.05

Note: * — $p \le 0.05$ compared to control.

The data shown in Table 1 demonstrate, that the investigated preparations 'Orgasept', 'Biochlor', 'Asept' after 3 minutes began to suppress the absorption of oxygen by cells. At the same time, for *E. coli* cells, this indicator decreased by 81% ('Orgasept'), by 75% ('Biochlor') and by 80% ('Asept').

At the study of *S. aureus* cells in 3 minutes after the administration of the preparations, the rate of absorption of oxygen decreased by 73% ('Orgasept'), 79% ('Biochlor') and 78% ('Asept'). The data obtained indicate that all of the above mentioned disinfectants directly affect the energy metabolism of *E. coli* and *S. aureus.*

The second series of experiments were aimed to determine bacteriostaticity, based on the study of the rate of absorption of oxygen by bacterial cells after incubating them with disinfectants.

Incubation of cells with the test substances for 30 minutes led to significant inhibition of oxygen absorption.

Thus, for example, 'Orgasept' suppressed absorption of oxygen by cells *E. coli* by 89%, and 'Biochlor' and 'Asept' by 91% and 89% respectively (Table 2). In 30 minutes of incubation the above mentioned disinfectants also significantly inhibited the absorption of oxygen by *S. aureus* cells. 'Orgasept', 'Biochlor', and 'Asept' reduced this indicator by 91%, 92%, and 92% respectively, as compared to control (Table 2).

Attention should be paid to the incubation of *E. coli* and *S. aureus* cells with 2.0% 'Biolong'. The ratio of the disinfectant solution to the quantity of cells (by volume) was also 1:6. Such incubation for 30 minutes practically did not affect the rate of absorption of oxygen by cells (Table 2).

Table 2 — The rate of absorption of oxygen by bacterial cells after 30 minutes of influence of disinfectants (oxygen nano atoms/min/10^8 cells; n = 5)

Disinfectants, %	*E. coli*	*S. aureus*
Control (without disinfectant)	0.85±0.01	1.05±0.07
'Orgasept', 0.1	0.09±0.01*	0.09±0.01*
'Biochlor', 0.1	0.08±0.01*	0.08±0.01*
'Asept', 0.5	0.09±0.01*	0.08±0.01*
'Biolong', 2.0	0.78±0.06	0.85±0.03

Note: * — $p \le 0.05$ compared to control.

Incubation of *E. coli* and *S. aureus* cells with test solutions for 60 minutes led to the same inhibition of oxygen absorption as the 30-minute incubation (Table 3).

Thus, for example, 'Orgasept', 'Biochlor', and 'Asept' inhibited *E. coli* cells' respiration by 89%, 90%, and 91% respectively. The respiration of *S. aureus* cells was also suppressed by the drugs 'Orgasept', 'Bioclor', and 'Asept'.

In addition, the degree of inhibition of the rate of absorption of oxygen by above indicated drugs was the same and was about 92%.

Table 3 — The rate of absorption of oxygen by bacterial cells after 60 minutes of influence of disinfectants (oxygen nano atoms/min/10^8 cells; n = 5)

Disinfectants, %	*E. coli*	*S. aureus*
Control (without disinfectant)	0.81±0.09	0.98±0.08
'Orgasept', 0.1	0.09±0.01*	0.08±0.01*
'Biochlor', 0.1	0.08±0.01*	0.08±0.01*
'Asept', 0.5	0.07±0.01*	0.08±0.01*
'Biolong', 2.0	0.52±0.05*	0.59±0.06*

Note: * — $p \le 0.05$ compared to control.

Incubation of cells for 60 minutes with the preparation 'Biolong' also led to a reliable inhibition of the absorption of oxygen. However, the percentage of inhibition was significantly lower (approximately 3 times) than at the use of drugs 'Orgasept', 'Biochlor', and 'Asept'.

Conclusions. Disinfectant solutions 0.1% 'Orgasept', 0.1% 'Biochlor', and 0.5% 'Asept' may be effective disinfectants for *E. coli* and *S. aureus* bacteria. Adding these drugs to a cell suspension practically in the first minutes led to a violation of the process of oxygen absorption by them.

Contact of cells with disinfectants for 30 and 60 minutes caused almost complete inhibition of their breathing. The results of the research also indicate that the use of 'Biolong' in this concentration is less effective than the use of the drugs 'Orgasept', 'Biochlor', and 'Asept'. For the effective use of disinfectants in order to prevent the development of resistance, it is necessary to follow the relevant recommendations.

References

Frank, G. M. (ed.) (1973) *A guide to the study of biological oxidation by the polarographic method [Rukovodstvo po izucheniyu biologicheskogo okisleniya polyarograficheskim metodom]*. Moscow: Nauka. [in Russian].

Kotsiumbas, I. Ya., Serhiienko, O. I., Kovalchyk, L. M., Khomiak, R. V., Kopiichuk, H. T. and Starchevskyi, M. K. (2010) 'Modern means of veterinary disinfection' [Suchasni zasoby veterynarnoi dezinfektsii], *Veterinary Medicine of Ukraine [Veterynarna medytsyna Ukrainy]*, 1, pp. 36–38. [in Ukrainian].

Kovalenko V. L. (2008) 'Disinfectant preparations use actual problems' [Aktualni problemy zastosuvannia dezinfikuiuchykh preparativ], *Veterinary Biotechnology [Veterynarna biotekhnolohiia]*, 12, pp. 78–90. [in Ukrainian].

Kovalenko V. L. and Nedosiekov V. V. (2011) *Methodical approaches to the control of disinfectants for veterinary medicine [Metodychni pidkhody kontroliu dezinfikuiuchykh zasobiv dlia veterynarnoi medytsyny]*. Kyiv. [in Ukrainian].

Lakin, G. F. (1990) *Biometry [Biometriya]*. 4th ed. Moscow: Vysshaya shkola. ISBN 5060004716. [in Russian].

Rabotnova, I. L. and Pozmogova, I. N. (1979) *Hemostatic cultivation and inhibition of growth of microorganisms [Khemostatnoe kul'tivirovanie i ingibirovanie rosta mikroorganizmov]*. Moscow: Nauka. [in Russian].

Schnitzer, R. and Grunberg, E. (1960) *Drug resistance of microorganisms [Ustoychivost' mikroorganizmov k lekarstvennym veshchestvam]*. Moscow: Inostrannaya literatura. [in Russian].