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Development of a Disinfectant Composition Based on Hydrogen Peroxide

G. E. Alpysbayeva,[1] M. R. Turabekov,[1] B. B. Barakhov,[1] Zh. B. Myrzabekov,[1] P. Sh. Ibragimov,[1] O. O. Tagayev,[2] K. D. Alikhanov[1,*] D. D. Narbayeva[1] and A. A. Taipova[1]

Abstract

The article presents the results of laboratory tests of a new disinfectant developed based on hydrogen peroxide to disinfect veterinary inspection facilities. Purpose of the study-creating unique, highly effective, cheap, multifunctional, and environmentally friendly disinfectants is an essential area of research in veterinary sanitation and disinfection of veterinary inspection objects. Preparations of this type include a newly developed disinfectant composition based on hydrogen peroxide. Hydrogen peroxide is a broad-spectrum antimicrobial agent. The scientific novelty of this work lies in creating a disinfectant composition that works comprehensively against microorganisms, and the drug can be used in the presence of animals. As a result of the study, the effectiveness of the new disinfectant was determined by the high bactericidal activity by diffusion into agar (the diameter of the growth inhibition zones is 21 mm for E. coli and 20 mm for St aureus) and at a 0.1% concentration, complete death of E. coli occurs and Staphylococcus aureus for 60 minutes. One of the important criteria for assessing the disinfection effect is the phenolic coefficient, which, for a composition based on hydrogen peroxide, the bactericidal effect of the drug under study exceeds the bactericidal effect of phenol by 24.81 and 20.66 times about E. coli pcs. 1257 and St. aureus 209-P, respectively. The results obtained during the development of modes of use of the disinfectant in laboratory conditions made it possible to preliminarily determine the working concentrations and consumption of the drug per 1 m^2. The product under study amounted to 1-3% concentration with exposures of 30 minutes and at consumption of 0.5 l/m2.

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1. Introduction

Disinfection plays a leading role in the complex of veterinary and sanitary measures aimed at preventing and eliminating infectious diseases of animals and birds. Disinfection is the process of destroying pathogenic microorganisms using chemical or physical agents. Many disinfectants are used alone or in combination (*e.g.*, hydrogen peroxide and peracetic acid) in livestock buildings. These include alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophors, peracetic acid, phenols, and quaternary ammonium.[1-5]

Disinfectants are not interchangeable, and incorrect concentrations and unsuitable disinfectants can lead to excessive costs. Because occupational illnesses among cleaners have been associated with certain disinfectants (*e.g.*, formaldehyde, glutaraldehyde, and chlorine), precautions (*e.g.*, gloves and proper ventilation) should be used to minimize exposure. The main disadvantage of conventional disinfectants is that they are carcinogenic, mutagenic, and teratogenic and cause serious health problems.[6-9]

The effectiveness of disinfection measures depends on the provision of veterinary practice with modern, environmentally friendly disinfectants. However, the volume of preparations for disinfection of objects of veterinary supervision is limited, and their supply to livestock farms is meager.[10-13] Developing new, highly effective, cheap, multifunctional, and environmentally friendly disinfectants is an essential area of

[1] *JSC, Kazakh National Agrarian Research University, Almaty city, Abai Avenue 26, A15C8A3, Republic of Kazakhstan.*
[2] *JSC, West Kazakhstan Agrarian-Technical University named after Zhangir Khan, Uralsk city, Zhangir Khan street, 51, 090009, Republic of Kazakhstan.*
**Email:* kuantar.alikhanov@kaznaru.edu.kz (K. D. Alikhanov)

research in veterinary sanitation and disinfection of veterinary inspection facilities.

It has been determined that disinfectants developed based on only one of the existing chemical groups have yet to have prospects for their wide practical use due to a narrow range of bactericidal properties. Only complex disinfectants have a broad spectrum of antimicrobial action and acquire antitoxic and anticorrosive properties. Based on this, an urgent task of sanitary science is the development of new disinfectants that are equal in their technological features and quality parameters to foreign analogs, have a wide range of antimicrobial effects, are environmentally friendly, and are affordable. Today, a promising direction is the development of disinfectants based on hydrogen peroxide. According to foreign experts, drugs in this group belong to the disinfectants of the 21st century. Using peroxide preparations with increased biocidal activity, a short exposure time, and the ability to decompose into water and oxygen allows you to quickly return premises and equipment to the production cycle without additional neutralization and washing, which provides a significant economic effect.[14,15]

Hydrogen peroxide is a broad-spectrum antimicrobial agent widely used for many years as a preservative, antiseptic, disinfectant, and sterilant. It provides many desirable properties of a microbicide with a unique balance of antimicrobial effectiveness and safety for various applications in human, veterinary, food processing, *etc*. Hydrogen in peroxides (mixtures with buffers, surfactants, *etc*.) can demonstrate enhanced antimicrobial activity, even in lower concentrations than peroxide solutions in water. The non-toxic environmental impact of using peroxide is another advantage because it quickly breaks down into water and oxygen. Aqueous solutions, formulations, and the gaseous form of hydrogen peroxide can exhibit significant differences in their antimicrobial effects, particularly in the various macromolecules that make up microbial structures (such as proteins, nucleic acids, and lipids). The overall effect of hydrogen peroxide significantly reduces any risk of developing resistance to the biocide over time, unlike many other types of anti-infective drugs or even biocides.[14-16,18]

Microbial resistance to peroxide, as with other disinfectants, is primarily due to differences observed in the growth and survival of microorganisms but can be overcome through the correct process and use of products containing hydrogen peroxide. Biocide concentration is an essential variable in these cases. The many advantages of using hydrogen peroxide for antimicrobial applications make it attractive for future and optimal development. The study's objectives were to test a new disinfectant based on hydrogen peroxide and develop technology for its use for preventive disinfection of veterinary inspection objects in laboratory conditions.

2. Materials and research methods

The experiments and methods used for researching laboratory animals comply with the requirements of biological safety and ethical principles of experimentation on animals set out in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1987) (Opinion of the Bioethics Commission Non-Commercial Joint Stock Company "Kazakh National Agrarian Research University" dated October 7, 2021).

The objects of research were known disinfectants and compositions under development. To accomplish the assigned tasks, a set of research methods was used, including the determination of the biocidal properties of the pieces.

Determination of the biological concentration of test microorganisms in a bacterial suspension. Cultures of test microorganisms are subjected to quality control. In particular, immediately before using test cultures for research purposes, it is necessary to ensure that the test strains grown on the nutrient medium are not contaminated with foreign microflora. Each test tube is visually inspected to assess the growth of test strain cultures, and the nature and massiveness of growth and changes in the color of the nutrient medium are taken into account. Microscopy of a Gram-stained smear of grown cultures is performed.

A working suspension of test cultures is prepared from a culture of a given test strain grown on a solid nutrient medium (MPA or casein agar) at 37 °C for 18-24 hours. The culture is washed off from the agar with sterile drinking water to prepare a bacterial suspension. The resulting suspension of microbes is filtered through a cotton-gauze filter and diluted with clean drinking water to a concentration corresponding in turbidity to the optical turbidity standard N20 (corresponding to 2 × 10 microbial bodies in 1 ml).

Determination of the biological concentration of test microorganisms is carried out by the method of serial tenfold dilutions of a suspension of test microorganisms in sterile drinking water, followed by sowing the break into Petri dishes with a dense nutrient medium (casein agar, Endo agar, MPA). After a specific incubation time at the appropriate temperature, the grown colony-forming units CFU are counted, and the number of viable bacteria in one ml of suspension is determined.[17,19,20] *Several methods determined the bactericidal activity of the tested working compositions*: by diffusion into agar using wells and serial dilutions according to the Kulikovsky method.[20]

To determine the antimicrobial activity of the drugs, 1 ml of 2 billion suspensions (in physiological sodium chloride solution) of a culture of microorganisms was added to sterile Petri dishes with a dense nutrient medium. 20-30 minutes after the diffusion of microorganisms, holes with a diameter of 8 mm were cut out on the surface of the inoculated medium at a distance of 4 cm from each other. 0.1 ml of the tested disinfectant was added to the wells. After keeping the cultures of microorganisms in a thermostat at 37 °C for 18-24 hours, the results were taken into account in terms of the size of the zone of growth inhibition of microorganisms around the disks. The effects were assessed as "resistant" (the organism is resistant to the drug's action) when the zone of growth inhibition did not exceed 10 mm; insensitive – 11-14 mm; sensitive - 15-24 mm; highly sensitive – more than 25 mm.

To obtain objective data on the survival of bacteria after exposure to disinfectants, we used the methodological techniques outlined in the work of A.V. Kulikovsky. Author-based research determined the survival rate of the microbial population (in vitro) with the preparation of the corresponding curve.

To determine the bactericidal properties of the drug, 9 ml of 2 billion were taken. Suspensions of microorganisms and 1 ml of a pre-prepared disinfectant solution (0.001%, 0.01%, 0.1%, *etc*.) were added. The rest was periodically stirred in a flask with 99 ml of 0.85% sodium chloride solution (10 2 dilutions), shaken thoroughly, and diluted to 10 4, 10 6. Dilution was carried out to make it convenient to count colonies on Petri dishes. From the last dilution, 1 ml of suspension was taken and placed in a sterile Petri dish (for more excellent reliability, 1 ml per 3 cups) and filled with molten meat-peptone agar, pre-cooled to +45 °C (the words were carefully rotated to distribute the culture evenly). The inoculation of microorganisms was kept in a thermostat. A direct count of the grown colonies was carried out after 48 hours on a light background in passing daylight, and the arithmetic mean was determined. The resulting number was multiplied by the dilution, and the total number of cells was determined.

Based on the results of counting colonies of microorganisms on Petri dishes, a survival curve was constructed, the determination of which allows us to show the dynamics of cell death under the influence of this drug.

Bacterial survival was calculated using the formula:

$V = O \times 100 / K$,

Where:

V – bacterial survival in % of control;

O – number of colonies after treatment with bactericidal preparations;

K is the number of colonies in the control.

Determining the optimal consumption of the disinfectant began with a low drug concentration. To develop disinfection regimes and to test a disinfectant as close as possible to natural conditions, test objects made from structural materials such as wood, galvanized iron, rubber, plastic, glass, and ceramic tiles were used in experiments.

The drug was tested in different concentrations with exposure periods of 10, 15, 20, and 30 minutes, while to study the consumption of the drug, test objects were irrigated at the rate of 0.25 and 0.5 liters per 1 m^2. The criterion for the effectiveness of a disinfectant in disinfecting surfaces is 100% death of test cultures of microorganisms.

The disinfection quality was controlled by examining swabs from test surfaces for the presence of a given test culture. To isolate E. coli, Koda and Endo nutrient media were used, and staphylococcus - 6.5% salt agar. The disinfectant concentration that ensured the disinfection of all test surfaces used in the experiments in the presence of growth in crops on the control test surfaces was considered effective.[18,20]

3. Results and its discussion

Disinfectant compositions have been created using dodecyldimethylbenzylammonium chloride with hydrogen peroxide and sodium hydroxide in various quantitative ratios. The ratio of components and their bactericidal activity are given in Table 1.

According to the research results given in the table, it can be seen that the second test composition with a concentration of up to 37 μg/ml suppressed the growth of Escherichia coli up to 96.6% and staphylococcus up to 97.6%.

As the chemical content increased to 44 μg⁄ml, the suppression of E. coli and staphylococcus growth reached up to 99%. Thus, for further research, the optimal composition of the composition was selected in the ratio of dodecyldimethylbenzylammonium chloride, sodium hydroxide, and hydrogen peroxide - 10.0: 5.0: 22.0 per 100 ml of distilled water. As a result of the experiments, the developed composition was named "Disinfectant composition based on hydrogen peroxide."

Bactericidal property is the main criterion in the development and evaluation of disinfectants and requires careful and comprehensive study. The bactericidal activity of the drug depends on several factors: concentration of the active substance, temperature of the working solution, exposure time, *etc*., during exposure depends on the effectiveness of the disinfectant on a given type of microorganism, the ability to form spores, and other physicochemical factors; An increase in temperature increases

Table 1. Bactericidal activity of a composition based on hydrogen peroxide at various concentrations.

Name of chemicals	Conc. chemical substance. µg/ml	E. coli (1257 pieces)		St. Aureus (piece 209-P)	
		Qty colonies, thousand	% survival	Qty colonies, thousand	% survival
Dodecyldimethylbenzylammonium chloride (80%)	8.0	69.1	83.1	64.7	83.4
	10.0	63.4	76.2	62.4	80.5
	12.0	60.7	73.0	57.6	74.3
Sodium hydroxide (40%)	3.0	54.3	65.3	54.1	69.8
	5.0	49.1	59.0	51.5	66.4
	7.0	43.4	52.2	43.2	55.7
Hydrogen peroxide (40%)	20.0	39.6	47.6	38.3	49.1
	22.0	32.1	38.6	32.5	41.9
	25.0	25.2	30.3	27.8	35.8
Dodecyldimethylbenzylammonium chloride + Sodium hydroxide + Hydrogen peroxide (8:3:20)	31	8.1	9.7	9.6	12.3
Dodecyldimethylbenzylammonium chloride + Sodium hydroxide + Hydrogen peroxide (10:5:22)	37	2.9	3.4	1.9	2.4
Dodecyldimethylbenzylammonium chloride + Sodium hydroxide + Hydrogen peroxide (12:7:25)	44	1.1	1.3	0.9	1.1
Control (saline solution)	-	83.1	100	77.5	100

the rate of growth of microorganisms and their death due to the action of chemical disinfectants; with increasing concentration of the disinfectant, the rate of death of microorganisms increases; And the activity of antimicrobial compounds, as a rule, depends on the pH of the environment. For example, chlorine and iodine-containing disinfectants lose their activity as the pH of the environment increases.

The bactericidal activity of disinfectant compositions was determined by diffusion into agar using wells, according to Kulikovsky. At the initial stage of studying bactericidal activity, studies were carried out to assess the sensitivity of the test microbes being studied to various concentrations developed by diffusion into agar using wells (Table 2, Figs. 1 and 2).

The bactericidal effect was studied using the following test microorganisms:

-Escherichia coli;

- Staphylococcus aureus 209- P (St. aureus).

A daily culture of working strains of test microorganisms was sown on slanted MPA and grown in a thermostat at 37 °C for 18-24 hours.

Table 2. Bactericidal activity of drugs based on hydrogen peroxide using the agar diffusion method.

Test drugs	Concentration, %	Diameter of growth inhibition zones (including holes), mm (M±m)	
		E.coli	St aureus
Disinfectant composition based on hydrogen peroxide	0.1	16±0.1	15±0.2
	0.2	21±0.3	20±0.3
	0.3	28±0.2	27±0.1
Ecodez	0.1	15±0.3	15±0.1
	0.2	20±0.2	19±0.2
	0.3	26±0.7	25±0.5
Control (saline solution)		-	-

Note – "-" absence of growth retardation zone.

According to the data given in Table 1 and Figs. 1 and 2, it can be judged that the diameter of the zones of growth inhibition when using a 0.1% concentration of the drug was 16

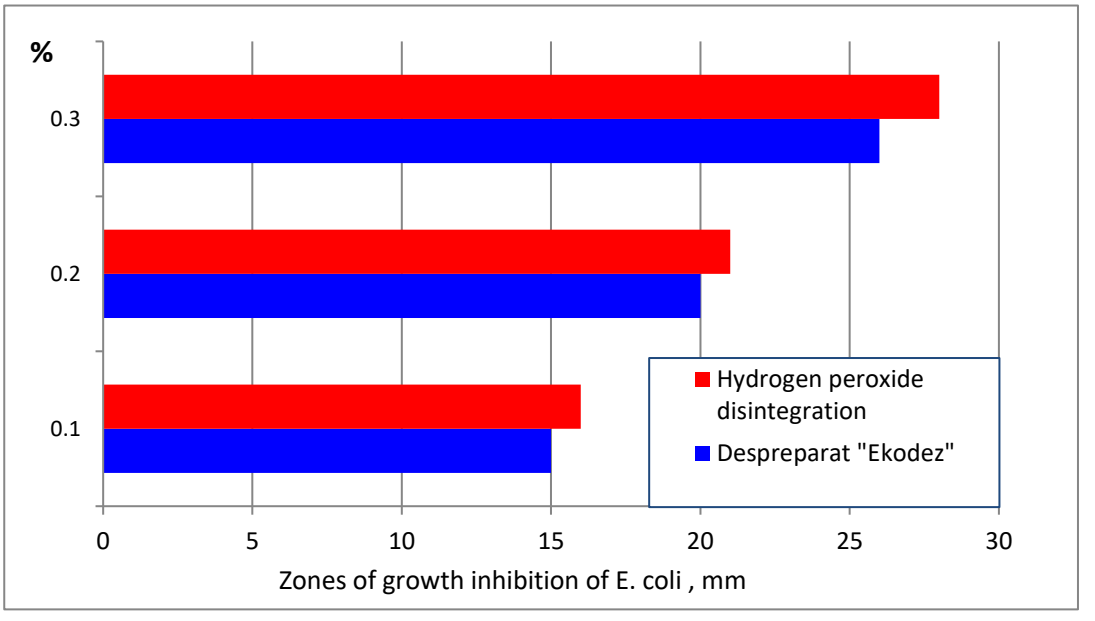

Fig. 1 Bactericidal activity of disinfectants against E. coli.

and 15 mm for Escherichia coli and Staphylococcus aureus, respectively. When using the "Ecodez" medicine, these figures were 15 mm for Escherichia coli and Staphylococcus aureus. The bactericidal activity of a disinfectant composition based on hydrogen peroxide is manifested in a 0.2% concentration, where the diameter of the growth inhibition zones is 21 mm for E. coli and 20 mm for St aureus, and at a 0.3% concentration; these indicators are for intestinal rods were 28 mm, Staphylococcus aureus - 27 mm.

According to the comparative analysis, it is clear that the composition being developed is equal in bactericidal activity to its analog, which indicates the feasibility of further research. In studies on the survival of bacteria after exposure to disinfectants, we used the methodological techniques outlined in the work of A.V. Kulikovsky. Author-based research laid down the definition of the survival of a microbial population (in vitro). The minimum inhibitory concentration of drugs was determined in vitro experiments by serial dilutions followed by plating on meat peptone agar. Table 3 and Figs. 3 and 4 show the results of bactericidal activity.

As a result of bacteriological studies, we have established different degrees of survival of Escherichia coli and Staphylococcus aureus when exposed to the drugs “Disinfectant composition based on hydrogen peroxide” and “Ecodez.” In this case, the drugs were used in 0.1% concentration. Thus, as a result of a 10-minute exposure to a

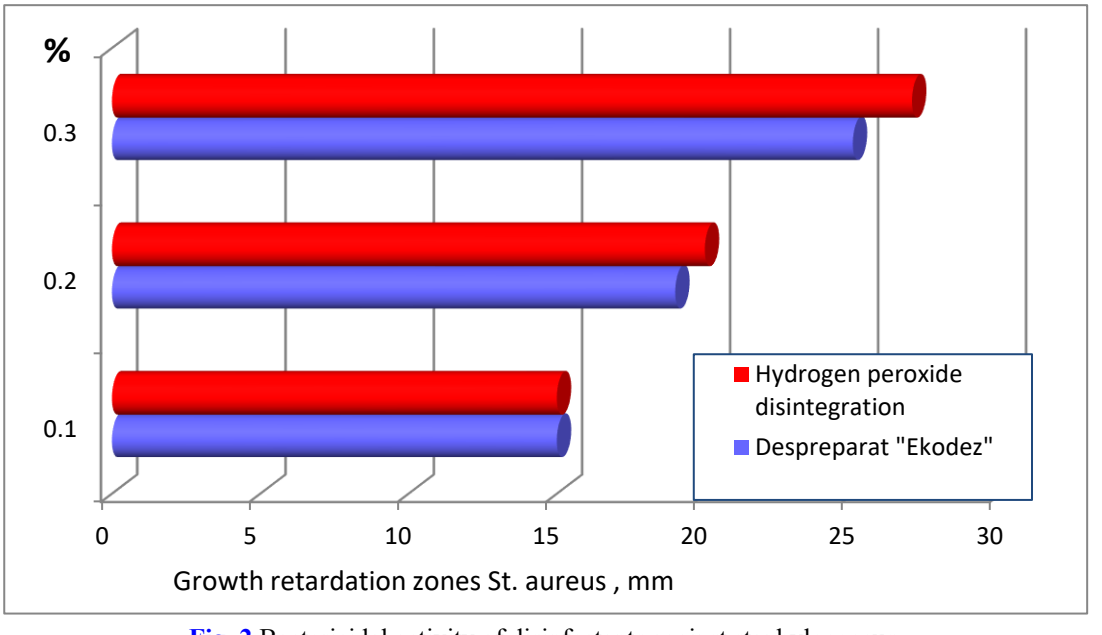

Fig. 2 Bactericidal activity of disinfectants against staphylococcus.

Table 3. Bactericidal activity of drugs based on hydrogen peroxide according to the Kulikovsky method.

Time, min.	Survival of E. coli when exposed to a 0.1% solution		Survival of Staphylococcus aureus when exposed to a 0.1% solution	
	Number of colonies in dilutions 10^{6}, thousand, (M ±m)	Survival rate, %	Number of colonies in dilutions 10^{6}, thousand, (M ±m)	Survival rate, %
Disinfectant composition based on hydrogen peroxide				
10	23.6 ±1.4	45.8	26.6 ±1.5	52.2
30	2.9 ±1.1	5.6	3.7 ±0.6	7.2
60	0 ±0	0	0 ±0	0
Control	51.5 ±2.1	100	50.9 ±2.4	100
Ecodez				
10	30.7 ±2.8	51.3	33.6 ±1.9	57.2
30	4.9 ±1.2	8.2	6.7 ±1.3	11.4
60	2.2 ±0.1	3.7	2.7 ±0.5	4.6
CONTROL	59.8 ±3.1	100	58.7 ±2.8	100

"Disinfectant composition based on hydrogen peroxide," the number of surviving E. coli colonies was 45.8%; after 30 minutes – 5.6%, and after a 60-minute exposure, not a single one survived Colonies of Escherichia coli.

The survival rate of E. coli as a result of exposure to Ekodez is as follows: after a 10-minute orientation, a relatively large number of colonies survived - 51.3%; after a 30-minute exposure - 8.2%, and after 60 minutes, 3.7% of the colonies retained their viability coli.

The bactericidal effect of the studied drugs on Staphylococcus aureus also manifested itself differently and depended on the exposure time. Thus, when exposed to an experimental composition based on hydrogen peroxide, the number of surviving staphylococcus colonies was: after 10 minutes - 52.2%, after 30 minutes - 7.2%, and after 60 minutes, not a single colony of Staphylococcus aureus survived.

The drug "Ecodez" on a culture of Staphylococcus aureus showed that after 10 minutes of exposure, 57.2% of the colonies retained their viability. After 30 - 11.4% and 60 minutes, bacterial survival remained in 4.6% colonies.

Based on the data obtained, survival curves for Escherichia coli and Staphylococcus aureus were compiled, shown in Figs. 3 and 4.

From the given survival curves of microorganisms, the time of initial action (T_{10}) of the drug "Disinfectant composition based on hydrogen peroxide" was found, which was 3-5 minutes for E. coli and for staphylococcus 5-7 minutes and lethal (T_{90}) – 50- 55 minutes for both microbial cultures.

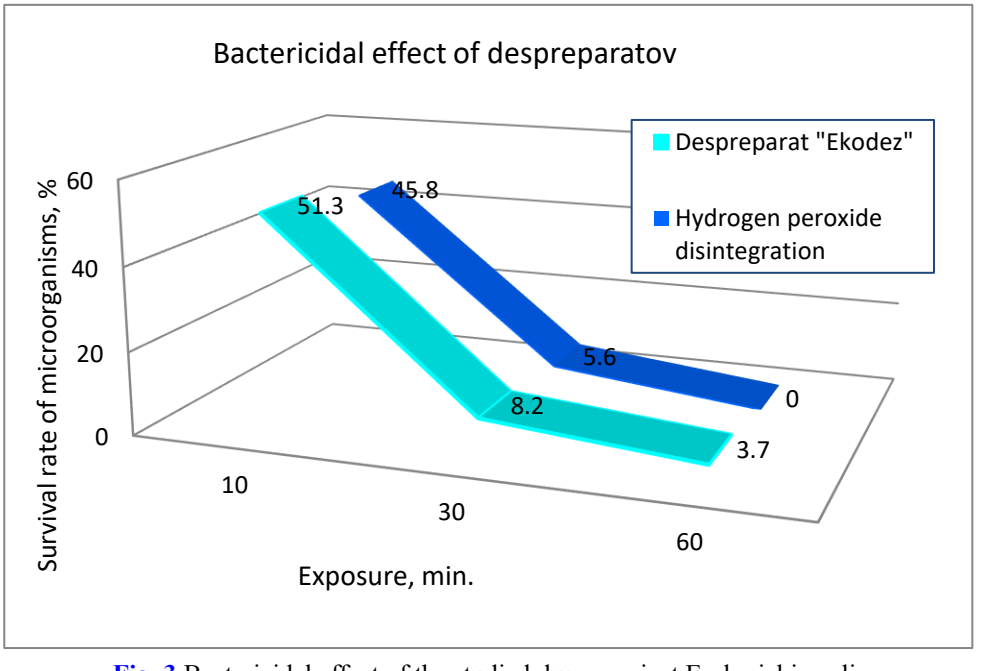

Fig. 3 Bactericidal effect of the studied drugs against Escherichia coli.

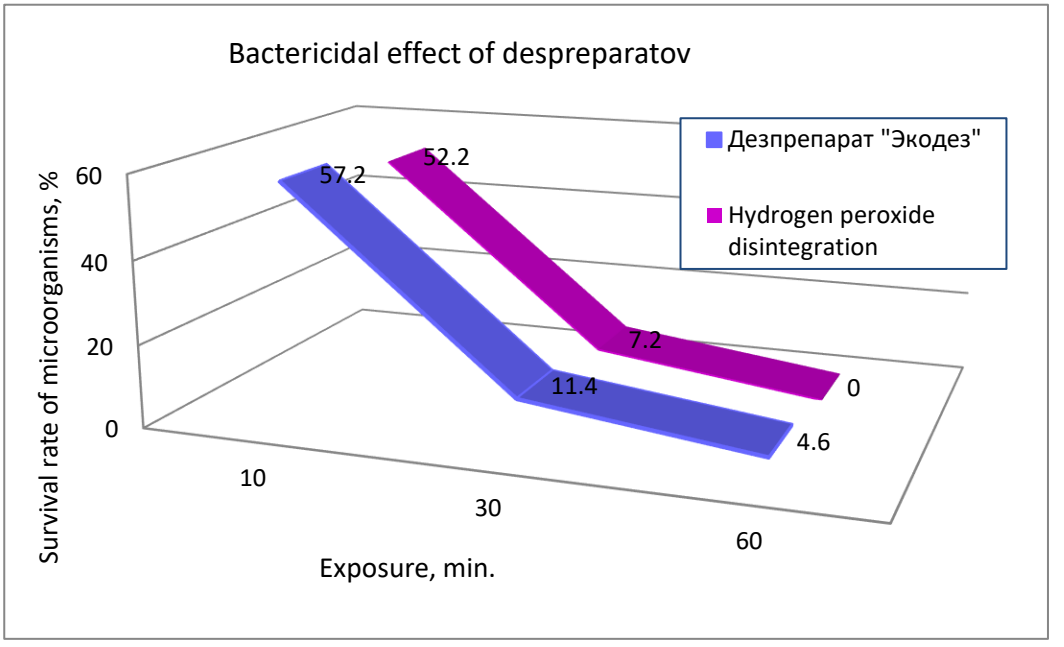

Fig. 4 Bactericidal effect of the studied drugs against staphylococcus.

The initial action time (T_{10}) of Ecodez is 7-9 minutes for E. coli and for staphylococcus - 9-10 minutes, and lethal (T_{90}) - more than 60 minutes for both microbial cultures.

When exposed to the test composition, complete death of E. coli and Staphylococcus aureus occurs within 60 minutes. When exposed to Ekodez at the same concentration, the total end of the bacterium is not achieved within 60 minutes.

Summarizing the data in Table 2 and Figs. 3 and 4, it can be stated that after 3-5 minutes, the drug being developed begins to have a destructive effect on microorganisms, and after 50-55 minutes, 100 % death of both cultures is observed. The picture of the survival of E. coli under the influence of the drug “Disinfectant composition based on hydrogen peroxide” at 10, 30, and 60-minute exposures are shown in Figs. 5, 6, 7, and 8.

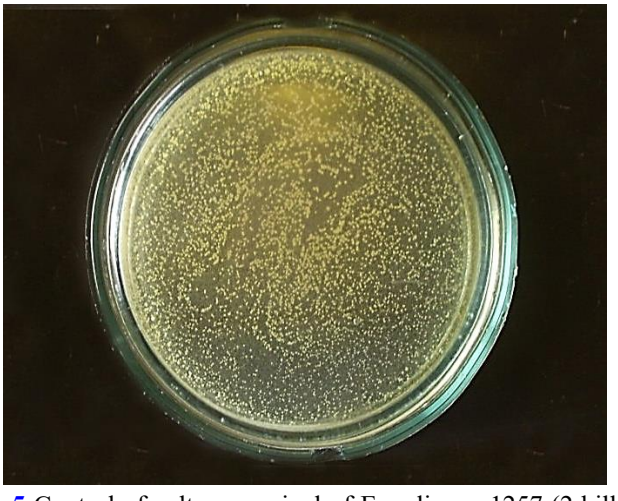

Fig. 5 Control of culture survival of E. coli pcs. 1257 (2 billion suspension).

Fig. 6 Survival rate E. coli pcs. 1257 (2 billion suspension) under the influence of the drug “Disinfectant composition based on hydrogen peroxide” after a 10-minute exposure.

The phenolic coefficient is one of the essential criteria in developing new disinfectants, which reflects the ratio of the concentration of the solution of the test drug to the attention of phenol, which has a bactericidal effect in an equal period and at the same temperature. The method for determining the phenolic coefficient is similar to deciding bactericidal dilution. To obtain accurate data, the experiment was repeated three times with the calculation of the average bactericidal dilution of phenol and the test product at 10 and 30 minutes of exposure. The results of the study are shown in Table 4.

Table 4. The value of the phenolic coefficient of a disinfectant composition based on hydrogen peroxide.

Test culture	Exposure, min	Test drug	Phenol	Phenolic coefficient	Average phenolic coefficient
Intestinal wand	10	1:2834.7	1:137.2	20.66	24.81
	30	1:5566.0	1:192.1	28.97	
Golden staphylococcus	10	1:2024.8	1:98	20.66	20.66
	30	1:3968.6	1:192.1	20.66	

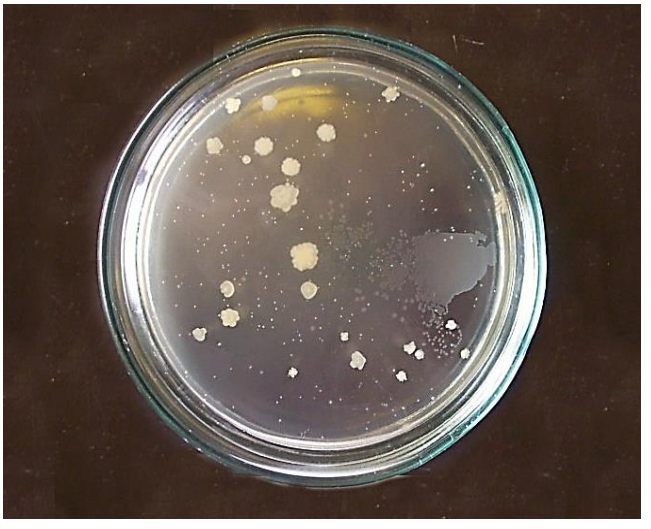

Fig. 7 Survival rate E. coli pcs. 1257 (2 billion suspension) under the influence of the drug "Disinfectant composition based on hydrogen peroxide" after 30 minutes of exposure.

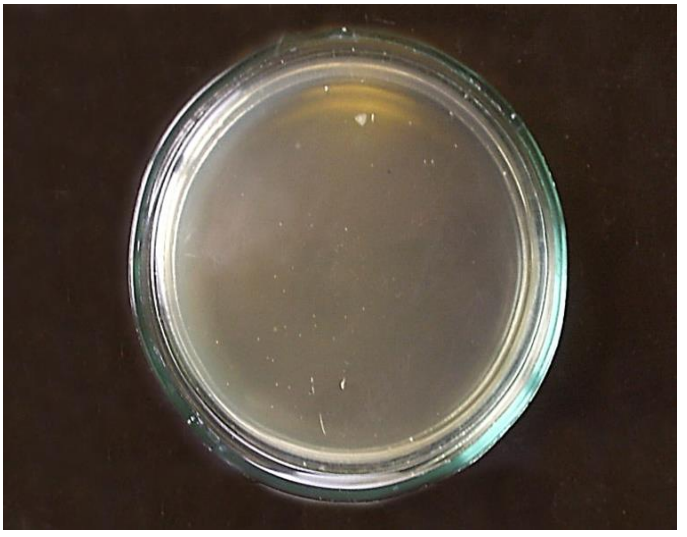

Fig. 8 Survival rate of E.coli pcs. 1257 (2 billion suspension) under the influence of the drug "Disinfectant composition based on hydrogen peroxide" after 60 minutes of exposure.

According to the table, it can be seen that phenol exhibits bactericidal activity against E. coli in dilutions of 1:137.2 and 1:192.1 at ten and 30-minute exposures, respectively, while the bactericidal effect of the test drug against E. coli at ten and After 30-minute exposures, dilutions of 1:2834.7 and 1:5566.0 were observed, respectively.

Hence: $FC_{(10')} = \frac{2834,7}{137,2} = 20,66$; $FC_{(30')} = \frac{5566,0}{192,1} = 28,97$;

$FC_{(average)} = \frac{20,66+28,97}{2} = 24,81$

The bactericidal effect of the tested composition against E. coli exceeds the bactericidal effect of phenol by 24.81 times.

A similar picture is observed in the study of determining the phenolic coefficient in relation to St. aureus – 1:2024.8 and 1:3968.6 at 10 and 30 minute exposures, respectively.

Hence: $FC_{(10')} = \frac{2024,8}{98} = 20,66$; $FC_{(30')} = \frac{3968,6}{192,1} = 20,66$;

$FC_{(average)} = \frac{20,66+20,66}{2} = 20,66$,

The bactericidal effect of the "Disinfectant composition based on hydrogen peroxide" against St. aureus exceeds the bactericidal effect of phenol by 20.66 times.

Fungicidal activity of the disinfectant against Candida spp. presented in Table 5.

Table 5. Fungicidal activity of the disinfectant against Candida spp.

Concentration, %	Exposure, min	Culture sowing rate, CFU/ml
		Candida spp.
Control	5	abundant growth
	15	abundant growth
	30	abundant growth
0.1	5	8980±124.7
	15	6720±103.2
	30	4540±85.5
0.2	5	3560±80.4
	15	2080±56.8
	30	950±42.2
0.3	5	60±5.2
	15	no growth
	30	no growth

Research results indicate that the proposed disinfectant has a pronounced fungicidal effect against Candida spp. At a concentration of 3%, exposure for 15 minutes, fungistatic at 1%, and direction for 5 minutes.

Studies were carried out to determine the preliminary

concentrations of the drug proposed for preventive disinfection to develop regimes for its use in laboratory conditions on test objects.

Experiments were conducted on test objects used in production (wood, galvanized iron, rubber, plastic, glass, ceramic tiles) infected with a two-billion-dollar suspension of 18-hour Escherichia coli and Staphylococcus aureus cultures. Contaminated test objects treated with sterile saline under similar conditions served as controls. After the specified exposure, swabs were taken from the surface of the materials using sterile swabs, which were then placed in test tubes with clean tap water. After 10 minutes, the contents were subjected to bacteriological examination.

The results of developing regimes for using a new disinfectant are presented in Tables 5-6.

When using a 1% concentration (solution flow rate - 0.25 l/m2), the disinfecting effect of the tested disinfection composition begins under the influence of a 30-minute exposure on test objects: galvanized iron, glass, ceramic tiles. Under the result of a 3% concentration, disinfection activity appears with a 20-minute orientation on some surfaces. With a 30-minute exposure, all test objects are disinfected, and the solution is consumed in a volume of 0.25 l/ m2.

Disinfection efficiency increased when the volume of the test product was doubled (solution consumption - 0.5 l/m2). When exposed to a 3% concentration on smooth surfaces (galvanized iron, glass, tiles), a disinfectant effect is observed within 15 minutes of consuming the drug. The results are presented in Table 7.

Table 6. Parameters for using a disinfectant based on hydrogen peroxide against E. coli on test objects.

Test objects	Concentration, %	Presence of microorganism growth (+/-)*							
		10 min		15 min		20 min		30 min	
		Consumption of the drug, l/m^2							
		0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5
Tree	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	-	-	-
	Control	+	+	+	+	+	+	+	+
Galvanized iron	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	-	-	-
	3	+	+	+	-	-	-	-	-
	Control	+	+	+	+	+	+	+	+
Plastic	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	-	-	-
	Control	+	+	+	+	+	+	+	+
Rubber	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	-	-	-
	Control	+	+	+	+	+	+	+	+
Ceramic tiles	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	-	-	-
	3	+	+	+	-	-	-	-	-
	Control	+	+	+	+	+	+	+	+
Glass	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	-	-	-
	3	+	+	+	-	-	-	-	-
	Control	+	+	+	+	+	+	+	+

*Notes: “+” - presence of growth;

“-” - lack of growth of microorganisms.

The studies were carried out at room temperature.

Table 7. Parameters for using a disinfectant based on Staphylococcus aureus hydrogen peroxide on test objects.

Test objects	Concentration, %	Presence of microorganism growth (+/-)*							
		10 min		15 min		20 min		30 min	
		Consumption of the drug, l/m^2							
		0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5
Tree	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	-
	Control	+	+	+	+	+	+	+	+
Galvanized iron	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	-	-	-
	Control	+	+	+	+	+	+	+	+
Plastic	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	-
	Control	+	+	+	+	+	+	+	+
Rubber	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	-
	Control	+	+	+	+	+	+	+	+
Ceramic tiles	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	-	-	-
	Control	+	+	+	+	+	+	+	+
Glass	0.5	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	-	-	-
	Control	+	+	+	+	+	+	+	+

*Notes: "+" - presence of growth;

"-" - lack of growth of microorganisms.

The studies were carried out at room temperature.

As a result of studies carried out in laboratory conditions, it was established that the disinfecting effect of aqueous solutions of a disinfectant is observed after processing all contaminated test objects starting from 3% concentration with an exposure of 20 minutes and at a drug consumption rate of 0.25 l/m^2 for staphylococcus test cultures.

4. Conclusion

Considering the significance of the problem under study, we have designed various combinations of hydrogen peroxide disinfectants ("Disinfectant composition based on hydrogen peroxide"). The bactericidal activity of different chemical compounds and their combinations against Escherichia coli and Staphylococcus aureus were studied to determine the synergism of the constituent components of the developed disinfectant compositions.

When studying the bactericidal properties of the drug using the method of diffusion into agar, the activity of a disinfectant composition based on hydrogen peroxide is manifested in a 0.2% concentration, where the diameter of the growth inhibition zones is 21 mm for E. coli and 20 mm for St aureus, and when using the drug Ecodes these figures were 20 mm for Escherichia coli and 19 mm for Staphylococcus aureus.

Studies on the survival of bacteria after exposure to disinfectants have established varying degrees of survival of Escherichia coli and Staphylococcus aureus when exposed to experimental drugs and their analogs. When exposed to an experimental disinfectant composition at a 0.1% concentration, complete death of E. coli and Staphylococcus aureus occurs within 60 minutes. When exposed to the well-known analog drug "Ecodez" at the same attention and time, an average of 3% of the test retained its viability - microorganisms.

One of the essential criteria for assessing the disinfecting effect is the phenolic coefficient, which was 24.81 for a composition based on hydrogen peroxide about E. coli and 20.66 for Staphylococcus aureus, *i.e.*, The bactericidal effect of the studied drug exceeds the bactericidal effect of phenol by 24.81 and 20.66 times about E. coli pcs. 1257 and St. aureus 209-P, respectively. The phenolic coefficient is the value of the concentration of a solution of any disinfectant per

concentration of a solution of chemically pure phenol, which has a detrimental effect on test microorganisms over an equal period and at the same temperature. Using the phenolic coefficient, it is determined how many times the disinfectant effect of the test product is stronger or weaker than phenol. It has been established that the developed disinfectant exhibits fungicidal activity against Candida spp. At a concentration of 0.3% with exposure for 15 minutes.

The results obtained during the development of modes of use of the disinfectant in laboratory conditions made it possible to preliminarily determine the working concentrations and consumption of the drug per 1 m^2. The product under study amounted to 1-3% concentration with exposures of 30 minutes and at consumption of 0.5 l/ m^2.

Laboratory studies have established that the product based on hydrogen peroxide is an effective disinfectant and can be recommended for further production tests. This study and the results obtained are a good starting point for discussion and other research. For carrying out preventive and forced disinfection in livestock, poultry, fur farms, and road and railway transport while monitoring its quality for isolating coliform bacteria and staphylococci and for forced disinfection at veterinary inspection facilities. For infectious diseases of bacterial and viral etiology and hazardous infections.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

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