The research work has been carried out in the Lithuanian Veterinary Academy, in 1997-2004. Dissertation is presented for equivalent examination.

The right to confer doctoral degree was given to Lithuanian Veterinary Academy together with LVA Veterinary Institute of decision of Government of Lithuanian Republic, No. 926 on 15th July 2003.

Dissertation is written in Lithuanian.

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The abstract of doctoral dissertation has been send on 08th January of 2007 according to confirmed address list.

This dissertation is available at the libraries of the Lithuanian Veterinary Academy and LVA Veterinary Institute.
LIETUVOS VETERINARIJOS AKADEMIIA

Aidas Grigonis

BENZALKONIO IR METENAMINO ANALOGŲ ANTIMIKROBINIO AKTYVUMO NUSTATYMAS IN VITRO IR JO PRIKLAUSOMYBĖ NUO AEROZOLIO DALELIŲ KRŪVIO

Daktaro disertacijos santrauka
Biomedicinos mokslai, veterininė medicina (12B)

Kaunas, 2007
INTRODUCTION

Disinfection is one of the key points in the system of sanitary, anti-epizootic and anti-epidemic measures. It is especially important when a big number of animals are kept indoors. Using antimicrobial agents for animals and birds treatment leads to gradual development of resistant microorganisms that circulate within the farm and cause diseases that are hard to cure.

There is a poor choice of agents that are effective, minimally toxic, environmentally safe after disintegration and can be used when animals are presented in the premises. Earlier halogens (chlorine and iodine compounds), fenols, aldehydes, alcohols, acids and alkali were used for disinfection. Some of the above agents (chlorine compounds, aldehydes, alkali) are good disinfectants but are rather toxic and cause corrosion, which limits their use.

In 1915-1916 quaternary ammonium compounds were first mentioned by W.A. Jackobs. In 1935 G. Domagc described the characteristics of the first synthesized quaternary ammonium salt dodecyldimethylbenzylammomium chloride. But for a long time the compounds of this group were not used. Approximately 20-25 years ago quaternary ammonium compounds gained recognition due to their high disinfecting activity, little toxicity and relatively low self-cost. At present these compounds are considered as disinfectants of new generation becoming more popular than other disinfectants. They are less toxic than halogens and aldehydes and cause significantly less corrosion. Lately attempts are made to synthesize new quaternary ammonium compounds that would possess especially low toxicity and broad activity spectrum.

Quaternary ammonium compounds are used not only as disinfectants, but also as atiseptics and preservative. It is very important that due to lipophylic radicals and positive charge of the molecule they are surface active compounds. Quaternary ammonium salts possess not less than two aliphatic radicals of different length. Changes of percentage of these salts lead to changes of antimicrobic activity spectrum.

For more than two years the laboratory of experimental and clinical pharmacology of Lithuanian Veterinary Academy uses methenamine (hexamethylentetramine) to synthesize new promising quaternary ammonium compounds, evaluates their antimicrobial activity and toxicity.

That's why the aim of this study is to determine the antimicrobial activity of new methenamine (hexamethylentetramine) quaternary ammonium compounds in vitro and its dependence upon the chemical structure of the compound. To determine the efficiency of quaternary ammonium compound-based solutions for disinfection.

The aim of the study
To determine the antimicrobial effect of new methenamine (hexamethylentetramine) quaternary ammonium compounds in vitro and its dependence upon the chemical structure of the compound. To determine the efficiency of quaternary ammonium compound-based solutions for disinfection.

Goals of the study
1. To evaluate the antimicrobic activity in vitro of newly synthesized quaternary ammonium compounds against microorganisms. To determine the parameters of activity and efficiency of the most effective compounds upon the chemical structure of the compound.
2. To compare the antimicrobic effect of newly synthesized quaternary ammonium compounds and benzalkonium chloride.
3. To determine toxicity parameters of the selected most effective compounds and to compare them with benzalkonium chloride.
4. To create quaternary ammonium disinfecting solutions and to determine their effectiveness for disinfection of premises using aerosols and electro-aerosols.

Novelty and practical importance of the study
Antibacterial effectiveness in vitro of the original quaternary ammonium compounds that were synthesized in the Laboratory of Biologically active substances was determined and the dependence of this activity upon chemical structure of the compound was established. Also generalisations have been made concerning the regularity of this dependency, the effectiveness of the compounds was compared to benzalkonium chloride, their advantages and disadvantages were discussed. Acute toxicity of the most effective compound was established and compared to that of benzalkonium chloride.

It was found that these compounds showed good antibacterial activity against Gr+ and Gr- bacteria and low toxicity, thus this original data was summarized in the patent Nr. 4712.

For the first time it was found that upon disintegration of quaternary methenonium compounds new quaternary ammonium compounds, aldehydes and ammonia are produced. The first two of the three show further antimicrobial activity.

Using quaternary ammonium salts and chlorhexidine a biocide for disinfection was created. The created biocide was tested for effectiveness when used for disinfection of air in the premises, horizontal and vertical surfaces. The compound was used in the form of aerosols and electro-aerosols. The research data showed that strong concentrations of Dezinfektas IV are necessary (up to 30%), but small amount of the solution per volume is enough
(20-30 ml/m³). Ten times higher concentration is needed for destruction of Gr- bacteria, than for destruction of Gr+ bacteria.

Company „Ruvera“ is planning to manufacture the biocide.

The structure and size of the study
The work is written in Lithuanian. It consists of introduction, bibliography, evaluation of methods and data, the results of own investigation, their discussion and conclusion. Bibliography includes 239 publications (101 patents among them). 14 tables and 20 pictures are presented in the work.

RESEARCH METHODS

Time, location and conditions of the research
The scientific research was performed in 1997-2004 at the laboratory of experimental and clinical pharmacology of Lithuanian Veterinary Academy, vivarium of Lithuanian Veterinary Academy and Veterinary Institute of Lithuanian Veterinary Academy. Evaluation of newly synthesized quaternary ammonium compounds in vitro was performed. The most effective compounds were selected and compared to benzalkonium chloride. Acute toxicity of the most effective compound was determined. Effectiveness of disinfectants (Dezinfektas II and Dezinfektas IV) for disinfection of air and surfaces in the premises was evaluated.

Materials and compositions of test solutions
Quaternary hexamethylenetrammonium compounds with acetylen fragment (U-10), aminoacetamide fragment (U-99) and oxymethylated group of aminoacetamide fragments (U-77).

Dezinfektas II solution consists of alkyltrimethylbenzylammonium chloride (40.0 g), eugenol (5.0 g), benzylbenzoate (10.0 g), formaldehyde (100.0 g), isopropyl alcohol (405.0 ml) and purified water (ad 1000.0 ml).

Dezinfektas III solution consists of alkyltrimethylbenzylammonium chloride (40.0 g), tymol (5.0 g), benzylbenzoate (10.0 g), formaldehyde (100.0 g), ethanol (405.0 ml) and purified water (ad 1000.0 ml).

Dezinfektas IV solution consists of alkyltrimethylbenzylammonium chloride (15.0 g), chlorhexydine bigluconate (15.0 g), emulgator of non-ionic origin (15.0 g), eugenol (2.5 g), ethanol (150.0 ml) and purified water (ad 1000.0 ml).

For evaluation of antimicrobic effectiveness of the compounds the cultures of E.coli ATCC 25922, Salmonella cholerae suis, Salmonella enteritidis Gartneri, Streptococcus agalactiae, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa (strains L001, L002, L003, L004) were used.

Methods in vitro
Sensitivity of microorganisms to the tested compounds was performed using the agar gel diffusion method. Plates with microorganism broth cultures were kept in a thermostat at the temperature of +37°C for 18-24 hours. Antimicrobial effectiveness was evaluated by measuring the diametre of the sterile zone in millimeters.

Acute toxicity testing method
White mice of both sexes with a weight of 20-22 g were used to determine parameters of the acute toxicity of the test substances. The mice were divided into groups, 6 animals in each. With the help of the tube different amounts of substance were administered to the stomach of mice of each group. The mice were monitored for 5 days after the oral administrations, the time of dying was registered and the dead mice were dissected. Acute toxicity parameters of the test substances were calculated using Leachfield – Wilcoxon method.

Method of room air disinfection
When evaluating the effectiveness of the Dezinfektas II we didn’t spray bacteria in the air for pre-contamination – we examined changes of natural air microflora taking samples prior and after the disinfection. We used R. Koch method for investigation. The solution of Dezinfektas II was sprayed in the form of polydisperse aerosol, the size of aerosol particles being 9-150 µm. The solution was sprayed by means of general-purpose electro-aerosol device UEA-5 (Dobilas, 2001) through electro-aerosol atomizer UEP-2 (copyright certificate No. 854402, Dobilas, 1981) as componential part of this device. For evaluation we estimated the number of bacteria per 1m³ of air using teh following formula:

\[ n_0 = 636 \left( \frac{a \cdot t}{r^2} \right) \]

where:
- \(a\) - the number of bacterial colonies growing on the surface of the medium in the Petri plate;
- \(t\) – time in minutes;
- \(r\) – radius of the Petri plate, cm.

The disinfecting activity of Dezinfektas IV can be evaluated via the testing of the bacterial impurity alterations in the closed room. Before the disinfection the premises were sprayed over with bacterial biomass, conformity made up 4 McF. Biomass density was determined with the help of colorimeter MCI – 5 (Latvia). To spray over the premises the active biomass solutions of E. coli ATCC 25922 and S. aureus ATCC 25923 was used a general-purpose electro-aerosol device UEA – 5 with electro-aerosol atomizer UEP -2. The room disinfection was performed using 9-150 µm particle
electro-aerosols with the help of electro-aerosol device UEA-5 and electro-aerosol atomizer UEP-2. The electro-aerosol particles of the desinfectant were charged with positive and negative charges. The evaluation of air bacterial contamination before and after disinfection was performed using Krotov’s device for bacterial sedimentation. An electric pump absorbs the air through the opening and it passes through the air measurer. This makes possible to estimate the amount of air that passes by a Petri plate that is placed inside the device. The increased amount of colonies of 1 m³ of air is being calculated in accordance with the following formula:

\[ h_b = \frac{(a \times 1000)}{V}, \]

where

- \( h_b \) – the increased bacterium colonies amount of 1 m³ of air;
- \( a \) - bacterium colonies amount, increased in Petri plate;
- \( V \) - The amount of air in litres, pumped through the device;
- 1000 – Constant figure, necessary for the recounting.

At the beginning of pumping process the measurer was fixed so that 30 litres of air were being pumped within a minute.

The air was sampled at once after the bacteria were sprayed and just at once after that the Dezinfektas IV solution was sprayed. After the Dezinfektas IV solution was sprayed over the premises the air was tested after 30 minutes and after an hour has passed.

McConkey agar is used for the experiments with *E. coli*, and peptone meat agar is used for the experiments with *S. aureus*, which contains 8,5% sodium chloride. On the same day Petri plates with the samples of tested air were put into the thermostat for 24 hours with the temperature of 37ºC. The result can be evaluated by the amount of grown bacterial colonies in Petri plate.

**Methods of surface disinfection**

Using the Dezinfektas II and Dezinfektas IV room surfaces were artificially contaminated spraying bacteria by means of general-purpose electro-aerosol device UEA-5 through electro-aerosol atomizer UEP-2. The biocide Dezinfektas II was tested using only *E. coli ATCC 25922* biomass, while testing Dezinfektas IV was performed using *E. coli ATCC 25922* and *Staphylococcus aureus ATCC 25923* biomass.

Prior to disinfection and 2 hours after it sterile cotton swabs were used to collect samples from the surfaces and these samples were examined in the laboratory, counting growing colonies on the medium.

**Statistical analysis**

The results were evaluated using computer programs “Microsoft Exel 97” and Graph Prism TM version 2.0”. Arithmetical averages (X), average error margins (Sx) and reliability quotient of average discrepancies (p) were estimated. Discrepancy of arithmetical averages was considered reliable with p<0.05.

**RESEARCH RESULTS**

**Examining methenamine quaternary ammonium compounds in vitro**

Antimicrobial effectiveness of 48 newly synthesized quaternary ammonium compounds was established and compared to benzalkonium chloride. Several groups of compounds were examined (code names of the groups were “X”, “P”, “S”, “U”), but the group of methenamine (hexamethylenetetramine) quaternary ammonium salts (code name “U”) was the most promising.

Once it was established that the examined compound of the “U” group had poor antimicrobial activity (had antibacterial effect only in concentrations of 5-10%) we stopped the production and testing of the compound. We chose 0.5% and 1% concentrations to survey the antimicrobial activity of a substance. Out of 20 compounds of the “U” group we selected the most effective ones – U-10, U-77 and U-90. We used them for further research. We used benzalkonium chloride as a control for comparison.

![Activity of 0.5% solutions in vitro](image)

1 pic. Activity of 0.5% solutions *in vitro*

Research with the selected 0.5% solutions revealed (pic. 1) that *E. coli* growth was the best inhibited by benzalkonium chloride, the worst - by U-
10 compound, the action on the latter being by 29.9% weaker than that of benzalkonium (p<0.05). U-77 inhibited *E. coli* growth by 12.7% worse and U-90 - by 13.1% worse than benzalkonium chloride (p<0.05). *Streptococcus agalactiae* growth was the best inhibited by U-77 (p<0.05). The activity of U-10 against *Streptococcus* was by 10.2% and of benzalkonium chloride by 12.3% weaker than that of U-77 (p<0.05). The least sensitivity of *Streptococcus* was displayed to U-90 compound – the latter inhibited their growth by 39.1% less than U-77 (p<0.05).

U-77 was the best for inhibiting *S. aureus* growth (pic. 1). U-10 and benzalkonium chloride showed slightly lesser effect. U-10 inhibited *S. aureus* growth by 0.4% less than U-77 (p<0.05). The activity of benzalkonium was 9.8% less than that of U-77 (p<0.05). Like *Streptococcus agalactiae*, *S. aureus* was the least inhibited by U-90 - the effectivity of the latter was by 17.8% less than that of U-77.

*Salmonella* spp. growth was the best inhibited by U-77. Benzalkonium chloride inhibited growth of *Salmonella cholerae suis* by 51.7% and *Salmonella enteritidis Gartnerii* – by 50.5% less than U-77 (p<0.05). The inhibiting effect of U-90 against *Salmonella cholerae suis* and *Salmonella enteritidis Gartnerii* growth was by16.8% less than that of U-77 (p<0.05). The inhibiting effect of U-10 was by 23.2% (*Salmonella cholerae suis*) and by 33.6% (*Salmonella enteritidis Gartnerii*) less than that of U-77.

The research using 1% quaternary ammonium salts showed that U-77 had the best effect against all bacteria species (pic. 2). U-77 inhibited *E. coli* growth by 1.9% better than benzalkonium chloride. U-10 inhibited *E. coli* growth by by 20.7% and U-90 – by 7.8% less than U-77 (p<0.05). U-77 inhibited *Streptococcus agalactiae* growth better than U-10 by 13%, than benzalkonium chloride by 15.2% and than U-90 by 31.3% (p<0.05).

U-77 and U-10 had equal effect on *S. aureus* (pic. 2). Benzalkonium chloride had lesser effect on staphylococcus by 9% and U-90 - by 11.4% compared to U-77 and U-10. Effect of benzalkonium chloride against *Salmonella cholerae suis* was by 47.5% lesser than that of U-77, by 29.7% lesser than that of U-10 and by 41% lesser than that of U-90 (p<0.05). Benzalkonium chloride inhibited growth of *Salmonella enteritidis Gartnerii* by 47.5% less than U-77, by 38.8% less than U-10 and by 39.2% less than U-90 (p<0.05).

The aim of the further research was to evaluate the effect of U-10, U-77 and U-90 upon growth of *Pseudomonas aeruginosa*. Testing *in vitro* was performed using 4 strains of *Pseudomonas aeruginosa*. We used 0.5-1% aqueous solutions of U-10, U-77 and U-90 and benzalkonium chloride. The results showed that quaternary ammonium salts *in vitro* had moderate effect on *P. aeruginosa*.

The results of the research indicated that the compound U-77 had the strongest antibacterial action, thus we performed its toxicity analysis and established the parameters of its acute toxicity. U-77 compound (N-oxyethylated carbamoilmethylhexamethylenetrammonium chloride) has an oxyethylated amide group that ensures strong and supposedly prolonged antibacterial action. Quaternary ammonium compounds are classified as medium toxicity, and the newest ones even as low toxicity substances. (Šurkus, Kajokas, 2002). U-77 LD_{50} for white mice after intragastric administration is 2890 mg/kg (table 1). This means that U-77 demonstrates 3-19 times less acute toxicity than benzalkonium chloride (LD_{50} for white mice orally 150-1000mg/kg, Wade, Weller, 1994).

Table 1. Parameters of acute toxicity of U-77 for white mice

<table>
<thead>
<tr>
<th>Dose, mg/kg</th>
<th>Animals in the group/of then died</th>
<th>Dose of 20% solution ml/per animal</th>
<th>Toxicity parameters, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>6/0</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>6/2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>3000</td>
<td>6/3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td>6/4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>6/6</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>LD</em>{sub}50* and reliability margin</td>
<td>2890 (2094.2-3988.2)</td>
</tr>
</tbody>
</table>
Based on the research data and requirements of pharmacopoeia and chemotherapy we can admit that U-77 belongs to the group of low toxicity compounds because its LD<sub>50</sub> is within 500-5000 mg/kg range, thus meeting the criteria for this group.

**Testing the Dezinfektas II in vitro**

Research of the Dezinfektas II in vitro showed that concentration of 1:59 was the smallest that had satisfactory inhibitory effect on bacteria (table 2).

<table>
<thead>
<tr>
<th>Dilution of solution</th>
<th>E. coli</th>
<th>S. cholerae suis</th>
<th>S. agalactiae</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:59</td>
<td>15.7±0.3</td>
<td>16.1±0.4</td>
<td>17.1±0.7</td>
<td>17.5±0.4</td>
</tr>
</tbody>
</table>

**Disinfection of room air using the Dezinfektas II**

Two experiments were performed using the Dezinfektas II for room air disinfection. We used 1:30 solution of the biocide because 1:59 proved to be ineffective for room air disinfection.

**Table 3. Effectiveness of room air disinfection**

<table>
<thead>
<tr>
<th>Number of colonies, vnt</th>
<th>Before disinfection</th>
<th>After disinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment I</td>
<td>Experiment II</td>
</tr>
<tr>
<td>211.2±12.8</td>
<td>72.8±9.4</td>
<td></td>
</tr>
<tr>
<td>98.7±21.3</td>
<td>34.3±10.3</td>
<td></td>
</tr>
</tbody>
</table>

The data (table 3) revealed that bacterial contamination of room air after disinfection decreased by 65.2-65.5% (p<0.05). The results were considered satisfactory and no more experiments were performed with the Dezinfektas II solution.

**The effectiveness of the Dezinfektas II solution when used for disinfection of surfaces indoors**

Prior to disinfection the surfaces were artificially contaminated with E. coli ATCC 25922 biomass using the method described above. The first two experiments were performed using 1:30 solution of the Dezinfektas II. During the first experiment the disinfecting solution was sprayed in the room air 20 ml/m³. Samples from the surfaces were collected prior to disinfection and 2 hours after it. The research data showed that aerosol disinfection reduced the number of bacteria on horizontal surfaces by 77.7% and on vertical surfaces by 52.4% (p<0.05).

During the second experiment we sprayed the same 1:30 solution 82.48 ml/m³. The results are shown in pictures 3.

![Graph showing bacterial reduction](image.png)

3 pic. The effectiveness of disinfection using the Dezinfektas II 1:30 solution aerosol

The data obtained after examining samples collected prior to and after disinfection showed (pic. 3) that aerosol disinfection reduced the number of bacteria by 87.5% on horizontal surfaces and by 43.9% on vertical surfaces (p<0.05). The analysis of the results of the first two experiments revealed the necessity to increase the concentration of the solution. That’s why performing the third experiment we sprayed a smaller amount of the Dezinfektas II solution (57.7 ml/m³) but used a more potent concentration (1:20).

The data of the third experiment showed that disinfection reduced the number of bacteria by 70.2% on horizontal surfaces and by 65.7% on vertical surfaces (p<0.05).

During the fourth experiment the surfaces were treated with 1:9 solution of the Dezinfektas II solution was sprayed 90.7 ml/m³ in the air. The results of the fourth experiment are shown in the pictures 4.

Analysis of the samples collected prior to and after the disinfection showed (pic. 4) that disinfection reduced the number of bacteria by 84.6% on horizontal surfaces and by 77.8% on vertical surfaces (p<0.05).
The decision to stop investigating the Dezinfektas II solution was influenced by the fact that the compound contained formaldehyde which is rather toxic and thus rarely used. Lately glutaric aldehyde is used as a part of disinfecting solutions because it is more effective and less toxic than formaldehyde. But since glutaric aldehyde also possesses significant toxicity most manufacturers abandon aldehydes as a constituent of their products.

After completing experiments with the Dezinfektas II started experimenting with Dezinfektas III solution, but since it showed low effectiveness in vitro, the research was stopped.

We decided to improve the Dezinfektas II solution by reducing its toxicity (removing aldehydes) and searching for a blend of active ingredients that would exhibit a pronounced and steady antimicrobial action. After two years of intensive work the Dezinfektas IV solution was ready. It demonstrated good antimicrobial effect, so it was investigated further.

**Investigating the Dezinfektas IV solution in vitro**

Based on the research results of the Dezinfektas II in vitro we chose dilution 1:59 for Dezinfektas IV as a start because this concentration of Dezinfektas II showed satisfactory antimicrobial effect, having in mind that Dezinfektas IV includes more ingredients with antimicrobial action.

<table>
<thead>
<tr>
<th>Dilution of solution</th>
<th>Antibacterial effect of the solution and the diameter of the sterile zone, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>1:59</td>
<td>20.2±0.9</td>
</tr>
</tbody>
</table>

The data (table 4) showed that 1:59 solution of the Dezinfektas IV has good effect against *E. coli* and *Staphylococcus aureus*, but *Salmonella enteritidis Gartneri* demonstrated pronounced resistance – they were less sensitive than *E. coli* by 39.6% and than *S. aureus* by 48.9% (p<0.05).

Our previous research showed that *Salmonella* spp. were rather resistant in vitro to classical quaternary ammonium compounds (alkyltrimethylbenzylammonium and didecyltrimethylammonium salts).

**Disinfection of room air using the Dezinfektas IV**

At the beginning of the experiments with *S. aureus ATCC 25923* firstly 1:9 and 1:16 Dezinfektas IV solution aerosol was used. In the process of this experiment it became clear that 1:9 and 1:16 Dezinfektas IV solution aerosols have the same deteriorating influence on bacteria. That is why for further experiment only 1:16 Dezinfektas IV solution aerosol and electro-aerosol was used. After the disinfection (table 5) with 1:16 Dezinfektas IV solution electro-aerosols with positive and negative charges and aerosol without any charge the amount of *S. aureus* in the air significantly was lowered (p<0.05).

Still the greater difference between the influences of Dezinfektas IV solution electro-aerosol with various charges and aerosol are not indicated. More significant difference between the activity of electro-aerosols and aerosols becomes noticeable after 30 minutes have passed from the moment the disinfection was completed. The growth of bacteria is on the lowest level after the disinfection of premises is done with Dezinfektas IV solution aerosols, which are without any charge. In this case the samples of the air *S. aureus* was not spotted. The same results were received after the disinfection was performed with 1:16 Dezinfektas IV solution electro-aerosols with negative charge (table 5). Bacteria amount in the air, comparing to the situation before the disinfection, decreased 99.99% (p<0.05).

The influence of Dezinfektas IV solution electro-aerosols with positive charge (table 5) on the bacteria was a little bit smaller, but the percentage of disinfection activity constituted 99.99% (p<0.05). After an hour passed from the moment the disinfection was performed, it was determined that the effectively of disinfection did not decrease (table 5) and stayed the same all the time 99.9% (p<0.05).
Table 5. The effectiveness of room disinfection with Dezinfektas IV aerosol and electro-aerosols

<table>
<thead>
<tr>
<th>Dilution of Dezinfektas IV solution</th>
<th>The number of colonies of S.aureus 1m³ of air after the disinfections</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes after the disinfections</td>
<td>One hour after the disinfections</td>
</tr>
<tr>
<td>161400±8652 1:16 without charge</td>
<td>0±0</td>
</tr>
<tr>
<td>111500±39300 1:16 with positive charge</td>
<td>44.3±11.3</td>
</tr>
<tr>
<td>1049000±22490 1:16 with negative charge</td>
<td>5.7±5.7</td>
</tr>
<tr>
<td>116600±5461 Lizoformin® Spezial 1:16 without charge</td>
<td>22.3±5.3</td>
</tr>
</tbody>
</table>

After all the results were evaluated, it became clear that the greater influence on Gr+ S. aureus microorganisms possesses Dezinfektas IV solution electro-aerosols with negative charge and aerosol without any charge. The Dezinfektas IV solution electro-aerosols with positive charge influence is weaker.

For comparison we performed disinfection of air pre-contaminated with S. aureus, using 1:16 solution of Lizoformin® Spezial aerosol without electric charge (table 5). Bacteria amount in the air 30 minutes and one hour after disinfection decreased 99.99% (p<0.05).

In the process of the experience with E. coli ATCC25922 was used 1.5:1 Dezinfektas IV solution electro-aerosols with positive and negative charge and aerosol without charge. The bigger concentration was chosen at this time for the reason, that E. coli Gr- bacteria are more resistant to quaternary ammonium compounds then Gr+ staphylococci.

The results after the disinfection with 1.5:1 Dezinfektas IV solution aerosol and electro-aerosols showed that the amount of E. coli bacteria noticeably decreased (table 6). The sampled air proved that bacteria growth, just after the disinfection, is much less then before the disinfection 99.99% (p<0.05).

The best results were received after the usage of Dezinfektas IV solution electro-aerosols with positive and negative charge. The effectively of aerosol without any charge was a little bit weaker. The air samples taken after 30 min. and an hour after the disinfection (table 6) proved the effectiveness of the disinfection – 99.99% (p<0.05).

Table 6. The effectiveness of room disinfection with Dezinfektas IV aerosol and electro-aerosols

<table>
<thead>
<tr>
<th>Dilution of Dezinfektas IV solution</th>
<th>The number of colonies of E. coli 1m³ of air after the disinfections</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes after the disinfections</td>
<td>One hour after the disinfections</td>
</tr>
<tr>
<td>1436000±218800 1.5:1 without charge</td>
<td>61.3±53.1</td>
</tr>
<tr>
<td>1297000±7709 1.5:1 with positive charge</td>
<td>16.7±9.5</td>
</tr>
<tr>
<td>125600±7220 1.5:1 with negative charge</td>
<td>5.7±5.7</td>
</tr>
<tr>
<td>1239000±20450 Lizoformin® Spezial 1.5:1 without charge</td>
<td>77.7±43.4</td>
</tr>
</tbody>
</table>

For comparison we performed disinfection of air pre-contaminated with E. coli, using 1.5:1 solution of Lizoformin® Spezial aerosol without electric charge (table 6). Bacteria amount in the air 30 minutes and one hour after disinfection decreased 99.99% (p<0.05).

Effectiveness of the Dezinfektas IV solution for room surfaces disinfection

For investigation of effectiveness of the Dezinfektas IV aerosols and electro-aerosols we used E. coli ATCC 25922 and Staphylococcus aureus ATCC 25923 biomass for artificial contamination of surfaces. For experimenting with S. aureus initially we used 1:16 solution.

Disinfection using 1:16 solution of the Dezinfektas IV in aerosol and electro-aerosols form reduced the number of bacteria by 15.6-52.7% on horizontal surfaces and by 9.3-26.9% on vertical surfaces (p<0.05). For comparison we used 1:16 solution of Lizoformin® Spezial aerosol solution for surface disinfection. The results revealed that both compounds were equally active, but their activity was not quite satisfactory. Thus we increased the concentration...
of the Dezinfektas IV solution and for further experiments we used 1:4 solution in the form of aerosol and electro-aerosols.

The data revealed that the least effective for horizontal surface disinfection against *S. aureus* was the aerosol of the biocide without electric charge (pic. 5). Samples collected from the surfaces prior to disinfection and after it showed that using aerosol without electric charge decreased the number of bacteria by 54.6% (p<0.05).

Significantly better results were obtained when disinfecting electro-aerosol with negative charge was used (pic. 5). Samples collected from the surfaces prior and after disinfection showed that electro-aerosol with negative charge reduced the number of bacteria on horizontal surfaces by 84.7% (p<0.05).

The best results were obtained performing disinfection with the Dezinfektas IV using electro-aerosol with positive charge (pic. 5). It reduced the number of bacteria on horizontal surfaces by 99.3% (p<0.05).

The least effect on vertical surfaces against *S. aureus* was obtained using electro-aerosols with positive charge (pic. 6). Samples collected from contaminated surfaces prior to and after the disinfection showed that such electro-aerosol decreased the number of bacteria by 78.2% (p<0.05).

The best results were obtained using electro-aerosol with positive charge (pic. 6). It reduced the number of bacteria by 88.6% (p<0.05).

For comparison we performed disinfection of surfaces pre-contaminated with *S. aureus*, using 1:4 solution of Lizoformin® Spezial aerosol without electric charge. The results revealed that the effect of 1:4 solution of the Dezinfektas IV and Lizoformin® Spezial against *S. aureus* on artificially contaminated surfaces was almost similar.

For experiments with *E. coli* ATCC 25922 we used 1.5:1 solution of the Dezinfektas IV in the forms of aerosol without electric charge and electro-aerosols with negative and positive charge. The reason is that our previous experiments showed that smaller concentrations had unsatisfactory inhibiting effect on *E. coli* growth.

Aerosol without electric charge showed the best activity against *E. coli* on horizontal surfaces (pic. 7). Samples collected prior to disinfection and after it showed that aerosol without electric charge reduced the number of bacteria on horizontal surfaces by 99.9% (p<0.05).

Similar results for horizontal surfaces were obtained using electro-aerosol with negative charge (pic. 7), it reduced the number of bacteria by 97.2% (p<0.05).

The least effect for horizontal surfaces contaminated with *E. coli* was obtained using electro-aerosols with positive charge (pic. 7). Samples collected from contaminated surfaces prior to and after the disinfection showed that such electro-aerosol decreased the number of bacteria by 78.2% (p<0.05).
The weakest effect for vertical surfaces against *E. coli* was obtained using the biocide solution electro-aerosol with negative charge (pic. 8) – it reduced the number of bacteria by 30.6% (p<0.05).

Much better results were obtained using electro-aerosol with positive charge. This resulted in bacterial number decrease by 99.3% (p<0.05).

For comparison we performed disinfection of surfaces pre-contaminated with *E. coli* using 1.5:1 solution of Lizoformin® Spezial without electric charge. The data revealed that the effect of the Dezinfektas IV and Lizoformin® Spezial was almost identical.

The data in literature about the influence of electric charge upon bacteria is somewhat controversial. Some investigators [Ahsan et al., 2002; Beers et al., 2002] point out that it doesn’t influence the interaction of bacteria with various substances, others indicate [Nomura et al., 1995; Gottenbos et al., 2001; Thiele et al., 2001; Oblak et al., 2002; Carmona - Ribeiro et al., 2006] that particles with positive charge stick to bacteria much easier (quaternary ammonium compounds and chlorhexidine have powerful and steady electrical charge). Some scientists express the opinion that increasing negative charge increases interaction between bacteria and other substances [Magnusson et al., 1980] and that decreasing the negative charge decreases such interaction [Magnusson et al., 1979].

Summing up the research results using the Dezinfektas IV we found that despite the negative charge of bacteria the biocide electro-aerosols with negative charge had better inhibiting effect on G+ and G- bacteria in room air than the electro-aerosol with positive charge. The aerosol without electric charge had slightly stronger effect on G+ and G- bacteria and was slightly less effective than the electro-aerosol with negative charge.

For disinfection of horizontal and vertical surfaces indoors the best results were obtained using electro-aerosol of the Dezinfektas IV with positive charge. Although the aerosol without electric charge showed good activity against *E. coli* on various surfaces, but it killed *S. aureus* on vertical surfaces unsatisfactorily. The least effect for surfaces was obtained using electro-aerosol with negative charge.

**CONCLUSIONS**

1. After investigating antibacterial activity in vitro of 48 newly synthesized quaternary ammonium compounds it was established that the most effective were quaternary hexamethylenetramonium compounds having acetylene fragment (U-10), aminoacetamide fragment (U-90) and especially oxymethylated amodic group of aminoacetamide fragment (U-77).
2. U-77 had the best effect against G+ and G- bacteria and had a broader antibacterial spectrum than benzalkonium chloride. Compared to benzalkonium chloride U-77 was 5 times less toxic.

3. 1:30 solution of the Dezinfektas II reduced bacterial contamination of room air 3 times. Depending upon concentration (1:9; 1:20; 1:30) the biocide solution reduced the number of *Escherichia coli* on pre-contaminated horizontal surfaces 3-8 times and on vertical surfaces 2-4.5 times.

4. After performing disinfection with Dezinfektas IV (1:5:1) aerosol and electro-aerosols of the premises artificially contaminated with *Escherichia coli* the number of bacteria in the air decreased from 20 000 to 100 000 times. The best effect was obtained using electro-aerosol with negative charge. The number of bacteria on vertical and horizontal surfaces decreased correspondingly from 322 to 827 times, the best effect was obtained using the aerosol without electric charge.

5. After performing disinfection with Dezinfektas IV (1:16) aerosol and electro-aerosols of the premises artificially contaminated with *Staphylococcus aureus* the number of bacteria in the air decreased from 10 500 to 200 000 times. The best effect was obtained using electro-aerosol with negative charge. The number of bacteria on vertical and horizontal surfaces after the disinfection using the biocide (1:4) decreased correspondingly from 9 to 153 times, and the electro-aerosol with positive charge showed to be the most effective.

6. Ten times higher concentration is needed for destruction of Gr- bacteria, than for destruction of Gr+ bacteria.

7. Using aerosols and electro-aerosols of Dezinfektas IV for disinfection it is necessary to use small dilution (i.e., potent concentration - up to 30%) solution, but small amount of substance per volume (20-30 ml/m³).

**RECOMENDATION**

The research data revealed that using aerosols and electro-aerosols of the Dezinfektas IV for disinfection of air and surfaces it’s necessary to are smoll dilution (i.e., potent concentration, up to 30%) disinfecting solution, but smoll amount of the solution per volume is enough (20-30 ml/m³). Ten times higher concentration is needed for destruction of Gr- bacteria, than for destruction of Gr+ bacteria.

**PUBLICATIONS**


REZIUMĖ

Lietuvos veterinarijos akademijos Klinikinės ir eksperimentinės farmakologijos laboratorijoje iš 48 susintetinių naujų kietvinių amonio darinių didžiausiu antagonistinio aktyvumo ir efektyvumo U-grupė, t. y., kietvinių heksametilentetraminio (metenamino) druskos. Heksametilentetraminio (metaenamino) dariniai lengvai skyla į formaldehidą ir amoniaką, susidaro amonio karbonato. Taigi, medžiagos skilimo produktai taip pat yra naudingi, nes pasižymi antimikrobiniu veikimu.


Rezultatų in vitro analizė parodė, kad naujų susintetinių kietvinių amonio darus (U-10, U-77 ir U-90) antagonistinio aktyvumo dažnai lenkė aukštesnį aukštesnį dezinfekcijos efektyvumą.

Įsitikiname, kad aerozolinės dezinfekcijos efektyvumui lemiančius veiksnius patvirtina ir literatūroje pateikiamos duomenys. Vykdant trečią ir ketvirtą bandymus Dezinfekto II tirpalo koncentracija buvo žymiai didesnė, negu pirmų dviejų bandymų metu, ir dezinfekcijos rezultatai geresni.

Sprendimą nutraukti tolesnius bandymus su Dezinfekto II variantu lūkesčio darbdavė ir ketvirtoji laboratorijos (bioželdėje). Heksametilentetraminio (metenamino) druskos. Heksametilentetraminio (metenamino) dariniai lengvai skyla į formaldehidą ir mažiau į formaldehidą. Tačiau, formaldehidą ir glutarino aldehidą, todelė rezultatai geresni, tačiau ir glutarino aldehidą todelė rezultatai geresni.


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zinfekcijos bakterijų ore rasta 99,99-100% (p<0,05), o praėjus 1 valandai po dezinfekcijos – 99,99% (p<0,05) mažiau, negu buvo iki dezinfekcijos.

Išanalizuot rezultatus nustatėme, kad biocido elektroaerozolis, turintis neigiamą elektros krūvį, *S. aureus* bakterijas ore naikino geriau už aerozolį be elektros krūvio ir elektroaerozolį, turintį teigiamą elektros krūvį. Bet pastebėtas skirtumas buvo nežymus (p>0,05).

Pradėję vykdyti patalpos paviršių dezinfekcijos bandymus su Dezinfekto IV tirpalo nustatėme, kad 1:16 skiedimo biocido tirpalas, efektyviai naikino *S. aureus* patalpos ore, o 99,99-100% (p<0,05), o praėjus 1 valandai po uždarymo ir tik nežymiai efektyvumą atsiliko nuo elektroaerozolio, turintių teigiamą ir negi namą elektros krūvį. Bet pastebėtas skirtumas buvo nežymus (p>0,05).

Prieš tai dezinfekavus patalpos paviršių dezinfekcijos bandymus su Dezinfekto IV tirpalo nustatėme, kad biocido elektroaerozolis, turintis neigiamą elektros krūvį, *S. aureus* patalpos ore, yra neefektyvus dezinkruojant šiomis bakterijomis užkėrstęs pavipiršius. Dezinfekcija 1:16 skiedimo tirpalo aerozolu ir elektroaerozoliais bakterijų kiekį ant horizontalių pavipiršių sumažino 15,6–52,7%, o ant vertikalių – 9,3–26,9%.

Biocido koncentracija buvo padidinta ir bandymams su stafilokokais naudojant 1:4 skiedimo Dezinfekto IV tirpalo aerозолис ir skirtingo elektros krūvio elektroaerozoliais. Bandymo rezultatai rodo, kad po dezinfekcijos aerozolu ir elektroaerozoliais *S. aureus* ant horizontalių pavipiršių rasta 54,6–99,3% (p<0,05), o ant vertikalių pavipiršių – 23,5–88,6% (p<0,05) mažiau, negu buvo iki dezinfekcijos.

Užkėstų *E. coli* bakterijomis pavipiršių dezinfekcijai naudojome 1:5:1 skiedimo Dezinfekto IV tirpalo aerozolį ir skirtingų elektros krūvū elektroaerozolodus. Išvertė bandymų rezultatus nustatėme, kad po dezinfekcijos *E. coli* bakterijų ant horizontalių pavipiršių rasta 78,2–99,9% (p<0,05), o ant vertikalių pavipiršių – 30,6–99,7% (p<0,05) mažiau, negu buvo iki dezinfekcijos.

Apie elektros krūvio įtaką bakterijoms literatūroje pateikiama prieštarbingi duomenys. Vieni tyrelėje nurodė [Ahlan et al. 2002; Beers et al., 2002], kad jis neturi jokios įtakos bakterijų sveikai su įvairiomis medžiagomis, kiti teigia [Nomura et al., 1995; Buck, 2001; Gottenbos et al., 2001; Thiele et al., 2001; Oulak et al., 2001; Carmona – Ribeiro et al., 2006], kad prie bakterijų daug greičiau prilimpa teigiamą elektros krūvį turinčios medžiagos (ketvirtiniai amonio junginiai ir chlorheksidinas turi stiprių ir stabilių teigiamų elektros krūvį). Kai kurie mokslininkai yra tos nuomonės, kad įvairių medžiagų sveika su bakterijomis stipriai didėjant neigiamam krūvui [Magnusson et al., 1980] ir silpnėja neigiamam krūvui mažėjant [Magnusson et al., 1979].

Apibendrinami visų bandymų patalpoje su Dezinfekto IV varianto tirpalu duomens galime teigti, kad nepaisant neigiamo bakterijų elektros krūvio, biocido tirpalo aerobacolas, turintis neigiamą elektros krūvį, tiek Gr-, tiek Gr+ bakterijas patalpos ore veikė geriau už elektroaerozolį, turintį teigiamą elektros krūvį. Tiriama tirpalo aerobacolas be elektros krūvio Gr+ ir Gr- bakterijas patalpos ore veikė šiek tiek geriau už elektroaerozolį su tie-
desnės Dezinfekto IV tirpalo koncentracijos, negu naikinant *Staphylococcus aureus*.

7. Atliekant dezinfekciją Dezinfekto IV aerozoliu ir elektroaerozoliiais reikia naudoti mažo skiedimo laipsnio (t. y. didelės, iki 30%, koncentracijos) tirpalą, bet užtenka nedidelio tirpalo kiekio tūrio vienetui (20–30 ml/m³).

**PASIŪLYMAI**

Tyrimų rezultatais nustatyta, kad dezinfekuojant patalpų orą ir paviršių reikia naudoti didelės, iki 30%, koncentracijos Dezinfekto IV tirpalą, bet užtenka nedidelio tirpalo kiekio tūrio vienetui (20–30 ml/m³). Gr+ bakterijų išaktyvavimui patalpoje reikia iki 10 kartų mažesnės biocido tirpalo koncentracijos, negu išaktyvuojant Gr- bakterijas.

**TRUMPOS ŽINIOS APIE AUTORŲ**


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Už teksto turinį ir redagavimą atsakingas autorius

Spausdino LVA Spaudos ir leidybos skyrius
Tilžės g. 18, LT-47181 Kaunas
Tiražas 50. 1,87 sp.l. Užs. Nr. 2d. 2007