ANTISEPTIC SPRAY BASED ON STABILIZED SILVER PARTICLES: AN ANALYSIS OF ANTISEPTIC PROPERTIES AND COMPREHENSIVE COMPARISON

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Introduction

The problem of microorganism's multidrug resistance today is very relevant. The nature and mechanism of the emergence of multiresistance are studied in many Universities of the West World [1], [2], [3]. Researchers consider that the formation of multidrug resistance is a protective reaction of microorganisms to human use of antibiotics and various antiseptics. The use of some alcohol-based antiseptics for hands hygiene, without following the instructions, leads to the formation of multiresistance strains of microorganisms on the hands, and it's may be possible way to spreading such strains in public places. Note, that according to the data of the authors [4], in 28% of users of public transport in the UK on hand was present fecal microflora. Based on these data, it can be assumed that even in Developed countries at least two dangers to public health are exist: the emergence of microorganism's multidrug resistance and the danger of contamination by such microorganisms in public places. Obviously, to reduce the level of the threat of infection, one should observe the hygiene of the hands. The aim of this study is to create an antiseptic for hand hygiene that can't contribute to the formation of multiresistance pathogenic microorganisms, but at the same time isn't inferior in effectiveness to antiseptics based on alcohols. It's well known, that silver is often used as an antiseptic: there are many examples of the use of silver nanoparticles [5], colloidal [6], and ionic [7] silver. Areas of exertion of antiseptic properties of silver are also diverse [8]. The impact of silver on the organism of animals and humans has been studied [9]. Thus, silver is a promising means for hand hygiene, devoid of many disadvantages of alcohol-based antiseptics.

Materials & Methods

The purpose of this study was testing the action of antiseptic "Dew" on four different test-strains. Also, we were compared the antiseptic effect of Dew with the antiseptic action of colloidal silver, nanosilver and antiseptically solution based on isopropyl alcohol. All materials and methods, that were used in our study, are given below.

Preparation of the antiseptic based on atomic silver

The prototype of device, based on electrochemical principle, we were obtained from Modern Biochem Technologies, Ltd (Ukraine). The Prototype was developed according to the patent [10]. The Prototype generates an antiseptic Dew on a water basis. For the preparation of antiseptic, drinking water from well-known brand was flooded into the Prototype. The chemical composition of water was marked by the manufacturer on the label (Table 1):

Table 1. Chemical composition of water for the preparation of antiseptic

Dry residue	100 – 300 ppm	Magnesium	40 ppm
Total alkalinity	4 mmole/L	Sodium	150 ppm
Total hardness	5 mmole/L	Phosphorus	0.3 ppm
Potassium	40 ppm	Iodine	20 ppb
Calcium	75 ppm		

Antiseptic Dew consists of water (Table 1) and dissolved atomic silver. Antiseptic is generated in the special chamber of the prototype. The prototype is equipped with a mechanical push-button pump for spraying Dew in the form of aerosol.

Antiseptics for comparison

For the comparative test 1-b, antiseptics certified in Ukraine were used (Table 2):

Table 2. Antiseptics for comparison

Name of the antiseptic and its manufacturer	The normative document according to which the antiseptic was made	Composition of the antiseptic, specified by the manufacturer
1. Solution with antiseptic properties DE-SEPT on the basis of colloidal silver with lemon TM Whirl (Ospices Ukraine, Ltd)	State standard of Ukraine (SSTU) 4093-2002	Water, colloidal silver, lemon hydrolat
2. Antiseptic solution based on isopropyl alcohol "Spray for hand disinfection TM Premium" (Ukrprofmed, Ltd)	Technical conditions of Ukraine (TC U) 20.4-38993983-002:2015	Isopropyl alcohol, prepared water, glycerin, D-panthenol, mixture "ChAS", flavor "Cherry".
3. Complex antiseptic additive "Silver colloid "Colloidal Nano Silver " (CNS)" (V.S. Revival, Ltd)	Technical conditions of Ukraine (TC U) 24.2-37079343-001:2001	3.1 Water, silver nanoparticles 10 ppm
		3.1 Water, silver nanoparticles 20 ppm

Test-strains

In all microbiological tests, test strains from the American Type Culture Collection were used: E. Coli ATCC 25922, Staphylococcus Aureus ATCC 25923,

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Candida Albicans ATCC 885-653 and Proteus Vulgaris ATCC 4636 with a billion concentration of colony forming units in 1 ml (10^8 - 10^9 CFU/ml, ln CFU/mL = 19.57...20.72).

Preparation and sanitation of the infected surface

The infections contaminated surface was prepared as follows: in a sterile Petri dish, one strain was introduced with a sterile cotton swab so that it was evenly distributed over the entire surface area of 63.5 cm^2. Surface treatment was carried out as follows: 1 ml of freshly prepared APP antiseptic or antiseptic was applied to the infected Petri dish for comparison; Aerosol was applied from a distance of 10 cm from the infected surface.

The smear for analysis was screened off with a sterile loop to the nutrient medium; the screened sample was placed in a thermostat at 37 °C (99 °F) for 24 hours.

All microbiological tests were carried out in the Laboratory of Biochemistry and Biotechnology of the Mechnikov Institute of Microbiology and Immunology National Academy of Sciences of Ukraine (Kharkov, Ukraine).

Determination of silver concentration in Dew

The silver concentration in the Dew was determined thuswise: 1 ml of Dew in the form of aerosol was introduced into a sterile container of polyethylene terephthalate; the solution was diluted of 10 ml bidistilled water; the solution was stirred for 1 minute; 1 ml of the solution was taken and was diluted of 10 ml bidistilled water; the solution was again stirred for 1 minute; the solution was acidified with 0.1 M nitric acid to pH = 2; 0.5 ml of the sample was taken for analysis. The analysis was carried out on the MGA-915MD atomic-adsorption spectrometer in the laboratory of the Analytical Ecological Studies of Karazin Kharkov National University (Kharkov, Ukraine).

Results & Discussion

Test 1-a "Test of Prototype working efficiency" results

The first test of the Prototype was performed on a test-strain of gram-negative bacteria Proteus Vulgaris ATCC 4636. The purpose of the first test was to determine the operability of the prototype and the efficacy of Dew against this test-strain. The treatment was carried out according to the described procedure. The smear was taken with a sterile loop after 1 minute (Fig.1, 2) and after 10 minutes (Fig. 1, 3) after treatment. The results of the first test are on the Figure 1:

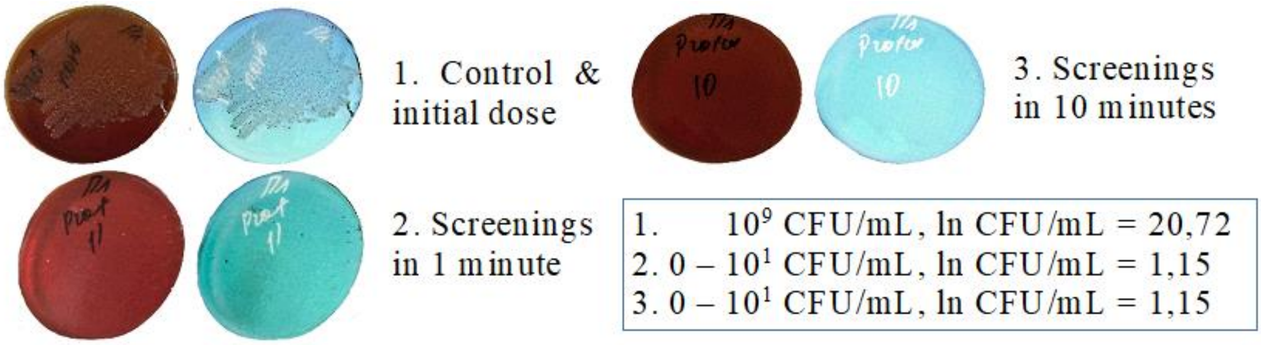

Figure 1. Effectiveness of Dew against test-strain Proteus Vulgaris ATCC 4636

The results of test 1-a were confirmed the efficacy of Dew against gram-negative bacteria Proteus Vulgaris ATCC 4636. 1 ml of Dew, that was applied on the infected surface in the form of aerosol, completely neutralized a billions contamination of test-strain.

Test 1-b "Comparative test" results

The test 1-b was conducted to comparing the efficacy of Dew with antiseptics of comparison. The test was carried out on the four test-strains, that given above. Dew was tested twice (Table 3, Dew # 1, Dew # 2). The smear was taken with a sterile loop for 10 seconds after the antiseptic was applied to the infected surface. The test was carried out according to the procedure described above. The temperature of antiseptic solutions was 21 ° C (70 ° F). The test results are below:

Table 3. Results of test 1-b

Strain	Control & initial dose	Dew #1	DE-SEPT	CNS 10 ppm	CNS 20 ppm	Dew #2	Isopropyl Alcohol
CFU/mL (ln CFU/mL)							
Staph. Aureus	10^9 (20,72)	10^2 (4,61)	10^4 (9,21)	10^3 (6,91)	10^3 (6,91)	10^1 - 10^2 (3,45)	10^1 - 10^2 (3,45)
E. Coli	$10^8 – 10^9$ (19,57)	10^2 (4,61)	10^4 (9,21)	10^3 (6,91)	10^3 (6,91)	10^2 (4,61)	10^3 (6,91)

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Candida Albicans	$10^8 – 10^9$ (19,57)	10^2 (4,61)	10^3 (6,91)	10^4 (9,21)	10^2 (4,61)	10^1 (2,3)	10^1 - 10^2 (3,45)
Proteus Vulgaris	10^9 (20,72)	$10^1 – 10^2$ (3,45)	10^4 (9,21)	10^5 (11,51)	10^4 (9,21)	0 -10^1 (1,15)	10^1 (2,3)

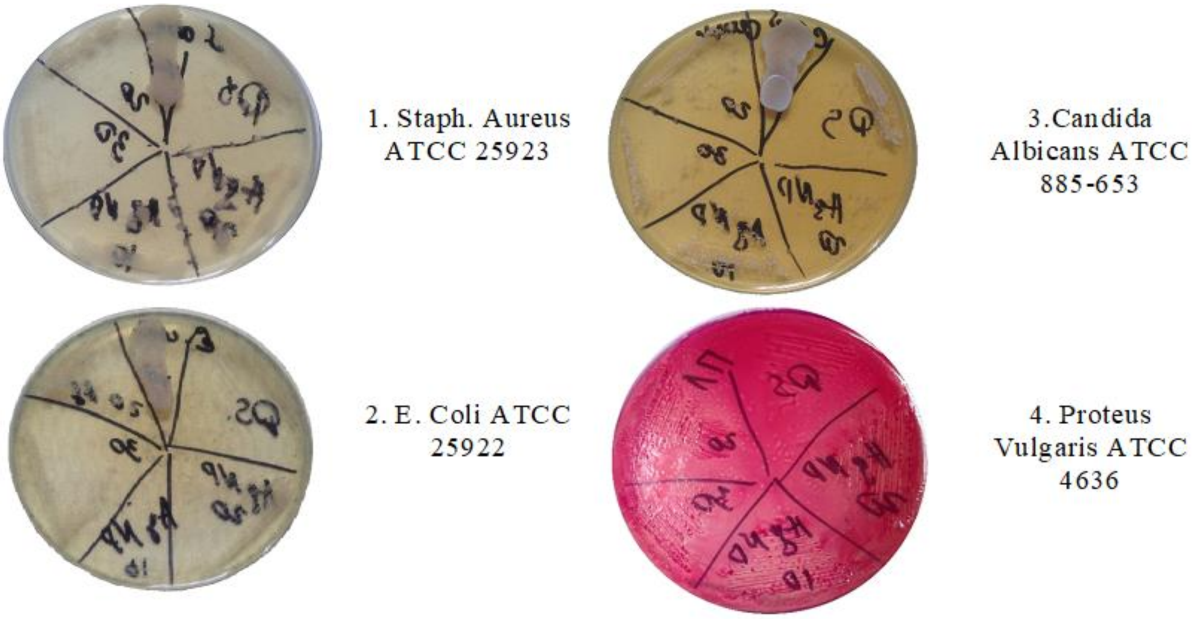

Figure 2. Results of the “comparative test”

Comparative test 1-b (Table 3, Figure 2) results are showed that Dew #1 antiseptic is superior to colloidal silver solution DE-SEPT, CNS 10 ppm and CNS 20 ppm (solutions of silver nanoparticles) in the efficiency against test-strains; Dew #2 is superior to the antiseptic, based on isopropyl alcohol, and all tested antiseptics, based on the colloidal and nanosilver (Figure 3).

Test 1-c “Additional tests of Dew effectiveness”

We were conducted the control tests on test strains of Staphylococcus Aureus ATCC 25923 and Candida Albicans ATCC 885-653. The purpose of the control tests was reaffirming of the efficiency of the Dew and determination of the optimal modes of operation of the prototype. In the context of test 1-c, data of test 1-b was affirmed. Test 1-c was performed in accordance with the procedure outlined above. The only change was the use of different modes of preparation of Dew, the description of which is beyond the scope of this article. The test results are shown in Figure 4.

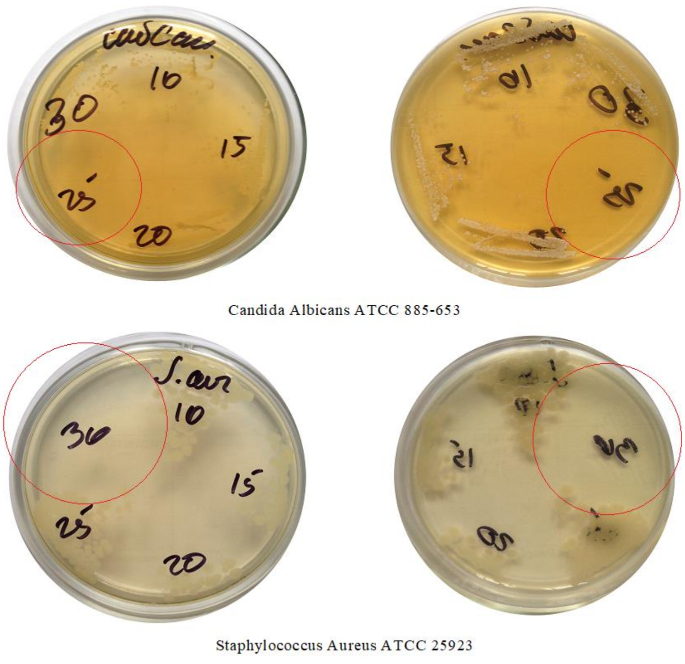

Figure 4. Results of additional test 1-c

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The test was carried out on the test-strains in 5 different working modes of prototype. The initial infection contamination in both cases was 10^9 CFU/mL. The microbial load after treatment (Figure 4, The circle limited by red-line) was reduced to 0-10^1 CFU/mL (ln CFU/mL = 1.15).

The results of the test 1-c convincingly prove, that Dew is able to neutralize a billionth infection contamination. Also, the test results allow us to assume, that the conditions for the preparation of Dew strongly and predictably affect the effectiveness of the antiseptic. The effect of prototype designs on efficiency was also determined.

Test 1-d "Determination of the concentration of silver in Dew" results

To determine the silver concentration in Dew, an analysis was performed on the MGA-915MD atomic adsorption spectrometer (Figure 5). The results of the test showed, that the range of true silver concentrations in the applied Dew solutions is in the range of 0.85 ppm to 1.45 ppm, i.e. up to 20 times less than in CNS by V.S. Revival LTD. Note, that the Dew used to sanitize polymeric surfaces contains 2.5 - 3 ppm of silver. In this concentration Dew is very effective against the formed biological film on polyethylene terephthalate [11].

Some characteristics of MGA-915MD

Working spectral range	190…800 nm
Spectral resolution	2 nm
Range of measurement of mass concentration of silver	from 0.005 to 0.5 ppm.
Relative expanded uncertainty for silver with a coverage factor of K = 2.	from 0.005 to 0.05 ppm, U = 20% from 0.05 to 0.5 ppm, U = 16%

Figure 5. Atomic adsorption spectrometer MGA-915MD

Discussion

According to the results of studies 1-a, 1-b and 1-c, we conclude that Dew is an antiseptic agent, that suitable for combating a wide range of microorganisms, including fungi. Undoubtedly, Dew needs to testing against multidrug-resistant and hospital strains. But even today, the data obtained suggest that Dew is not inferior, and even surpasses the effectiveness of antiseptics based on various forms of silver. These results indirectly indicate, that the main active agent of Dew isn't colloidal silver and isn't silver nanoparticles. Note, that Dew was tested in six complex tests, the results of one of which are presented above in the form of tests 1-a, 1-b and 1-c. All of tests were showed the accordance of the anticipation results and reproducibility of the results, and high efficacy of Dew against test-strains.

The nature of the Dew effect

Dew was prepared in a special cave of the Prototype of portable device. As a result of the action of the electric current, applied to the electrodes from biocidal metals, the cascade of electrochemical and chemical processes occurs inside this special cave. The target process is the generation of dissolution biocidal metals, the secondary - is the generation of molecular hydrogen and the emission of atomic hydrogen. The emission of atomic hydrogen is explained by the Russell effect, the main information about which may be found in the work of the authors [12].

We will continue to study the nature of the Dew effect. For the day of writing this article, we were formulated several theories, that explaining this effect. However, we haven't yet succeeded in confirming or refuting that theories by the confirmed facts. Ethics don't allow us to cite unconfirmed theories in the scientific article, but we can note that none of the theories goes beyond modern concepts of existing of particles in the water phase or of the mechanisms of electrode processes. All theories somehow or other are related to the fact, that in the special cave of the prototype the period of activity of metal particles is prolonged. By "activity" we mean not only antiseptic efficacy, but also the preservation of the amount of the particle charge or its charge density under the given conditions. Also, it has been experimentally confirmed that the efficiency of Dew directly depends on the frame of the prototype chamber, its configuration and location. Anyway, the high efficiency of Dew is verified and reproduced many times. And its means that the definition of the nature of this effect is only a matter of time.

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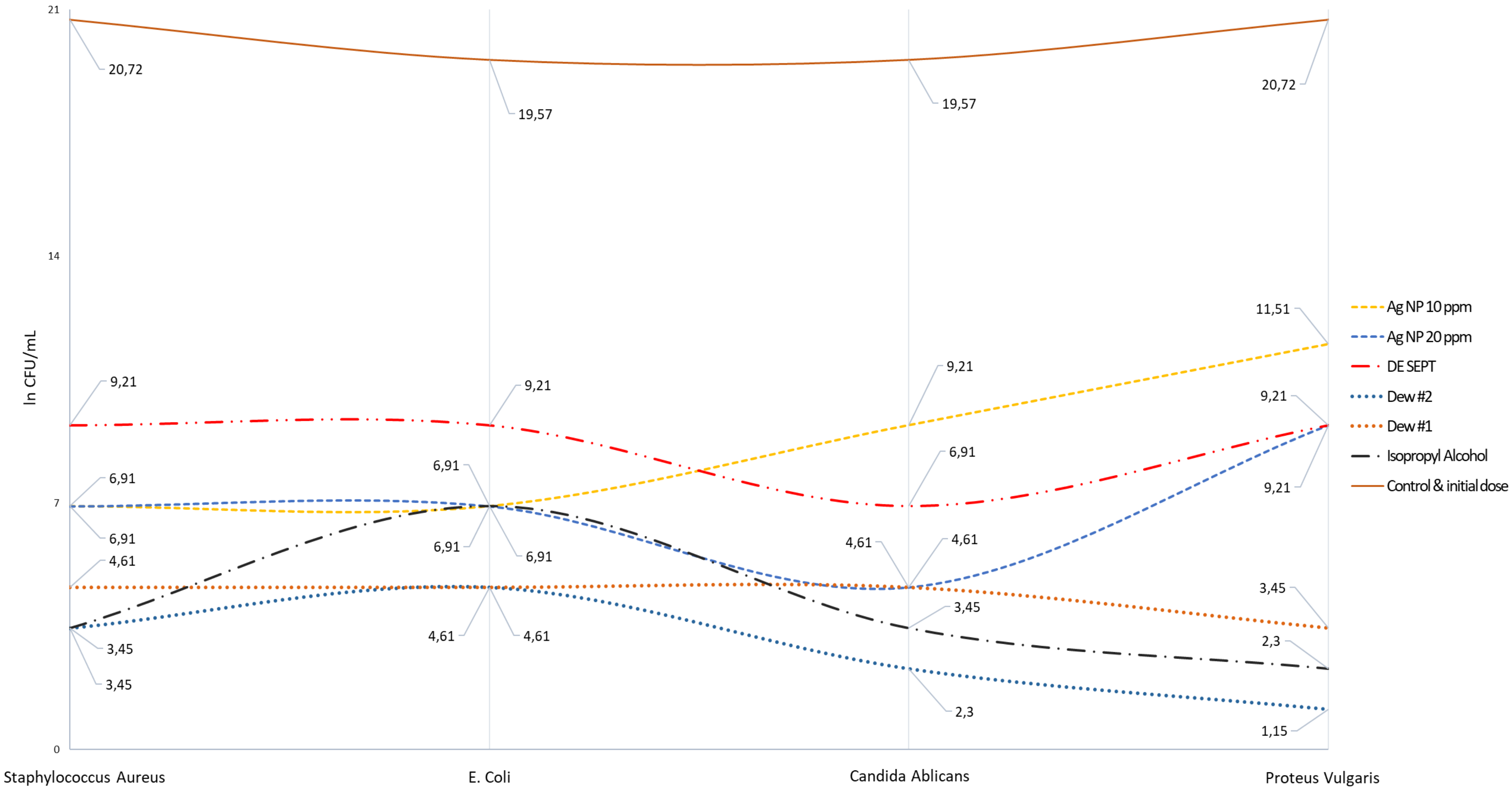

Figure 3. Graphical representation of the results of the "comparative test"

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Reasons for choosing a biocidal metal

The choice of biocidal metal for the creation of Dew was made in favor of silver. There are several reasons for it: first, the effect of in vivo silver compounds on the body as a whole, and on the skin in particular, has been sufficiently studied [9], [13]; secondly, there are expressly standards prescribing [14] or recommending [15] the safe and unsafe silver content in both drinking water and inhaled air; Thirdly, silver is successfully used in clinical practice for the treatment of burns [16], purulent wounds [17] and other injuries, i.e. there are many clinical data supporting both the effectiveness and safety of silver as an antiseptic agent. In favor of using of silver also loquitur our long-term experience of use silver-based disinfection systems, for disinfection of technical and recreational water. Note, that it's very likely to use other biocidal metals for the purpose of disinfecting the skin of hands or various surfaces. We don't exclude the possibility that copper-based Dew or copper alloys-based Dew exhibit similar or even identical antiseptic properties. In any case, our own research indicates this. However, the amount of data that we have received is not yet sufficient for final conclusions.

Conclusion

Summarizing, it can be concluded that the preparations of silver, in particular Dew, are a proven by time, and, in same time, promising antiseptics. The main active substance of Dew, atomic silver, demonstrates a multiply superior efficiency against test strains, in comparison with other silver compounds. Dew is also superior to an antiseptic, based on isopropyl alcohol, but atomic silver is devoid of many disadvantages of alcoholic antiseptics. For example, the formation of multidrug resistant strains of pathogenic microorganisms under the action of silver compounds is unlikely and is not described in the scientific literature; atomic silver hasn't restrictions to use, for example, it can be applied to the damaged skin, there is low risk of undesirable consequences when using Dew inwards, there is no danger of serious complications when the antiseptic hits the eyes and mucous membranes.

However, the final conclusion about the safety of Dew can be made after in vivo tests. We expect, that the results of done tests on laboratory animals will help us to get answers for not only the issue of safety of Dew, but also will help in determining the nature of the effectiveness of Dew.

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ANTISEPTIC SPRAY BASED ON STABILIZED SILVER PARTICLES: AN ANALYSIS OF ANTISEPTIC PROPERTIES AND COMPREHENSIVE COMPARISON

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Introduction. It's known that some antiseptic sprays based on alcohols can provoke the formation of multi-resistant strains of pathogenic microorganisms. In addition, alcoholic antiseptics has a number of restrictions to use, for example, they can't to be used in even the presence of micro-trauma on the skin, their ingression into the body and mucous membranes is unacceptable. Alternative can be natural antiseptics based on colloidal silver or silver nanoparticles, as well as silver in ionic form. However, such antiseptics has low efficacy against the most dangerous strains. Company Modern Biochem Technologies Ltd. announced the development of a portable device that generates a natural and safe antiseptic Dew, based on stabilized silver particles. Antiseptic Dew surpasses the vast majority of antiseptics based on silver, and is not inferior in effectiveness to antiseptics based on alcohols. This work is devoted to testing the declared characteristics of Dew and its comparison with antiseptics based on colloidal silver, silver nanoparticles and isopropyl alcohol. **Materials and methods.** To test the antiseptic effect of these agents, we used four test strains from the American Type Culture Collection: E. Coli ATCC 25922, Staphylococcus Aureus ATCC 25923, Candida Albicans ATCC 885-653 and Proteus Vulgaris ATCC 4636 with billion concentration of colony forming units in 1 ml ($10^8 – 10^9$ CFU/mL, ln CFU/mL = 19.57 ... 20.72). Sowing and screening of cultures were performed on sterile Petri dishes according to the standard procedure. Antiseptic Dew was prepared by prototype provided by Modern Biochem Technologies. The antiseptics of comparison were purchased in Kharkiv, Ukraine. The treatment of contaminated surfaces was performed using mechanical pump sprayers. In accordance with the internal protocol, 1 ml of antiseptic was sprayed from the distance of 10 cm onto the infected surface. To determine the silver content in the Dew, we were used atomic-absorption spectrometer MGA-925MD. Samples for analysis were prepared in accordance with the standard protocol for operation on MGA-925MD. **Results & Discussion.** For the research we conducted more than 200 microbiological tests. Based on the results of these tests, we can conclude that Dew is indeed superior to other silver-based antiseptics. Also, we can note that in one case, Dew surpassed the effectiveness of the antiseptic based on isopropyl alcohol, but in the second case was slightly less effective. Dew is effective against all four test strains, and the decrease in microbial load after treatment is very substantially - from 10^9 CFU/mL to 0-10^1 CFU/mL. The concentration of silver in Dew does not exceed 1.45 ppm and is on average 20 times lower than that of the other tested antiseptics based on silver. **Conclusion.** Based on the data obtained, we conclude that Dew is a promising antiseptic. Note that the prototype generated the antiseptic with stable characteristics throughout the study. Antiseptics of comparison were shown their effectiveness against test strains, however in one case Dew was surpassed them all in efficiency. In conclusion, we note that we recommend conducting in-depth tests, primarily aimed at determining the effect of Dew in vivo. We also recommend testing Dew on hospital-strains or other resistant strains.

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