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The research of antibacterial properties of decamethoxin, decasan, horosten

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Abstract

The study of Staphylococcus resistance to the antibacterial drugs Decamethoxin, Decasan, Horosten remains an important medical problem.

The aim of this study was to investigate the antistaphylococcal properties of Decamethoxin, Decasan, Horosten. It has been proven that qaterinary ammonium antiseptic drugs (Decamethoxin, Decasan, Horosten) have high antistaphylococcal properties. The bactericidal activity of Decamethoxin has been shown to be stable under adverse pH conditions of different microbial loading. Different concentrations of Decamethoxine have been shown to cause the formation of resistant variants of *Staphylococcus*, which lose the ability to form pigments and enzymes.

Key words: *Staphylococci*, antiseptics, properties, decamethoxin, decasan, horosten, resistance.

Background. Antibiotics, antiseptics are systematically used for the prevention and treatment of purulent-inflammatory diseases. The antimicrobial decamethoxine® belongs to Quaternary ammonium compounds (QACs), which is intensively studied by chemists,

microbiologists, and doctors of many specialties [1, 2]. is a part of Decasan® and Horosten® containe the main active substance decamethoxin, which is registered in Ukraine by the Ministry of Health and approved for the use in practices [3, 4].

The systematic use of antiseptic drugs is accompanied by the formation in various bacteria of resistance to antiseptics, which has increased into an urgent problem of medicine. Microbial resistance to antimicrobials has grown at an alarming rate over the past decade [5-8]. The research of antistaphylococcal properties of the antiseptic drugs Decamethoxin, Decasan, Horosten and substantiation of their effectiveness remains an important scientific direction [9-11].

The aim is to research of antistaphylococcal properties of Decamethoxin (DCM), Decasan, (DS), Horosten (HS).

Materials and methods. Decamethoxin. Chemical name: 1,10-Decamethylene bis (N, N-dimethylmethoxycarbonylmethyl) ammonium dichloride. White powder. DCM belongs to pharmacological group of "Antisaptis and desinfectants". Its ATC code is DO8A. The registration certificate number of powder (substance) of decamethoxin is UA/12180/01/01 in accordance with Order No. 341 of Ministry of Health of Ukraine since 29.03.2017. DCM is a surface active substance, which provides the violation of the integrity of the cell wall of bacteria, consisting of proteins and lipopolysaccharides. The drug DCM potentiates the action of antibiotics, and obtains a bactericidal, virucidal, fungicidal, anti-inflammatory, desensitizing effects. It leades to the elimination of resistance plasmids in bacteria.

Decasan® contains decamethoxin 0.2 g and sodium chloride 9.0 g per liter of solution. Product: 0.02% decamethoxin solution for 0.9% sodium chloride solution in 100, 200 and 400 ml vials. DS also belongs to pharmacological group of "Antisaptis and desinfectants". Its ATC code is DO8A. The registration certificate number of Decasan® No UA/5364/01/01 in accordance with Order of Ministry of Health of Ukraine scince 22.12.2016, No 1391. DS is practically not absorbed by mucous membranes, intact skin and wound surface.

Horosten® contains 0.25 mg of decamethoxin in 1 ml of the preparation such excipients as ethyl alcohol, glycerin, citral solution. HS has a bactericidal, fungicidal, antiviral, anti-inflammatory effect. HS

HS belongs to pharmacological group of "Antisaptis and desinfectants" (ATC code is DO8A). While usage of HS the susceptibility of antibiotic-resistant strains of Staphylococci reveals to antibiotics. The registration certificate number of the remedy of Horosten® as solution for external usage No UA/2048/01/01in accordance with Order of Ministry of Health of Ukraine scince 19.05.2014, No 340. HS is used for rubbing, lotions. This drug is

practically not absorbed by the mucous membrane, skin, or wound surface. DCM is also known to alter the enzymatic activity of bacterial cells by inhibiting enzyme systems.Hydrophobic radical of DCM promotes large-scale binding of the drug to the *Staphylococcus* cell membrane. The DCM cationic radical first reduces and then neutralizes the electrical charge of the microbial cell wall. Due to the presense of DCM, HS is highly active against antibiotic-resistant bacteria (penicillin, chloramphenicol, tetracycline, streptomycin, gentamicin, neomycin, oleandomycin, cephalosporins, fluoroquinolones, etc.).

The anti-staphylococcal bacteriostatic, bactericidal effect of DCM, DS, HS was studied in 130 museum and clinical strains of *Staphylococcus*, had been isolated from patients. Microbiological research was carried out at the Department of Microbiology, Virology and Immunology in National Pirogov Memorial Medical University, Vinnitsa. *Staphylococcus* strains had typical morphological, cultural, biochemical properties, and were characterized by different sensitivity to antibiotics and antiseptic drugs DCM, DS, HS. The experiments were performed on 16-20 hour *Staphylococcus* cultures. The bacterial suspension density was $1 \cdot 10^3$ - $1 \cdot 10^{10}$ CFU/ml. The experiments were accompanied by appropriate control samples.

The work was carried out within the framework of the scientiffic research "Study of the multi-vector properties of the medicinal antimicrobial drug decamethoxine®" (state registration number 0115U006000). Experiments on animals were performed in accordance with the basic bioethical rules of the Law of Ukraine "On the Protection of Animals from Cruelty" of 13.12.2010 № 3447-15, Resolution "Medicines. Good Laboratory Practice", approved by Order No. 95 of the Ministry of Health of Ukraine of February 16, 2009, taking into account the norms used in international practice. Studies on the formation of resistance to DCM, DS, HS were performed on *Staphylococcus* strains.

The susceptibility of *Staphylococcus* strains to DCM, DS, HS was determined by sequential serial dilutions in meat-peptone broth (pH 7.2). In each test tube titration row were sown 100 thousand CFU/ml of nutrient medium. At the same time, the experiments were accompanied by appropriate controls of the nutrient medium, culture of bacteria, antiseptic drugs. The tubes were incubated in a thermostat at 37° C for 24 hours. The smallest amount of the antiseptic, which caused a complete delay in the growth of *Staphylococci*, was taken as the minimum inhibition concentration (μg/ml). The bactericidal activity of the antiseptics DCM, DS, HS was determined after seeding from tubes on meat-peptone agar (MPA). The absence of growth of colonies of *Staphylococci* on cups with IPA was taken into account after 24-72 hours.

In the following series of experiments, the anti-staphylococcal activity of DCM, DS, HS was determined at different pH of the culture medium (pH 6.0; 7.2; 8.0). The study of the susceptibility of strains of *Staphylococcus* was carried out with a microbial load of $1 \cdot 10^3$; $1 \cdot 10^6$; $1 \cdot 10^9$ CFU/ml of nutrient medium.

While using antimicrobials in medical practice, the specialists have established the formation of resistance in clinical strains of *Staphylococcus* to antibiotics and antibacterial agents. To characterize the effectiveness of antiseptics, there was investigated the rate of resistance formation in three strains of *Staphylococcus* to DCM, DS, HS. For this, after determining the minimal anti-staphylococcal activity of DCM, DS, HS, the strains of these bacteria were screened in the presence of increasing sub-bacteriostatic concentrations of antiseptic drugs. In total, 35 passages of *Staphylococci* were performed. After every 5 passages, morphology, tinctorial, cultural, biochemical properties, susceptibility of *Staphylococcus* strains to DCM, DS, HS were studied. All experiments were accompanied by appropriate controls. The numerical results of the experiments were subjected to statistical processing using the methods of mathematical variational statistics of the "Statistica 6.0" computer program.

Results and discussion. There was presented anti-staphylococcal activity of DCM, DS, HS in the Table 1.

Table 1

Antimicrobial activity of DCM, DS, HS against clinical strains *S.aureus* (n 130)

Antimicrobials	MIC[x]	MBcC[xx]
	M±m, µg/ml	
Decamethoxin®	1,27±0,25	2,84±0,48
Decasan®	3,23±0,31	4,21±1,08
Horosten®	4,34±0,63	5,86±1,57

[x] – minimal inhibitory concentration;

[xx] – minimal bactericidal concentration.

In accordance to the data, presented in table 1, the inhibition of Staphylococci was registered, when DCM, DS, HS had been used in concentrations from 1,27±0,25 by 4,34±0,63 µg/ml. Bactericidal activity of the studied antiseptics varied in accordance to the antiseptics' MBcC 2,84±0,48 - 5,86±1,57 µg/ml. Clinical isolates of *Staphylococcus* were found to be susceptible to bacteriostatic (inhibitory) bacteriscidal concentrations of DCM. Clinical strains

of *Staphylococcus* were the most resistant to HS, that may be related with its chemical composition.

In conditions when nutrient media with pH 6,0 had been used, there was registered MIC of DCM against *S.aureus 209* above 0,97 μg/ml. Bactericidal activity of *S.aureus 209* in the same conditions was determined when 1,95 μg/ml of DCM had been used. In a slightly alkaline nutrient medium (pH 7.2), MBcC of DCM was 0.24 μg/ml, and MIC did not exceed 0.12 μg/ml. When the pH of the medium was 8.0, the antimicrobial activity of DCM did not differ in the medium with a pH of 7.2. Therefore, with increasing alkalinity of the nutrient medium, the antimicrobial activity of DCM did not change. The shift of the pH of the nutrient medium to the acidic side was accompanied by a slight decrease in the anti-staphylococcal activity of DCM.

In the study there was found, that *Staphylococcus* sensitivity to DCM was dependent on the number of bacterial cells per unit volume. However, the increase in the sowing dose of *S.aureus 209* 10 thousand times reduced MIC, MBcC of DCM 4 times.

Staphylococcus resistance formation to antimicrobials is a biological phenomenon, that ensures the preservation of *Staphylococci* in adverse environmental conditions. The *Staphylococcus* population has the ability to become resistant to antibacterial drugs. It was proved that the ability of *Staphylococci* to acquire resistance to antiseptics adversely affected their effectiveness in the process of antiseptic prophylaxis, antiseptic therapy of purulent-inflammatory diseases.

Antiseptic resistance in *Staphylococci* apparently causes changes in the bacterial cell genome due to mutations. The selective action of antiseptics on Staphylococcal cells is accompanied by the elimination of sensitive cells in the bacterial population, the survival and spread of resistant variants of these bacteria. Antiseptic resistant Staphylococcus variants are characterized by their slowdown by the rate of their reproduction and growth in nutrient media.

For the research of the resistance formation there were studied three strains of *Staphylococcus* (*Staphylococcus aureus* ATCC 25923, *S.aureus* 2531, *S.epidermidis* 5736). A pure *Staphylococcus* culture was isolated from one colony. Pure *Staphylococcus* cultures were characterized by typical morphological, tinctorial, cultural, biochemical properties. The morphology of the studied bacteria was investigated by a conventional technique using an immersion microscope. The cultural, biochemical characteristics of *Staphylococci* in control resistant to DCM, DS, HS variants of bacteria were studied in solid, liquid nutrient media.

The results of the study of resistance formation to DCM, DS, HS in strains of *Staphylococcus aureus* are presented in the Figure 1 and Table 2.

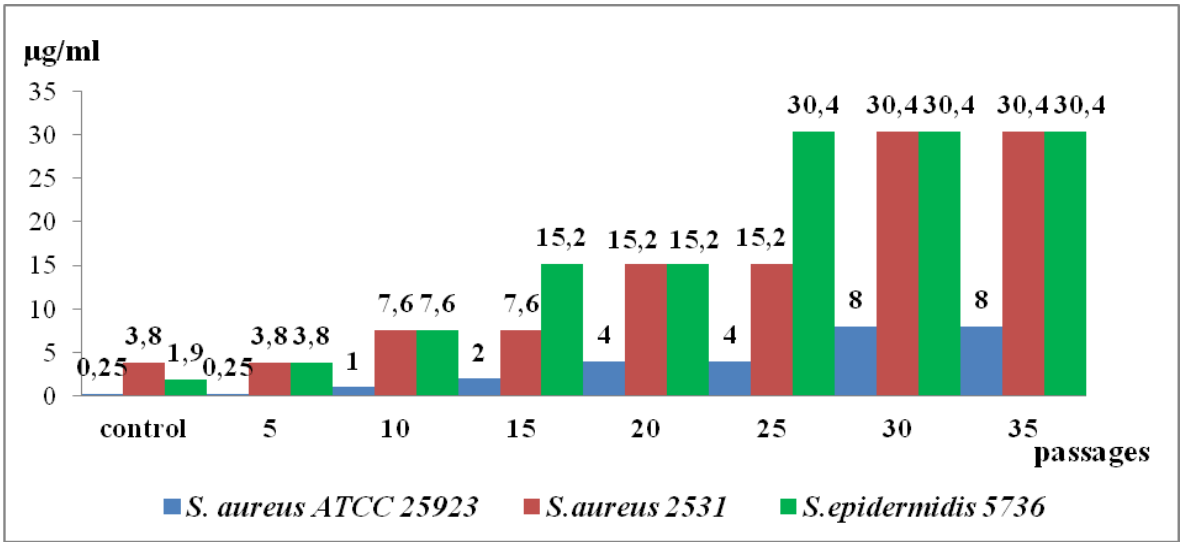

Fig. 1. Dynamics of resistance formation in strains of *Staphylococcus* to DCM.

In the next series of experiments, the formation of resistance in *Staphylococcus* strains to decasan was studied (Tabl.2).

In accordance to the received data, presented in the table 2, the formation of resistance to DCM in *Staphylococci* was characterized by the following indicators. Thus, in the control experiments the sensitivity of *Staphylococcus* strains to DCM ranged 0.25-3.80 µg/ml. After 10 passages of bacteria, the sensitivity to DCM increased and was 1-7,60 µg/ml. After 20 passages of cultivation the was found the resistance of *Staphylococci* to 4-15.20 µg/ml of DCM, depending on the individual properties of each strain. Over the next 35 passages of *Staphylococci* cultivation, bacterial resistance reached in *S.aureus ATCC 25923* to 8 µg/ml of DCM (increased 32-fold), in *S.aureus 2531* - to 30.40 µg/ml of DCM (increased 8-fold) and in *S.epidermidis 5736* - to 30, 40 µg/ml of DCM (increased 16-fold).

The study of the properties of *Staphylococcus* strains, which had obtained the resistance to antiseptics DCM, DS, HS, showed the following. During the formation of antiseptic resistance, changes in the morphology of *Staphylococci* were observed. These bacteria formed giant, tiny cells; lost their clunky location. On solid nutrient media, resistant strains formed atypical dwarf, rugged R-shaped colonies. Atypical variants of *Staphylococci* lost the ability to produce pigment. *Staphylococci*, while forming R-variants, lost their ability to hydrolyze sugars, polyhydric alcohols in comparison with control cultures samples.

Changes in the morphology of cultural biochemical traits during the formation of antiseptic resistance were accompanied by changes in their enzyme activity.

Table 2

Dynamics of resistance formation to DCM, DS, HS in strains of *Staphylococcus*

Passage of bacterial cultvation	*S.aureus ATCC* 25923		*S.aureus* 2531		*S.epidermidis* 5736	
	MIC	control sensitivity change	MIC	control sensitivity change	MIC	control sensitivity change
1	2	3	4	5	6	7
Decamethoxin						
Susceptibility of the bacterial strain (control)	0,25	-	3,80	-	1,90	-
5 passage	0,25	-	3,80	-	3,80	2
10 passage	1,0	4	7,60	2	7,60	4
15 passage	2,0	8	7,60	2	15,20	8
20 passage	4,0	16	15,20	4	15,20	8
25 passage	4,0	16	15,20	4	30,40	16
30 passage	8,0	32	30,40	8	30,40	16
35 passage	8,0	32	30,40	8	30,40	16
Decasan						
Susceptibility of the bacterial strain (control)	0,48	-	0,48	-	0,96	-
5 passage	1,90	4	1,90	4	1,90	2
10 passage	1,90	4	1,90	4	1,90	2
15 passage	3,80	8	3,80	8	3,80	4
20 passage	7,60	16	7,60	16	3,80	4
25 passage	15,20	32	7,60	16	7,60	8
30 passage	15,20	32	15,20	32	15,20	16
35 passage	30,40	64	30,40	64	30,40	32
Hororsten						
Susceptibility of the bacterial strain (control)	1,90	-	1,90	-	3,80	-
5 passage	1,90	-	3,80	2	3,80	-
10 passage	3,80	2	7,60	4	7,60	2
15 passage	7,60	4	7,60	4	7,60	4
20 passage	15,20	8	15,20	8	15,20	8
25 passage	15,20	8	15,20	8	15,20	8
30 passage	30,40	16	30,40	16	30,40	16
35 passage	30,40	16	30,40	16	30,40	16

Conclusions

1. Medicinal antiseptic drugs decamethoxine, decasan, horsten have high antistaphylococcal properties.

2. The minimal bactericidal effect of decamethoxin remaims its stabilty under unfavorable conditions (pH, microbial load) of *Staphylococcus* cultivation.

3. DCM, DS, HS inhibit the formation of resistant *Staphylococcus* variants with low initial resistance. *Staphylococci* on nutrient media in the presence of antiseptics form R-forms of bacteria, lose the ability to form pigment and enzymes. Therefore, resistant to DCM, DS, HS variants of *Staphylococci* have antipypic, morphological, cultural, biochemical properties, which should be taken into account during the microbiological diagnosis of diseases and in the course of the use of etiotropic treatment.

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