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SUBSTANTIATION OF OVERCOMING OF ANTIBIOTIC RESISTANCE IN *ACINETOBACTER BAUMANNII* CLINICAL STRAINS BY USAGE OF DECAMETHOXINUM

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In the manuscript there are presented the data of susceptibility of clinical strains of *A.baumannii* to antibiotics ampicillin/sulbactam, cefoperazone, cefoperazone/sulbactam, meropenem, imipenem, amikacin, ciprofloxacin, gatifloxacin and antiseptic decamethoxinum (DCM). The decreasing of susceptibility of Acinetobacteria was found to cephalosporins, carbapenems in 2011-2015 years. High resistance of *A.baumannii* to ciprofloxacin (96,1 %), gatifloxacin (95,8%) was found out. In the research *in vitro* the increasing of susceptibility of clinical strains of *A.baumannii* to antibiotics, when sub-inhibitory concentrations of DCM had been used, were proved.

Key words: antibiotics, acinetobacteria, decamethoxinum, resistance.

The role of non-fermrntive Gram-negative bacteria in the origin of difficult forms of medical care aquired infections stadily increases. The list of resistant to antimicrobial therapy opportunistic bacterial infections of didderent localiztion has been recently enriched by microbial complications related with *Acinetobacter baumanii*, which rapidly has found its prominent place among problematic pathogens (ESKAPE). *Acinetobacter*, as other representatives of this group of bacteria, causes difficult diseases, capable to generalization [1, 2].

Recommended for the therapy antibiotics lose their effectiveness against *A. baumanii*, casing the selection of resistant strains among them. Unfortunately, many years practice in struggling infections, caused by non-fermenting Gram-negative bacteria, shows that usage of new antibiotics, escalation of antimicrobial therapy gives the possibility temporarily solve the problem of resistance of these bacteria to antibiotics. The study of the resistance and search of the ways of its overcoming is among urgent tasks in microbiology [3, 4].

Aim – microbiological substantiation of the overcoming of resistance in clinical strains of *A. baumannii* on the basis of synergic antimicrobial activity of antibiotics and antiseptic decamethoxinum.

Materials and methods

The research was carried out on clinical strains of 190 *A.baumannii*, isolated from patients with burn disease, who underwent treatment in Burn Centre of Vinnytsya Regional Clinical Hospital named after N. I. Pirogov in 2011-2015 years. Clinical isolates of *A.baumannii* were received from patients with 2^{nd}b – 3^{rd} degree burns. They had total burned body surface area 30,0 - 85,0 %. All patients underwent surgery (early necrectomy during first three days after trauma and xenodermograft pastics). Also, they received complex general (balanced infusion and transfusion, antibacterial, symptomatic) and topical treatment with antiseptics and wound healing remedies according to the standard protocols of treatment of such patients. Microbiological research of biological material, which had been received from patients before antibiotics were administered, was done in the Bacteriological laboratory of the Department of Microbiology in National Pirogov Memorial Medical University, Vinnytsya.

The sensitivity of clinical strains of *A.baumannii* to such antibiotics as: ampicillin/sulbactam, cefoperazone, cefoperazone/sulbactam, meropenem, imipenem, amikacin, ciprofloxacin, gatifloxacin and antiseptic decamethoxinum (DCM; Registration certificate № UA/14444/01/01 since 24.06.2015. Order of Ministry of Health of Ukraine № 373) was determined [5]. Expediency of the use of mentioned antibiotics for the treatment of *A.baumannii* mediated infections was estimated according to the quantity (%) of sensitive, intermediate and resistant strains of total sample (n=190). The antimicrobial susceptibility testing of *A.baumannii* strains was done by using standard disk-diffusion and double serial dilution tests (Order of Ministry of Health of Ukraine №167 since 05.04.2007 p. "On approval of guidelines determining the sensitivity of microorganisms to antimicrobials"). While analyzing *A. baumanii* susceptibility to antibiotics we guided by EUCAST expert rules in antimicrobial susceptibility testing [6, 7].

The study of the influence of antiseptic decamethoxinum on the sensitivity of *Acinetobacteria* to antibiotics was carried out on clinical strains of 35 *A.baumannii*, which had been blind randomly selected from the total number of research samples. The influence of DCM on the sensitivity of *A. baumanii* to antibiotics was estimated while comparative analysis of minimal inhibitory concentrations (MIC) of an antibiotic, been received in double serial dilution test in the nutrient broth, with those ones, been obtained in the nutrient broth, containing sub-minimal inhibitory concentrations of DCM (MIC^{DCM}). For an objective assessment of the degree of reliability of the results variation-statistical analysis method was used in the research. The arithmetic mean (M), the average error of the arithmetic mean (m), the average error (t), the reliability of results the difference (p) were calculated by means of Statistica 6.0.

Results and discussion

Results of the research have shown low susceptibility in clinical strains of *Acinetobacteria*, received from patients in 2011-2015 years. (table 1). The changes of the profile of antibiotic susceptibility of *A.baumannii* has being found during five years of our study. In 2011 year the majority of our strains were found to be sensitive to cefoperazone/sulbactam (55,6%), imipenem (57,1%), meropenem (52,8%). At the same time the susceptibility to ampicillin/sulbactam and ciprofloxacin has been found in a small number of *A.baumannii* strains (3,4% and 2,7%, respectively). After five years of research there was found the decreasing of susceptibility of *Acinetobacteria* to all studied antibiotics except ampicillin/sulbactam.

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Table 1. The susceptibility to antibiotics of clinical strains of *A. baumannii* (n = 190), isolated from patients with severe burns in 2011-2015. (%)

Antibiotics	2011 p. (n= 38)			2012 p. (n= 40			2013 p. (n= 27)			2014 p. (n= 34)			2015 p. (n= 51)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin/sulbactam	3,4	0	96,6	40,0	16,7	43,3	52,4	23,8	23,8	58,6	24,1	17,3	70,21	10,64	19,15
Cefoperazone/ sulbactam	55,6	16,6	27,8	82,3	11,8	5,9	72,2	16,7	11,1	46,9	28,1	25,0	32,0	32,0	36,0
Imipenem	57,1	2,9	40,0	25,6	7,7	66,7	47,8	4,4	47,8	32,3	3,2	64,5	36,8	5,3	57,9
Meropenem	52,8	13,9	33,3	33,3	0	66,7	50,0	4,2	45,8	25,8	3,2	71,0	13,5	2,7	83,8
Amikacin	13,5	8,1	78,4	18,2	12,1	69,7	8,7	13,0	78,3	9,7	16,1	74,2	9,8	11,8	78,4
Ciprofloxacin	2,7	21,6	75,7	10,3	0	89,7	8,3	4,2	87,5	6,25	0	93,75	3,9	0	96,1
Gatifloxacin	32,4	2,7	64,9	18,7	9,4	71,9	16,7	8,3	75,0	6,3	3,1	90,6	4,2	0	95,8

Footnote: S – susceptible; I – intermediate; R – resistant

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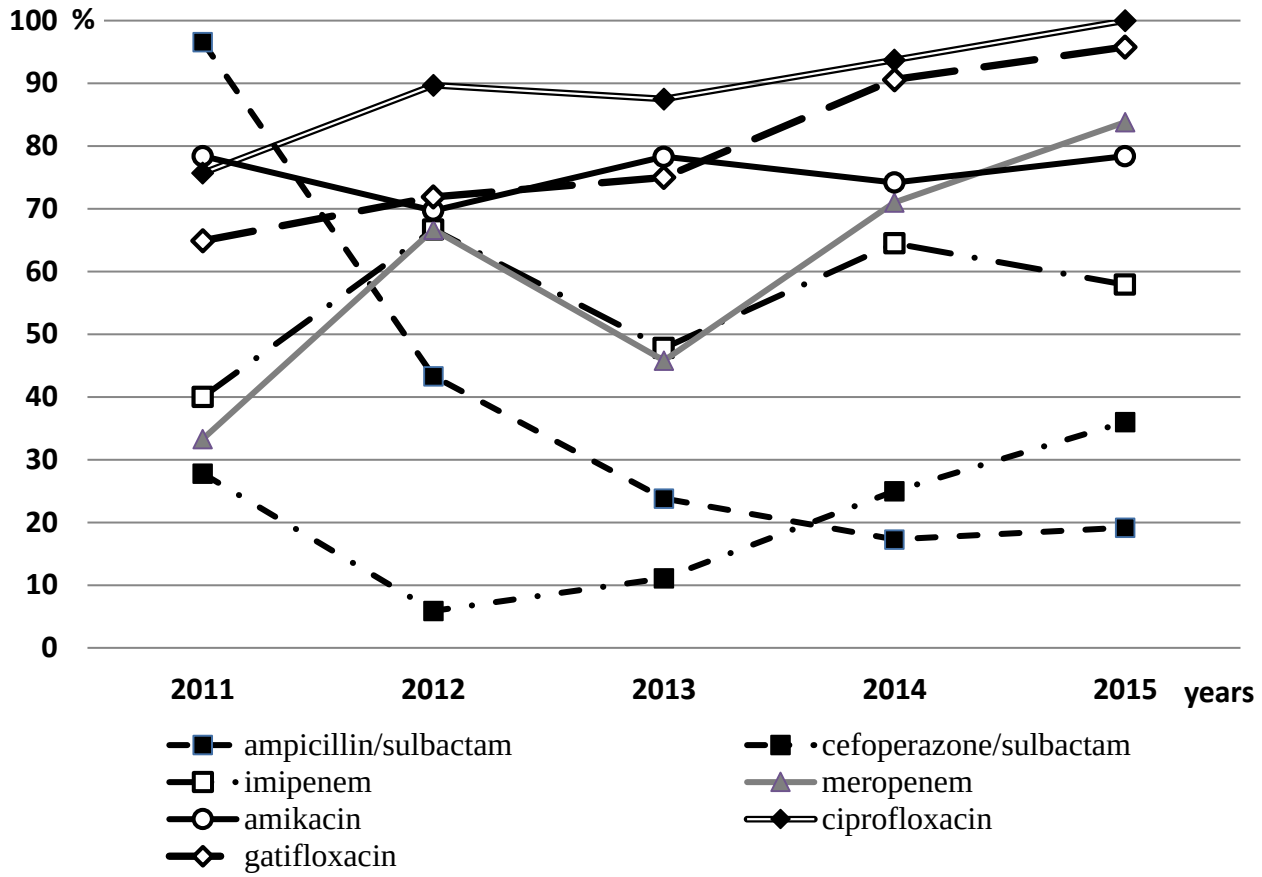

Fig.1. The dynamics of antibiotic resistance of clinical strains of *A.baumannii* in 2011-2015 (%).

Thus, the number of susceptible strains of *A.baumannii* reached to 70,2 %. However, it can be explained first of all, by the selective pressure of antibiotics, used for the treatment of infections caused by these microorganisms.

In dynamics (2011-2015 years) the increasing incidence of resistant strains of *A.baumannii* to antibiotics was found (fig.1). According to received data, antibiotics, widely recommended nowadays for the treatment of *Acinetobacter spp.* associated infections, have been losing their effectiveness gradually, as evidenced by the growing number of resistant strains by research period. Accordingly to the results of microbiological antimicrobial susceptibility testing the decreasing incidence of resistance among clinical strains of *A.baumannii* to ampicillin/sulbactam can be explained by the limited use of this drug in the treatment of wound infection in this clinic since 2011 year, when it efficacy was no more than 3,4 %.

Simultaneously, high resistance to fluoroquinolones (ciprofloxacin – 96,1%; gatifloxacin – 95,8%) in *Acinetobacteria* was determined in 2015 year. That relatively could had happened because of wide use of these antibiotics for treatment patients with burn infection. Alike dynamics was found in carbapenems. In particular, clinical strains of *A.baumannii* were resistant to meropenem (83,8%) and imipenem (57,9%). The received experimental data showed the tendency of changing antibiotic susceptibility profile in *A.baumannii* from multiresistant (MDR) to extensivelyresistant (XDR). This tendency is a serious problem in nowadays treatment of infections, caused by this group of microorganisms [6].

The search for possible ways of improvement of sensitivity in clinical strains of bacteria to antimicrobial therapy and the reducing of the rate of formation of resistance in microbial isolates to antimicrobials are among the most important tasks of nowadays medicine. For this purpose we have investigated *in vitro* combined antimicrobial activity of antiseptic remedy DCM and mentioned earlier antibiotics against 35 clinical strains of *A.baumannii*. These microbial isolates accordingly to their antibiotic susceptibility were subdivided into such groups as: resistant to 1 or 2 antibiotics, belonging to the same chemical group (11 %); multi-resistant (MDR) – obtained resistance to all of studied antibiotics except one of pharmacological class of antibiotics (60 %); and extensively resistant (XDR) strains – which demonstrated sensitivity only to one of antibiotics (29 %).

Results of the study of susceptibility of *Acinetobacteria* to antibiotics demonstrated the decreasing of MIC of drugs in 1,5-4 times in media, containing sub-MIC of DCM. This was particularly expressed in strains of resistant microorganisms (tabl. 2; 3). There were proved the revealing or increasing of the susceptibility rate in studied clinical strains of *A.baumannii* to antibiotics in the presence of DCM.

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Table 2. The antimicrobial action of antibiotics and DCM antiseptic against clinical strains of *A.baumannii* (n=35)

Antibiotic	Susceptible		Intermediate		Resistant	
	Number of strains (n)	MIC (M±m, mkg/ml)	Number of strains (n)	MIC (M±m, mkg/ml)	Number of strains (n)	MIC (M±m, mkg/ml)
Cefoperazone	2	7,8±0	13	22,8±2,25	20	322,4±76,9
Cefoperazone/ sulbactam	9	7,8±0	15	26,03±1,97	11	287,5±61,23
Ampicillin/ sulbactam	5	6,03±0,61	18	25,2±1,85	12	90,9±9,84
Meropenem	9	1,85±0,41	2	15,6±0	24	107,3±22,21
Imipenem	14	2,18±0,4	2	15,6±0	19	123,3±33,3
Amikacin	6	12,48±1,91	9	31,25±0	20	227,0±27,9
Ciprofloxacin	2	0,98±0	2	3,9±0	31	127,3±21,68
Gatifloxacin	4	1,22±0,24	8	4,39±0,49	23	125,0±27,28
Decamethoxinum	15	6,07±0,47	15	18,7±1,67	5	200±82,4

Table 3. The influence of sub-inhibitory concentrations of DCM on the susceptibility of *A.baumannii* to antibiotics (n=35)

Антибіотик	Susceptible		Intermediate		Resistant	
	Number of strains (n)	MIC^{DCM} (M±m, mkg/ml)	Number of strains (n)	MIC^{DCM} (M±m, mkg/ml)	Number of strains (n)	MIC^{DCM} (M±m, mkg/ml)
Cefoperazone	13	6,18±0,58	16	26,03±1,97	6	72,9±10,4
Cefoperazone/ sulbactam	22	4,92±0,59	10	26,03±2,6	3	62,5±0
Ampicillin/ sulbactam	17	5,85±1,13	17	19,5±1,75	1	62,5±0
Meropenem	11	0,73±0,17	14	11,4±1,12	10	59,4±11,8
Imipenem	15	1,09±0,28	13	12,35±1,16	7	89,3±29,3
Amikacin	18	9,41±1,06	6	31,25±0	11	79,5±8,8
Ciprofloxacin	4	0,4±0,08	7	3,25±0,4	24	32,5±5,73
Gatifloxacin	15	0,9±0,19	5	4,88±0,98	15	31,24±4,58

The data, representing results of overcoming bacterial resistance to antibiotics are shown at fig. 2; 3.

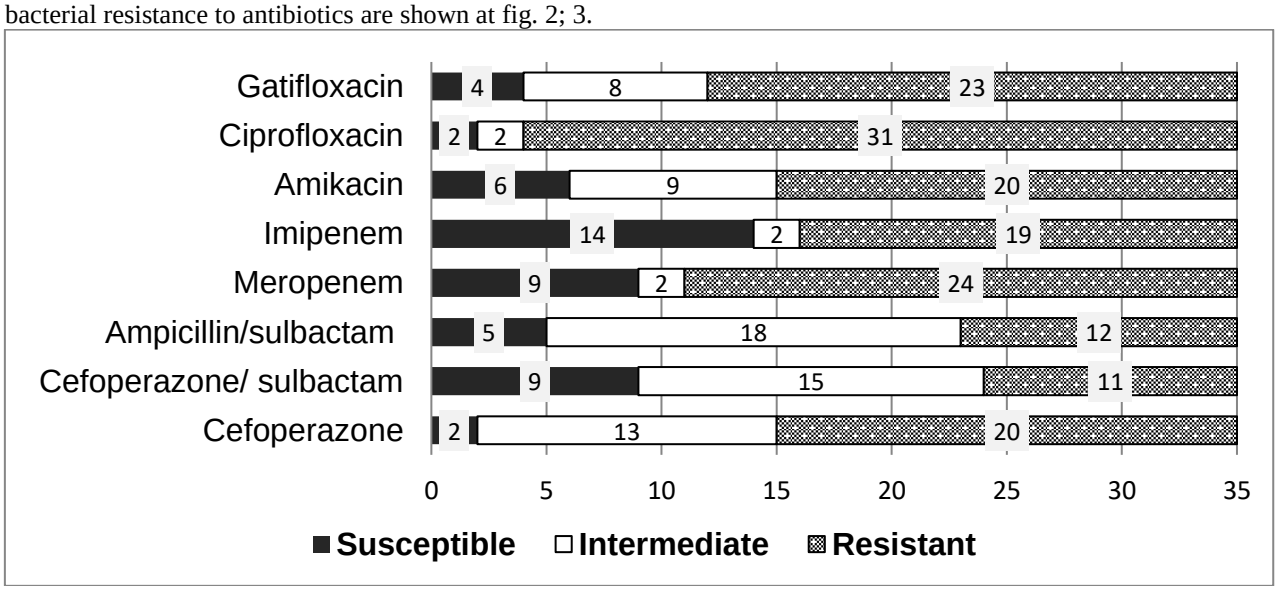

Fig. 2. The characteristics of susceptibility of clinical strains *A.baumannii* to antibiotics (n = 35, the absolute data).

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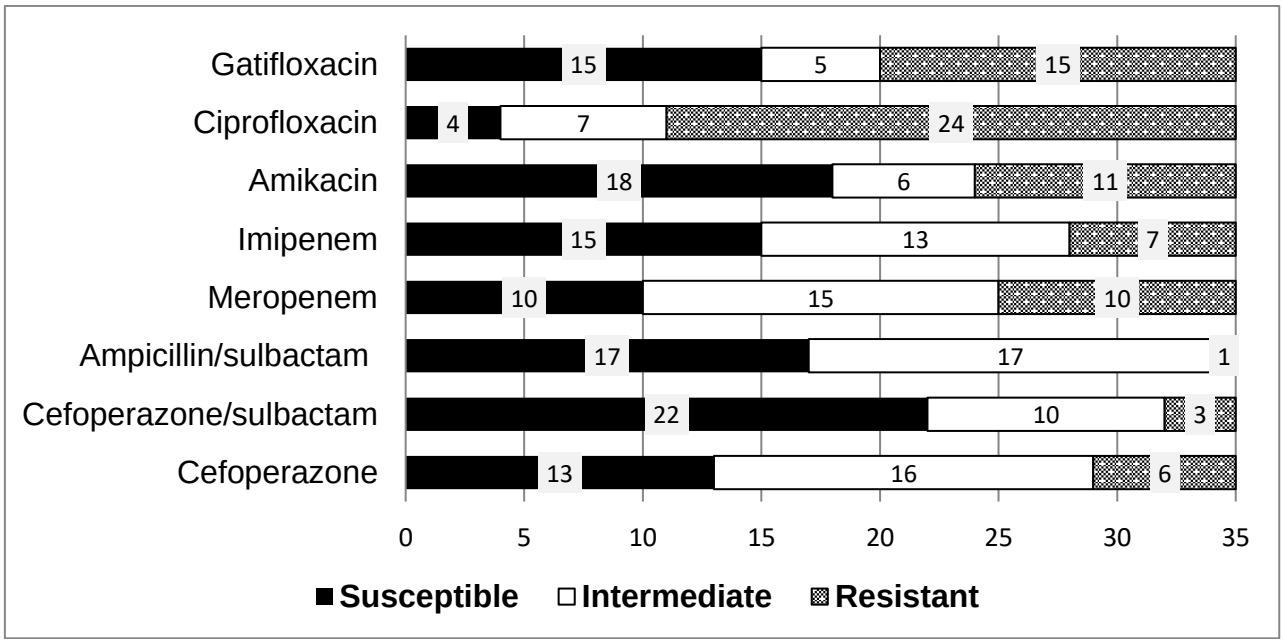

Fig. 3. The characteristics of susceptibility of clinical strains *A.baumannii* to antibiotics in the presence of decamethoxinum (n = 35, the absolute data).

There was found, that the incidence of susceptible strains of *Acinetobacteria* to all studied antibiotics increased, when DCM had been used. Even effectiveness of fluoroquinolones and cefoperaxone against resistant to them *A.baumannii* was optimized. Positive influence of sub-MIC of DCM on optimizations of susceptibility of *A.baumannii* to antibiotics happened due to various mechanisms of its antimicrobial effect (inducing the permeability of cell walls of bacteria, the inactivation of bacterial enzymes, elimination of resistance plasmids, etc.), which laded to overcoming the resistance antibiotics in *Acinetobacteria*.

Conclusions

1. Under the selective influence of antibiotic therapy with inhibitor-combined β-lactams, carbapenems, fluoroquinolones, aminoglycosides the increasing incidence of antibiotic resistance happens among *A.baumannii*, causative agents of infectious complications in patients with burn disease.
2. Antiseptic remedy decamethoxinum increases antibiotic sensitivity of strains *A.baumannii*, which obtain high innate resistance and have the ability for its rapid formation in hospital conditions. Such properties of decamethoxinum, while its use in complex treatment of patients, broaden prospects for improvement of the management of *Acinetobacter spp*. mediated complications of burn disease.

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SUBSTANTIATION OF OVERCOMING OF ANTIBIOTIC RESISTANCE IN *ACINETOBACTER BAUMANNII* CLINICAL STRAINS BY USAGE OF DECAMETHOXINUM®

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Introduction. Non-fermenting Gram-negative bacilli are known as one of the most frequent causative agents of hospital-acquired infections. *Acinetobacter baumanii*, as causative agent of infection complications of different localization, has obtained recently high resistance to antibiotics and has belonged to ESKAPE group of pathogens. Antimicrobials, recommended for the prophylaxis ant therapy of hospital-acquired infections, have been failing in their effectiveness and lead to selection of antibiotic resistant strains of *A. baumanii*.***The aim*** of this research was to substantiate the way of overcoming of resistance in clinical strains of *A.*

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baumannii, by means of synergic antimicrobial activity of antibiotics and antiseptic decamethoxinum®. **Material and methods.** The research was carried out on 190 clinical strains of *A.baumannii*, isolated from patients with burn disease in 2011-2015 years. The sensitivity of clinical strains of *A.baumannii* was determined to such antibiotics as ampicillin/sulbactam, cefoperazone, cefoperazone/sulbactam, meropenem, imipenem, amikacin, ciprofloxacin, gatifloxacin and antiseptic decamethoxinum® (DCM; Registration certificate № UA/14444/01/01 since 24.06.2015. Order of Ministry of Health of Ukraine № 373). The sensitivity of *A.baumannii* to antibiotics and DCM were done by means of disc-diffusion method and serial dilution one (Order of Ministry of Health of Ukraine №167 since 05.04.2007 year; EUCAST Expert rules). The research of the influence of antiseptic DCM on the sensitivity of acinetobacteria to antibiotics was studied on 35 clinical strains of *A.baumannii*, drafted from the general number of isolates enrolled in the research. For this, the sensitivity of *A.baumannii* to antibiotics in the presence of subminimal inhibitory concentrations (subMIC) of DCM was identified. The received experimental data were analyzed by «Statistica 6.0». **Results and discussion**. The changes of antibiotic sensitivity profile of *A.baumannii* during five years were shown. The sensitivity of *A.baumannii* to majority of antibiotics, selected for study, was found to decrease significantly. But the only ampicillin/sulbactam was found to have vice versa tendency. We found the rising quantity of antibiotic resistant strains of *A.baumannii*. At the same time, high resistance of acinetobacteria in 2015 year was found to fluoroquinolones (ciprofloxacin – 96,1%; gatifloxacin – 95,8%). The *in vitro* research of combined activity of antiseptic remedy DCM and early mentioned antibiotics against clinical strains of *A.baumannii* demonstrated the reveal antibiotic effectiveness. As follows, minimal inhibitory concentrations of antibiotics decreased in 1,5-4 times in the mediums, had contained subMIC of DCM. Especially vividly this tendency was found in resistant clinical strains. **Conclusion**. Under selective influence of antibiotic use of protected by inhibitors β-lactams, carbapenems, fluoroquinolones, aminoglycosides the antibioticresistance increases in *A.baumannii*, causative agents of infectious complications in patients with burn disease. The antiseptic remedy decamethoxinum® helps to improve antibiotic sensitivity in resistant *A.baumannii*.
Key words: antibiotics, acinetobacteria, decamethoxinum, resistance.

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