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BIOFILM FORMATION IN BOVINE MASTITIS PATHOGENS AND THE EFFECT ON THEM OF ANTIMICROBIAL DRUGS

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ABSTRACT

The ability of bacteria to produce a biofilm is considered an important virulent property in pathogenesis of mastitis. The purpose of studies is to investigate the ability to form biofilms, their density, to determine and compare the sensitivity to antibacterial drugs of planktonic and biofilm forms of the main bovine mastitis pathogens on dairy farms of the Western region of Ukraine.

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Diagnosis of bovine mastitis, selection of milk samples and secretions of the mammary gland, microbiological studies were carried out in accordance with generally accepted methods. The performed studies have established that among pathogens, both acute and chronic forms of mastitis, the most productive film-forming ability had *S. aureus* strains, which on average 1.5 times more often formed the biofilm than *Str. agalactiae* and *Str. dysgalactiae* strains. It was revealed that *S. aureus* strains, isolated from cows under the subclinical form of mastitis and at carriage, 2.0 times ($p < 0.05$) more often formed biofilms than in the clinical form of mastitis. The highest sensitivity of planktonic bacteria to pathogens of mastitis of streptococci and staphylococci was to ceftriaxone and doxycycline (100-80.9%). The least susceptible streptococci and staphylococci were to benzylpenicillin 32.3-45.4%, and the susceptibility of *S. aureus* strains was 19.0%. When determining the influence of antibiotics on biofilm forms of bacteria found that cells in the biofilm are more resistant to antibacterial drugs. It was found that antibiotic enrofloxacin completely inactivated streptococci and staphylococci in biofilms. Also, antibiotics ceftriaxone and doxycycline were also effective on bacteria in biofilms. At the same time, under the action of antibiotics penicillin's, aminoglycosides and macrolides, the amount of microbial cells that survived in a biofilm was about lg 5.3 CFU/cm^2 of area. Consequently, studies have shown that it is necessary to seek effective methods and develop new drugs that would influence the bacteria in biofilms to effectively treat bovine mastitis.

Keywords: biofilms, mastitis pathogens, antibiotic resistance

1. INTRODUCTION

Among the topical issues of veterinary medicine, the issue of improving the methods of diagnostics and treatment of mastitis of cows takes one of the leading places (RUEGG; PETERSSON-WOLFE, 2018). This is explained by the frequency of appearance of this disease and its complications, despite the implementation of a comprehensive set of preventive measures (KUKHTYN, et al. 2017; SALISBURY, et al. 2018).

Clinical features of infectious process of mastitis are largely due to the biological properties of microorganisms, namely – the presence of pathogenic and persistent potentials in them (LIU, et al. 2018). In the last decade, the study of

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mechanisms of bacterial survival is given special significance. It is established that 99% of microorganisms in natural ecosystems exist in the form of structured communities – biofilms (LIU, et al. 2018; KUKHTYN, et al. 2017).

Biofilm – a community of microorganisms attached to the surface and one to another, enclosed in the matrix of synthesized by them extracellular polymeric substances, which demonstrate a change in the phenotype, which is expressed in the change in parameters of growth and expression of specific genes (GOMES; SAAVEDRA; HENRIQUES, 2016).

The cells of bacteria in a biofilm have a complex polymorphic organization with a certain cytoarchitectonics. The multilayer topography affects the metabolism and physiological activity of cells. Within the biofilm, changes can occur that include the reaction of general stress, the stop of key metabolic processes and the induction of protective mechanisms. Reduced metabolism of microorganisms in a biofilm leads to the emergence of antibiotic resistance, since antibacterial drugs are most effective against metabolically active cells (FELIPE, et al., 2017; ASLANTAŞ; DEMIR, 2016).

In addition, the susceptibility of microorganisms in biofilm to antibacterial substances is due to the arbitrary presence of cells with a resistant phenotype (known as “persisters”) and/or poor penetration of antibiotics into the polysaccharide matrix (NEOPANE et al., 2018). Since, in order for nutrient and antimicrobial molecules to fall into microbial cells in biofilms, they must be diffused through a matrix of biofilm or mucus that is produced by the bacterium (BENGTSSON, et al., 2009).

This diffuse limitation may be the result of transport constraints (inability of antimicrobial molecules to diffuse through a polymer matrix), or inactivation of antimicrobial molecule by material of matrix. In addition, the extracellular matrix, which is required for the binding of bacteria to the biofilm, may consist of polysaccharides, proteins, and extracellular DNA (eDNA). Scientists have proved that eDNA functions as a matrix component and is responsible for antibiotic resistance of microorganisms in biofilms formed by Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus intermedius, and others. These protective mechanisms act synergistically, providing the overall increased resistance of the biofilm to antimicrobial compounds (HORIUK, et al., 2018; RUEGG, 2017).

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The abovementioned factors, to some extent, explain the inadequate effectiveness of treatment of mastitis in cows and the emergence of recurrent intramammary infections.

The purpose of research is to study the ability to form a biofilm, its density, to determine and compare the sensitivity to antibacterial drugs of planktonic and biofilm forms of the main pathogens of mastitis of cows in dairy farms of the Western region of Ukraine.

2. RESEARCH METHODOLOGY

182 specimens of cow secretion were selected from which 513 Staphylococcus aureus cultures, 263 Staphylococcus epidermidis cultures, 282 *S. agalactiae* cultures and 162 *S. dysgalactiae* cultures were identified and studied *for the ability to form microbial biofilms*. Samples were sampled at dairy farms in Ukraine.

Diagnostics of mastitis of cows, samples collection of milk and secretion of mammary gland, their delivery to the laboratory and microbiological studies were conducted in accordance with generally accepted methods. For isolation of microorganisms, culturing of samples for medium was carried out: Staphylococcus aureus – *BD Baird-Parker Agar* (HiMedia, India); Coliform bacteria – agar Endo (Pharmactive, Ukraine), streptococci – Streptococcus Selection Agar (HiMedia, India). Cultivations were carried out at temperature of 37^0C, the results were evaluated after 24-48 hours. The identification of pure cultures was carried out according to the morphological, tinctorial, culture, biochemical properties, which are described in the determinant of Bergy bacteria (STEPANOVIC, et al., 2000).

To determine the ability to form a biofilm, a pure culture of the isolated strain was seeded into wells of immunological plate in the amount of at least 10^5 CFU/ml. The plate was incubated at 37±1°C for 3 days. If during this period a biofilm was formed – surface or bottom growth in the well, which gave a film, which at the removal of the medium settled on the walls, then the strain was considered as film-forming (STEPANOVIC, et al., 2000).

96-well plastic plates were used to determine the density of formed biofilms. In the well was introduced 0.1 cm^3 of daily culture of microorganisms and was kept for 3 hours at room temperature. Then, 1 cm^3 of meat agar was added and incubated at

ISSN: 2236-269X
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37°C for 24 hours. After incubation, the wells were washed three times with phosphate buffer, dried and fixed biofilm. Then, they were painted with 0.1% solution of crystalline violet for 10 min, washed again with phosphate buffer and dried. 96^0 ethanol was added into each well and washed properly. The optical density of alcohol solution was measured for spectrophotometrically washing at a wavelength of 570 nm (STEPANOVIC, et al., 2000).

Electronic and microscopic studies of formed biofilms on abiotic surfaces were performed on an electron scanning microscope (REM 106 *И*, Ukraine).

Sensitivity of isolates to antibacterial drugs was determined by disc and diffusion method using antibiotic disks (Himedia, India). Mueller Hinton Agar was used during the method setting (Himedia, India). Preparation of microbial suspensions was performed according to the optical standard of turbidity of 1.0 units according to McFarland scale using Densi-LaMeter device (PLIVA-Lachema Diagnostika, Czech Republic).

The study of sensitivity of microorganisms in the biofilm form to antibiotics was carried out on daily microbial biofilms grown in Petri plastic dishes. After 24 h of culture incubation, the cups were washed three times with planktonic (unsaturated) microorganisms with sterile phosphate buffer and introduced 5 cm^3 of freshly prepared antibiotics.

After exposure, the antibiotics were poured out, the cups were washed three times with sterile phosphate buffer, 5 cm^3 of sterile 0.9% sodium chloride solution was added and a microbial biofilm was carefully washed off the walls and bottom of the cup with sterile tampon. From the cups, 1.0 cm^3 of suspension was taken, a number of ten-fold dilutions was prepared, seeding of 1.0 cm^3 of each breeding was performed in a Petri dish, poured with MPA and incubated at 37^0C for 24-48 h for the determination of the amount of bacteria.

Statistical processing of the results was carried out using methods of variation statistics using the program Statistica 6.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon's criteria, Mann-Whitney's criteria). The arithmetic mean (x), the standard error of the mean (SE) was determined. The difference between the comparable values was considered to be true for $P < 0.05$.

ISSN: 2236-269X
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3. RESULTS

The research was conducted to determine the ability to form microbial biofilms by pathogens of mastitis in dairy farms in the Western region of Ukraine.

It has been established that microorganisms *Streptococcus agalactiae, Str. dysgalactiae, Staphylococcus aureus and S. epidermidis* are the main causative agents of cows' mastitis in dairy farms (HORIUK, et al., 2018). The study of the formation of microbial biofilms in bacteria isolated from patients with various forms of mastitis and at carriage is presented in Table 1 and 2.

Table 1: Formation of biofilms by pathogens in different forms of mastitis

Forms of mastitis	Type of microorganism							
	Str. agalactiae		*Str. dysgalactiae*		*S. aureus*		*S. epidermidis*	
	n_1 (%)	n_2 (%)	n_1 (%)	n_2 (%)	n_1 (%)	n_2 (%)	n_1 (%)	n_2 (%)
subclinical, *n* = 84	184 (100)	120 (65.2)	94 (100)	65 (69.1)	214 (100)	207 (96.7)	117 (100)	100 (85.5)
clinical, *n* = 52	98 (100)	31 (31.6)	69 (100)	13 (18.1)	145 (100)	71 (48.9)	87 (100)	37 (42.5)
carriers, *n* = 46	–	–	–	–	154 (100)	154 (100)	59 (100)	50 (84.7)

n – number of investigated samples of the secretion of cow's nymph;
n_1 – number of studied cultures of microorganisms.
n_2 – number of cultures of microorganisms that formed biofilms.

From the data presented in Table 1, it is shown that the largest number of film-forming strains of *S. aureus* were isolated in the subclinical form of mastitis – 96.7%. In the clinical form of mastitis, the number of *S. aureus*, which formed biofilms, was 2.0 times ($p<0.05$) less.

A similar pattern was found in the study of other mastitis causative agents, which was characterized by the fact that in the subclinical form, the number of bacteria that formed the biofilm was 2.0-3.8 times ($p<0.05$) greater than in the clinical form.

Also, these data in tables indicate that strains *S. aureus*, which are pathogens of cow mastitis, 1.4-1.5 times more often form microbial biofilms than streams *Str. agalactiae and Str. dysgalactiae.* This indicates that treatment of bacterial mastitis of cows, the pathogen of which is *S. aureus*, will be more difficult than with streptococcus mastitis.

It is found (Table 2) that *Str. agalactiae and Str. dysgalactiae* formed a weak and average density biofilm in 86.0-94.5% of studied strains and only from 15.4 to 13.9% formed dense biofilms. At the same time, almost 100% of strains of *S. aureus*

ISSN: 2236-269X
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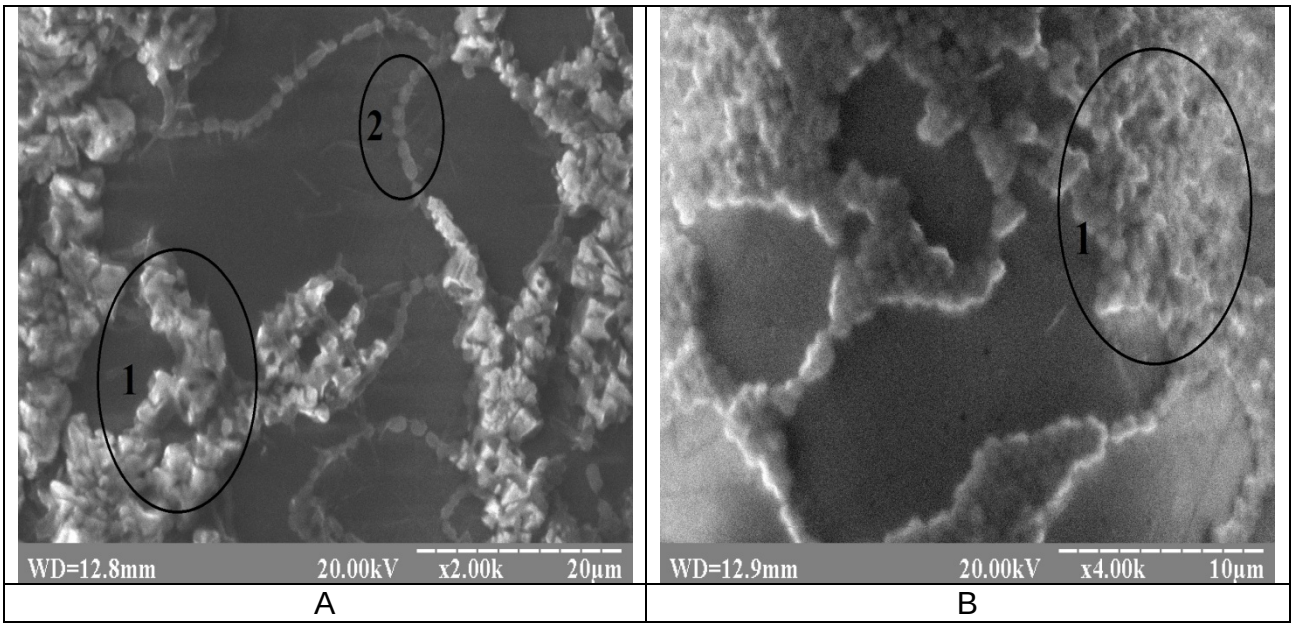
bacteria, isolated from the mammary gland of patients with cows mastitis, formed dense and average biofilms. In somewhat smaller quantities, average and dense biofilms were formed by strains *Str. epidermidis* in -75.8 ± 5.6% of cases. It was also found that *S. aureus* strains for 7-10 hours on abiotic surfaces formed dense biofilms.

Table 2: Density of biofilms of mastitis-induced pathogens, % (x ± SE, n = 324)

Biofilm density, CU	Number of studied cultures that formed biofilms			
	Str. agalactiae, *n* = 86	Str. dysgalactiae, *n* = 74	S. aureus, *n* = 98	S. epidermidis, *n* = 66
Weak, up to 0.50	61.6±4.2	37.8±3.5	–	24.2±3.4
Average, 0.51–0.10	24.4±2.7	56.7±5.4	12.2±2.3	57.6±4.2
Dense, more than 0.11	13.9±1.9	5.4±1.1	87.7±5.6	18.2±3.3

n – number of studied cultures that formed biofilms.

Figure 1 shows the results of electronic and microscopic studies of strains *S. aureus* and *S. agalactiae* in planktonic form and biofilm.



A B

Figure1: Microphotographs of mastitis causative bacteria formed in biofilm: A - *Str. agalactiae;* B - *S. aureus*; 1 - bacteria in a biofilm; 2 - bacteria without biofilm

The analysis of microphotographs, shown in the picture, showed that the bacteria, present in the biofilm, have a bulk surface and a solid matrix that protects against adverse factors.

Results of studies of sensitivity of pathogenic pathogens of cows' mastitis to antibacterial substances most common in veterinary medicine are given in Table 3 and 4.

ISSN: 2236-269X
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There were conducted comparative studies of sensitivity of isolated cows mastitis pathogens, located in planktonic forms (Table 3) and in formed biofilms (Table 4), to antibiotics.

Table 3: Sensitivity of planktonic forms of bacteria to antibiotics, %, (x ± SE, n = 124)

Name of antibiotic, amount of active substance	Number of studied cultures			
	Str. agalactiae, n = 34	*Str. dysgalactiae*, n = 22	*S. aureus*, n = 42	*S. epidermidis*, n = 26
Benzylpenicillin, 10 iU	32.3	45.4	19.0	42.3
Amoxicillin, 30 μg	41.2	68.2	35.7	57.7
Erythromycin, 15 μg	41.2	54.5	28.6	50.0
Streptomycin, 30 μg	23.5	45.4	23.8	30.7
Gentamicin, 30 μg	58.8	59.0	30.9	42.3
Lincomycin, 10 μg	38.2	59.0	47.6	57.7
Enrofloxacin, 10 μg	64.7	59.0	52.3	65.3
Ceftriaxone, 30 μg	100	100	95.2	100
Doxycycline, 30 μg	100	80.9	95.2	95.4
Tetracycline, 30 μg	23.5	22.7	11.9	26.9

Table 3 shows that the most effective among the studied antibiotics was cephalosporin of III generation – ceftriaxone. To which all isolated streptococci and epidermal staphylococci were susceptible, and the susceptibility of *S. aureus* strains was 95.2%.

Sensitivity of planktonic forms of bacteria to benzylpenicillin ranged from 32.3 to 45.4%, while *S. aureus* was more resistant, as the number of sensitive strains was only 19.0%. The antimicrobial activity of amoxicillin was higher than benzylpenicillin, so the number of sensitive streptococcal cultures ranged from 41.2 to 68.2%, and staphylococci from 47.6 to 57.7%.

The effectiveness of antibiotics from the group of aminoglycosides (streptomycin, gentamicin) was slightly different. The highest sensitivity of streptococcus was to gentamicin (58.8-59.0%), and to streptomycin the sensitivity was within the range of 23.5-45.5%. Staphylococci to the drugs of this pharmacological group were more stable than streptococci. Thus, the susceptibility of *S. aureus* strains did not exceed 30.9%, and the number of cultures of *Str. epidermidis*, which were sensitive to gentamicin, was 42.5%. Sensitivity to streptococci and staphylococci to erythromycin did not exceed 54.5%.

The drug enoforfloxacin exhibited a stable bactericidal effect on all streptococcal and staphylococcal strains, with a sensitivity of 52.3-65.3%. It should be noted that there is a fairly high antimicrobial activity in the antibiotic of tetracycline series – doxycycline. The number of susceptible to this antibiotic streptococci

ISSN: 2236-269X
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fluctuated within 80.9-100%, and the staphylococcal sensitivity was 95.2%. At the same time, the sensitivity of isolated bacteria of mastitis pathogens to tetracycline was 4-5 times less, compared with doxycycline.

Consequently, the results of determining the sensitivity of isolated microflora to antibiotics have an important clinical significance, since they allow us to justify the choice of a rational scheme of antibiotics.

Microorganisms are situated mostly in biofilms, and the planktonic form is intended for the colonization of other biotopes. The results of studies on the influence of antibiotics on bacteria that are formed in a biofilm are given in Table 4. The bacteria strains are used in the experiment, planktonic forms of which are susceptible to the determined antibiotics in Kirby-Bauer disc diffusion method.

Table 4. Influence of antimicrobial drugs on bacteria in biofilm (lg CFU/cm2, x ± SE)

Name of antibiotic, amount of active substance	Number of cells in biofilm							
	Str. agalactiae		*Str. dysgalactiae*		*S. aureus*		*S. epidermidis*	
	prior to action of antibiotics	after action of antibiotics	prior to action of antibiotics	after action of antibiotics	prior to action of antibiotics	after action of antibiotics	prior to action of antibiotics	after action of antibiotics
Benzylpenicillin, 10 iU	6.8 ± 4.3	5.5 ± 3.3	6.1±5.1	5.2±3.1	8.9±7.9	6.0 ±4.1	6.7±5.7	5.8 ± 3.8
Amoxicillin, 30 μg / ml	6.8 ± 4.3	4.8 ± 3.4	6.1±5.1	5.0 ± 2.9	8.9±7.9	5.1 ± 2.7	6.7±5.7	5.0 ± 3.2
Streptomycin, 30 μg / ml	6.8 ± 4.3	5.0 ± 3.1	6.1±5.1	5.1±2.7	8.9±7.9	5.3 ± 3.2	6.7±5.7	5.0 ± 3.1
Erythromycin, 15 μg / ml	6.8 ± 4.3	4.5 ± 3.3	6.1±5.1	4.7±2.5	8.9±7.9	4.9 ±2.9	6.7±5.7	4.7±2.7
Gentamicin, 30 μg / ml	6.8 ± 4.3	4.2 ± 3.1	6.1±5.1	4.5 ± 2.2	8.9±7.9	4.8 ±2.9	6.7±5.7	4.6±2.5
Lincomycin, 10 μg / ml	6.8 ± 4.3	4.7 ± 2.6	6.1±5.1	4.2 ± 2.0	8.9±7.9	5.1 ± 3.1	6.7±5.7	4.9 ± 2.2
Enrofloxacin, 10 μg / ml	6.8 ± 4.3	0	6.1±5.1	0	8.9±7.9	0	6.7±5.7	0
Ceftriaxone, 30 μg / ml	6.8 ± 4.3	1.7 ± 1.2	6.1±5.1	1.4 ± 0.3	8.9±7.9	1.9 ±1.1	6.7±5.7	1.7 ± 0.7
Doxycycline, 30 μg / ml	6.8 ± 4.3	2.3 ± 1.3	6.1±5.1	2.0 ± 1.1	8.9±7.9	2.5 ± 1.2	6.7±5.7	2.4 ±1.0
Tetracycline, 30 μg / ml	6.8 ± 4.3	2.5 ± 1.3	6.1±5.1	2.1 ± 1.2	8.9±7.9	2.8 ±1.4	6.7±5.7	2.6±1.3

As can be seen from the data in Table 4, antibiotics showed bactericidal action against microorganisms in a microbial biofilm, but microbial cells proved to be viable at levels above the “threshold of infection”. S. aureus cells were the most protected with biofilms, and from the investigated antimicrobials the best effect was on the cells in the biofilm enrofloxacin. After its action, streptococci and staphylococcus from the

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matrix of biofilm were not allocated. Antibiotics of penicillin series showed the weakest ability to influence bacteria in biofilms, after exposure to benzylpenicillin and amoxicillin, the number of live streptococcal cells ranged from lg 4.8 to 5.5 CFU/cm2 of biofilm area, while staphylococci were excreted in the amount of 5.0-6.0 mg CFU/cm2 of biofilm area.

Under the action of antibiotics of aminoglycosides and macrolides, the amount of microbial cells that survived did not exceed lg 5.3±3.2 CFU/cm2 of biofilm area. Antibiotics ceftriaxone and doxycycline were sufficiently effective on bacteria in biofilms. After exposure to ceftriaxone, the amount of surviving bacteria was 1.9±1.1 CFU/cm2 of biofilm area, and doxycycline lg 2.5±1.2 CFU/cm2.

Consequently, studies have shown that cows' mastitis bacteria, which are formed in biofilms, are more resistant to antimicrobial drugs than planktonic forms. Since, according to (RUEGG, 2017; ROYSTER; WAGNER, 2015), mastitis in cows is mostly chronic, it can be argued that microorganisms, isolated from patients with cows mastitis, are in biofilm and complicate antimicrobial therapy.

4. DISCUSSION

Mastitis remains a widespread disease of dairy herds around the world (RUEGG, 2017). Microorganisms are the main cause of mammary gland infection in cows and the ability of bacteria to produce biofilms is considered an important virulent property in the pathogenesis of mastitis (FELIPE, et al., 2017).

Our studies have shown that among the pathogens, both acute and chronic forms of mastitis, the most film-forming ability has strains of *S. aureus*, which in 1.4-1.5 times more often formed a microbial biofilm than streams *Str. agalactiae and Str. dysgalactiae*.

In addition, the ability to form a biofilm is determined not only by the type of pathogen, but also by the nature of infectious process in which the pathogen is involved. We found that *S. aureus* strains, isolated from cows with subclinical mastitis and at carriage, 2.0 times ($p<0.05$) more formed biofilms than in the clinical form of mastitis.

It is also found that *Str. agalactiae and Str. dysgalactiae* formed weak and of average density biofilm in 86.0-94.5% of examined strains, while almost 100% of strains of bacteria *S. aureus*, isolated from the mammary gland of patients with cows

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mastitis, formed the dense and average biofilms. Obviously, the presence of staphylococcus aureus in the formed biofilm in carriers, as well as in cows suffering the subclinical form of mastitis – to ensure its preservation, as a species, in a dairy farm. To cause illness – not the main task of microorganisms that are in the formed biofilm.

After all, the appearance of a subclinical form of mastitis is a manifestation of factor infection (GOMES; SAAVEDRA; HENRIQUES, 2016). It is well known that the interaction of a microorganism and a host depends on the resistance of the latter – the level of its local and general immunity (KEEFE, 2012). The biofilm form of microorganisms provides long-term survival of bacteria in cows-carriers and converts them into the reservoir of pathogen.

It is believed that the bacteria, present in the matrix of biofilm, are practically inaccessible to the action of antibiotics, despite the high sensitivity of the planktonic cells to these drugs. Thus, according to data (LIU et al., 2018), chronic inflammatory processes, in particular mastitis, are caused by microorganisms in a biofilm and traditional antibiotic therapy is ineffective. *S. aureus* has been reported to exhibit high ability to form a biofilm that is resistant to many antibiotics, including Methicillin (NEOPANE, et al., 2018).

Our studies have found that the highest sensitivity of planktonic bacteria to pathogens of mastitis of streptococci and staphylococci was to ceftriaxone and doxycycline (100-80.9%). Sensitivity of streptococcus to antibiotics of aminoglycosides and macrolides was within the range of 41.2-59.0%, and sensitivity of *S. aureus* was 23.8-30.9%.

Allocated bacteria exhibited sensitivity to enrofloxacin at the level of 52.3-65.3%. The least susceptible streptococci and staphylococci were to benzylpenicillin 32.3-45.4%, and the susceptibility of *S. aureus* strains was 19.0. Studies (SALISBURY, et al., 2018) also report high and moderate sensitivity of mastitis pathogens to antibiotics of various pharmacological groups.

Despite the significant sensitivity of planktonic forms of bacteria, isolated in mastitis, antibiotics do not always achieve a positive result during treatment (RUEGG, 2018), since in the pathogenesis of the subclinical form of mastitis, the leading role belongs to biofilm forms of bacteria. The conducted studies coincide with

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the numerical data on the need to determine the sensitivity of microflora to antibiotics during the treatment of mastitis.

When determining the influence of antibiotics on biofilm forms of bacteria it was found that cells in the biofilm are more resistant to antibacterial drugs. Of the tested antibiotics, enrofloxacin was most effective because of its low molecular weight and ability to penetrate through the pores and channels of biofilm to microbial cells. After the action of enrofloxacin on the biofilm, streptococci and staphylococcal cells were completely inactivated.

The fact that fluoroquinolones are easily diffused through biofilms and effectively reduce their growth and bactericidal action on microbial cells is reported by other scientists who have conducted *in vitro* experiments (STEPANOVIC, et al., 2000; LAGO; GODDEN, 2018). Also, antibiotics ceftriaxone and doxycycline were found to be effective on bacteria in biofilms. After exposure to ceftriaxone, the amount of surviving bacteria was 1.9±1.1 CFU/cm^2 of biofilm area, and doxycycline lg 2.5±1.2 CFU/cm^2.

At the same time, under the action of antibiotics penicillin, aminoglycosides and macrolides, the amount of microbial cells that survived amounted to about lg 5.3 CFU/cm^2 of biofilm area. Increased resistance of bacteria in biofilms to subclinical forms of mastitis, to antibiotics is noted in studies of other scientists (KOVALENKO, et al. 2018; NEOPANE, et al., 2018; RUEGG, 2018).

Thus, conducted laboratory microbiological studies indicate that the study of the laws of formation of biofilms by pathogens of cows mastitis are important for the implementation of effective anti-mastitis measures in dairy farms and the development of new anti-mastitis drugs with specific properties that will act on microorganisms in biofilms.

5. IMPLICATIONS AND CONCLUSION

Conducted studies show that the treatment of mastitis cannot currently be based on the plankton concept of microbiology. Knowledge about biofilm changes approaches to the treatment of infectious pathology, which affects the mechanisms of functioning of bacterial communities in the form of biofilms. Therefore, it is necessary to search for effective methods and develop new drugs that would

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influence the bacteria in biofilms for the purpose of effective treatment of cows' mastitis.

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