

ASSOCIATED MOVEMENT OF ESCHERICHIOSIS WITH ROTAVIRUS INFECTION OF CALVES IN EARLY POSTNATAL PERIOD.

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***Goal.** In early postnatal period calves often suffer from lesions of the digestive system of different infectious etiology that is clinically seen by diarrhea, which leads to the development of dehydration, toxemia, immune deficiencies, metabolic disorders and loss of livestock. Low effectiveness of treatment and preventive measures proves insufficient level of research in epizootiology, prevention and treatment of colibacillosis in calves, which determined the choice of the topic of this paper.*

***Materials and methods of research.** Epizootological and bacteriological methods were used for the research. The reports concerning the spread of escherichiosis and rotavirus infection in calves were applied.*

***Results.** The current system of anti-epizootic measures at colibacillosis at the farm was analyzed, bacteriological tests – tests of excrements of calves suffering from acute gastrointestinal tract and also from the corpses of dead calves were conducted, Escherichia coli in 100 %, Rota - virus and Corona - virus in 26 % of samples were defined. In addition, sensitivity of the selected pathogen to antibiotics was found.*

***Conclusions.** The complex treatment scheme of treatment of calves with neonatal diarrhea was made, which aimed to suppress pathogenic microflora, to normalize digestion processes, water-salt metabolism, toxicity reduction, elimination of dysbiosis that make it possible to save calves from 80 % to 100 %, depending on the complex of preventive measures, time of defining diagnosis and applied drugs.*

Keywords: escherichiosis, rotavirus infection, calves, epizootic and bacteriological methods of treatment and preventive measures.

UDC 599.735.5:616-08:616-056.2:579.845

THERAPY OF STAPHYLOCOCCAL MASTITIS IN COWS

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Mastitis therapy is conducted with aim to achieve complet recovery of mammary parenchyma -restitutio ad integrum- and elimination of causative agent. If tissue damage is more manifested, result of therapy is less benignant. Besides parenteral chemotherapy, intramammary application and thoroughly milking of infected quarter is also effective. In case of staphylococcal mastitis effect of therapy depends of stage of lactation, tissue damage and selection of antimicrobial drug. In this paper, antimicrobial susceptibility of Staphylococcus aureus was tested by disk diffusion method by Kirby Bauer. Results showed that the highest number (89.33 %) of isolates of Staphylococcus aureus is resistant to bacitracin, and all were sensitive to amoxicillin/ clavulanic acid, tetracycline, novobiocin and trimethoprim/ sulphamethoxazole. Resistance of Staphylococcus aureus to methicillin was also determined by PCR method in one of 75 Staphylococcus aureus isolates.

Nucleotide sequences encoding Staphylococcal protein A of isolates of Staphylococcus aureus originating from cows in our research, are deposited in GenBank under accession numbers from KJ023978 to KJ024046 at the US National Center for Biotechnology. In conclusion some recommendations are given to improve the efficiency of mastitis therapy.

Keywords: mastitis therapy, cow, Staphylococcus, antimicrobial resistance

For the determination of the treatment of mastitis and establishing goals of treatment we must take into account the following: certain forms of the disease correspond to different degrees of alteration of tissue, which allow only certain possibilities for structural and functional regeneration.

Such as:

- *Restitutio ad integrum*, including the elimination of causes. This is only possible with latent infection, subclinical mastitis, in acute mastitis or catarrhal mastitis;

- The total or partial re-establishment function (*restitutio ad function*) with the presence of irreversible chronic tissue changes, with often prolonged period of excretion causes an increased number of cells. This result is typical of catarrhal mastitis-suppurating and galaktoforomastitis;

- Dry off the quarter. The aim here is the utilization of healthy quarters for milk production. The loss of the district can occur spontaneously or by medication (due to unsuccessful therapy), as a result of chronic productive infection of the udder, or directly as a continuation of acute mastitis. Often the secretion of causes is continuing;

- *Restitutio ad vitam*. This objective is at the forefront with acute inflammation of the udder purulent type, acute or gangrenous mastitis with disorders that are life threatening due to septicemia, toxemia or piemia.

The effectiveness of treating the udder is estimated from the clinical standpoint based on the achieved degree of parenchymal regeneration of function in relation to disease duration, and frequency of recidivism may be increased by timely applied, science-based methods of treatment. In acute mastitis with every hour of the disease significantly reduces the chances of *restitutio ad integrum* and complete return to function.

In chronic process satisfactory results in terms of treatment is natural regeneration process of fine tissue in the dry udder.

Reserves to increase the effectiveness of treatment of mastitis are located next to the appropriate choice of treatment, and the timely introduction of therapies.

Methods of therapy. Therapeutic methods are divided into those that are directed against microbial udder pathogens (etiologial therapy) and those that are aimed at strengthening the immune system of the body and its defensive and regenerative potential (so-called supportive therapy). The importance of symptomatic therapy in practice is often neglected, and it is not applied sufficiently. By means of this method can be quantitative limited, and thus enhance the efficiency of antimicrobial therapy and chemotherapy. In this way, the losses are reduced in the milk production and the development of resistance to antibiotics in humans. Indicating the proper use of all the features of symptomatic therapy therefore deserves special attention of veterinarian. In this sense, we should expect a significant improvement in the modern study of mastitis, primarily in the area of non-specific and specific defense mechanisms against microorganisms in the udder as well as in pharmacological research. Acupuncture and homeopathy are also worth studying.

Milking therapy - Thorough removal of altered secretion of the udder during milking is the first measure that should be applied immediately after the determination of mastitis in lactating cows and in dried cows as well. Removal of bacteria, bacterial toxins and inflammatory products in this way, in most cases it is sufficient to start the healing process, particularly when performed repeatedly milking. Effects of intracisternally administered drugs are thus drastically improved.

Often thoroughly milking as a therapeutic method does not significantly affect the length of intracisternally applied antibiotics and sulphonamides. It has been found that a suitable blood screen, tissue and secretions image exceeds the maximum value after first 6 hours after the administration with no milking in the meantime. Thorough milking of treated mammary gland after this period of time, only slightly affects the therapeutically effective concentration of the antibiotics.

Fueling emptying of the udder by using oxytocin: before other therapeutic measures, exogenously added oxytocin (10 IU, IV and 20-30 IU, IM) leads to:

- Reinforcement effect of rinsing (which is performed manually milking) due to the ejection of the residual milk;

- Overcome the inhibition of milk ejection, which is caused by the painful condition of the udder of acute mastitis;

- Diagnostic demarcation of inhibition of ejection reflex from acute mastitis;

- Prognostic assessment of the degree of udder tissue damage in mastitis. If, after application of oxytocin can again get milk secretion character, damage is not affected all areas of the gland.

Chemotherapy. There are a number of different types of antibacterial drugs of different chemical structure. The agents are, taking into account the status of resistance, affecting with different chemotherapeutics, including: parenterally (IM or SC) and / or intramammary. Chemotherapeutics that are most commonly used are: β -lactams, aminoglycosides, lincosamines, tetracyclines, macrolides, polypeptides and combinations of trimethoprim and sulfonamides.

Recently, clinical trials are performed with the inhibitors of DNA gyrase (fluoroquinolones) in terms of their suitability for use in infectious mastitis. It is known that fluorinated quinolones are administered parenterally, especially norfloxacin are concentrated at the high level in milk (more than 10-fold in serum) and this advantage can be used for parenteral treatment of acute mastitis. Dapsone (difenilsulfon), an integral part of any modern preparations against mastitis is similar to the sulfonamides.

Indications for the application of this particular combination of a large spectrum of chemotherapeutics, depending on the properties of the established or suspected pathogen. This paper will present a general aspects that should be taken into account when applying these products.

Pharmacodynamic aspects. - Antibacterial chemotherapeutics act bactericidal or bacteriostatic. Bactericidal effect have especially penicillins, colistin and polymyxin B, where they affect the cell wall synthesis

and protein synthesis in the cell interior, or destroy cell wall. Bacteriostatic substances prevent the growth of bacteria: tetracyclines, streptomycin, erythromycin, sulfonamides, such as bacteriostatic effect suppressing constituents coenzyme F, p-aminobenzoic acid (PABA-e), and thereby inhibit the biosynthesis of folic acid by bacteria (substrate competition). Combining bacteriostatic and bactericidal substances is contraindicated, since they interact in collision. Antibacterial activity of chemotherapeutics are best expressed in the udder in most cases only when activation of the immune system of the body occurs which can be stimulated by supportive therapy.

The resistance of certain pathogens to antibacterial substances is either already present, or may develop during treatment. In this case, it is a natural, infectious or acquired resistance (transmission of the resistance factor, for example by plasmid, between the bacteria). Certain agents are often resistant to all representatives of a group of active substances, eg. to tetracyclines, penicillins (cross or parallel resistance).

However, those who apply chemotherapeutics must be aware that the antimicrobial therapy directed against one of the main causes of mastitis (causal therapy), but it does not affect predisposing factors for the occurrence of mastitis, or the tissue damage that may have been incurred.

Pharmacokinetic aspects - The concentration of the chemotherapeutic in milk, blood and tissue of the udder and its distribution in the tissue of the udder and its elimination from blood, milk and udder tissues, in terms of intensity and duration besides to the size of dose depends on the form of the drug and the nature of the carrier. The level in blood and in milk is quickly reached, but decreases rapidly with compounds that have a salt character, in contrast to those substances which are ester or otherwise associated with a large molecule. Aqueous carriers stimulate and oils and waxes inhibit decrease in drug concentration in the tissues and milk.

Concentration in blood serum, which is achieved upon the application of the substance intracisternally, increases with an increase in the solubility of the composition in the lipids (for example, stronger with erythromycin than penicillin). Substances in acid or ionized form are passing the blood-udder barrier exceptionally well.

Passing the blood-udder barrier is a prerequisite that after intracisternal treatment of certain quarter a substance found in the untreated quarters - though not in high concentrations and not for a long time. Accordingly, after intramammary as well as parenteral therapy of antibiotics and sulphonamides active substance is excreted through the neighborhood quarters. In addition, acutely inflamed quarters, due to increased permeability of the barrier, show higher concentrations in tissues and secretions than healthy districts of the same udder. In chronic mastitis, the situation is reversed.

The amount and duration of drug levels in tissues and milk depend on the stage of lactation, whereas in the fresh milking cows and cows with high milk production should be used higher doses than in cows with a long lactation and less productive cows. In dry off the udder is determined that presence of active ingredient for extremely long period of time.

Frequent milking shortens the duration of excretion of intracisternally applied substances. However, therapeutically effective level is not at the beginning particularly great under the influence of this procedure, where the antibiotic therapy and milking (milking first 6 hours after the administration of the medicament) are to be excluded.

Decisive for the effect of microbial preparations in the treatment of mastitis is their distribution in the udder.

For the intracisternal application the following factors have a major impact:

- The passing of milk canal in the alveolar cavity (Due to the swelling of milk canal and clogging the system with fibrin, cells and cellular detritus and bacteria, mobility is extremely reduced or completely disabled in all areas of udder quarter or in parts. This aspect is particularly represented in acute mastitis.)
- Strength and the age of barrier of connective tissue in inflammatory areas (abscess capsule interalveolar and interlobular septum), and changes of the milk canal wall (This aspect is particularly in acute and chronic mastitis and significantly limits the distribution of the active substance of medicament.)

For the parenteral application the following factors have a major impact:

- The degree of vascularity of the tissues of the udder, which is very high in acute mastitis, and encouraging the achievement levels of the drug in the tissue
- The age and strength of the tissue layers, which in the case of chronic mastitis (or diffused in the form of aggregates, ascends) prevent the transport of active substances in the invasion of germs and inhibit proliferation

For the pathogenicity of *Staphylococcus aureus* are primarily responsible four hemolysin and leukocidin (all extracellular toxins). The task of the antibodies is to neutralize the toxins that act as antigens. *Saphylococcus aureus*, and *Streptococcus* groups A, C and G, on its surface creating a special protein, which binds to fibronectin (glycoprotein) which is very prevalent in the liquid inside the organ. In this way, the binding of pathogens to the tissue surface is the first step of infectious process. On the model of mastitis in mice, it could be seen that with the vaccine, which is focused on the binding protein on the surface of the bacteria, reducing the risk of mastitis, or that the disease can be easily overcome.

The above examples clearly indicate the possibilities to study direction of immunoprophylaxis, consisting of a very clear definition of specific bacterial antigens in order to create also well defined and specific antibodies. Protective effects can be expected only in a relative increase in defense capabilities against certain bacterial species.

With the practical application of the vaccine, the eradication of bovine mastitis, still can not be achieved.

Material and methods. Our research included highly productive cows that produce over 5,000 kg of milk per lactation, from 46 dairy farms in Vojvodina and central Serbia in 2012. The occurrence of clinical mastitis on these farms was not frequent, however, an increased somatic cell count (over 400,000 SCC/mL) in bulk tank milk samples were recorded.

All cows were tested using California Mastitis Test according to the manufacturer's instruction.

Isolates of *Staphylococcus aureus* were biochemically confirmed using API Staph (bioMérieux, France) and by PCR targeting nuclease gene-characteristic for *Staphylococcus aureus*. A 255-bp fragment of the nuclease gene was amplified using the following primer pair:

nuc-F (TCAGCAAATGCATCACAACAG) and

nuc-R (CGTAAATGCACTTGCTTCAGG).

Susceptibility of *Staphylococcus aureus* to antimicrobial agents was tested by disk diffusion method by Kirby Bauer as directed by the European Committee for antimicrobial susceptibility testing (EUCAST), to amoxicillin 25 mg amoxicillin / clavulanic acid 20/10 mg, 10 mg ampicillin, bacitracin 0.04 IU, neomycin 30 mg, 5 mg novobiocin, penicillin G 10 IU, tetracycline 30 mg, 5 mg trimethoprim and trimethoprim/sulphamethoxazole 1.25/23.75 mg.

Resistance to methicillin -presence of *mecA* and *mecC* gene was determined using PCR method. PCR amplification of a 162-bp fragment of *mecA* gene and 138-bp fragment of *mecC* gene used primer pairs as follows:

mecA-f (TCCAGATTACAACCTTCACCAGG);

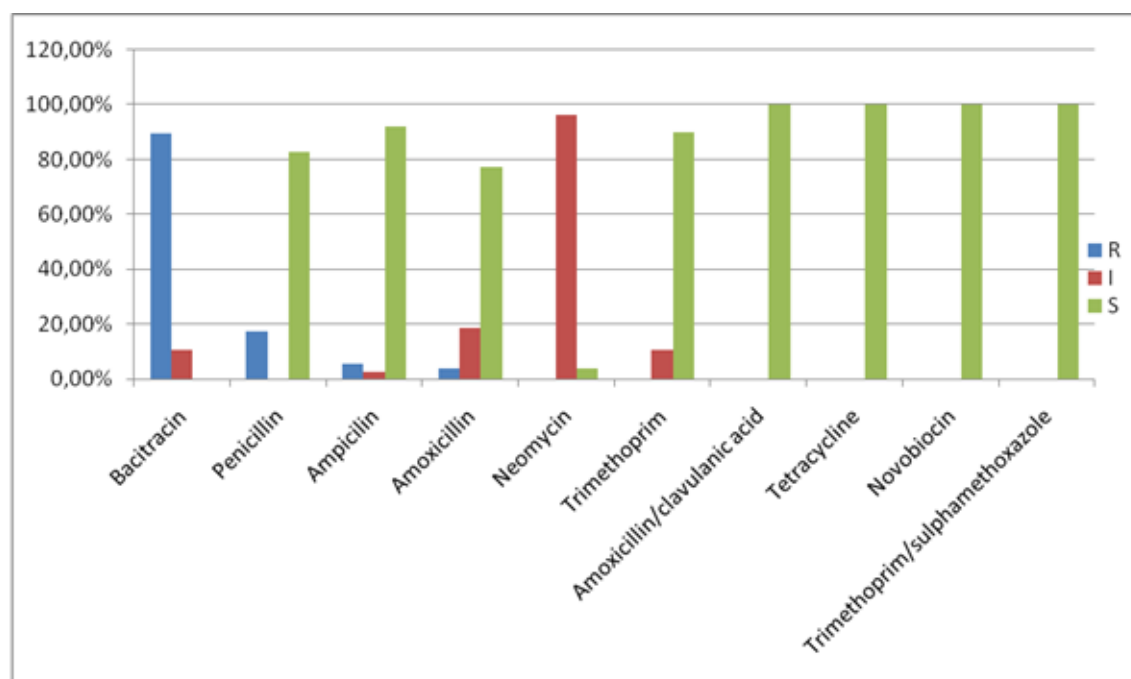
mecA-r (CCACTTCATATCTTGTAACG);

mecC-f (GAAAAAAGGCTTAGAACGCCTC);

mecC-r (GAAGATCTTTTCCGTTTTCAGC).

Nucleotide sequences encoding Staphylococcal protein A of isolates of *Staphylococcus aureus* originating from cows in our research, are deposited in GenBank under accession numbers from KJ023978 to KJ024046 at the US National Center for Biotechnology (<http://www.ncbi.nlm.nih.gov>).

Results. Antimicrobial susceptibility of *Staphylococcus aureus*, based on the diameter of zone of inhibition of *Staphylococcus aureus* have been referred to as sensitive (S) intermediately susceptible (I) or resistant (R) (Graph 1).



Graph 1. Antimicrobial susceptibility of *Staphylococcus aureus* based on disk diffusion method

The highest number (89.33 %) of isolates of *Staphylococcus aureus* is resistant to bacitracin (0.04 IU), and all were sensitive to amoxicillin/ clavulanic acid (20/10 mg), tetracycline (30 mg), novobiocin (5 mg) and trimethoprim/ sulfamethoxazole (1.25/23.75 mg) (Graph 1).

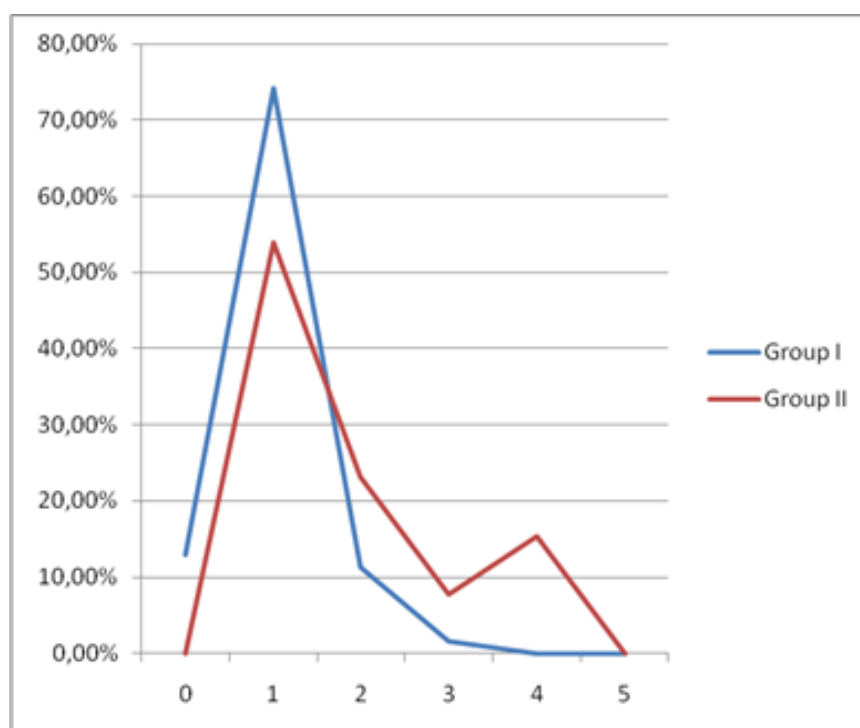
Isolates of *Staphylococcus aureus* originating from cow's udder with subclinical mastitis were resistant to fewer antimicrobials than isolates of *Staphylococcus aureus* originating from cow's udder with clinical mastitis on the basis of susceptibility to 10 antimicrobials.

The greatest number of isolates were resistant at the same time only to one of 10 antimicrobial drugs, 46 of the 62 (74.19 %) isolates from cows with subclinical mastitis and 7 of the 13 (53.85 %) isolates from cows with clinical mastitis (Table 1 and Graph 2).

Table 1. Number of *Staphylococcus aureus* isolates and the total number of antimicrobial drugs they are resistant to

The total number of antimicrobial drugs they are resistant to	Isolates of <i>Staphylococcus aureus</i>		
	Group I (62 isolates)	Group II (13 isolates)	TOTAL (75 isolates)
0	8 (12,90 %)	-	8 (10,67)
1	46 (74,19 %)	7 (53,85 %)	53 (70,67)
2	7 (11,29 %)	3 (23,08 %)	10 (13,33)
3	1 (1,61 %)	1 (7,69 %)	2 (2,67)
4	-	2 (15,38 %)	2 (2,67)

Groups: I - cows with subclinical mastitis, II - cows with clinical mastitis



Groups: I - cows with subclinical mastitis, II - cows with clinical mastitis

Graph 2. Percentage of *Staphylococcus aureus* isolates and the total number of antimicrobial drugs they are resistant to

Resistance of *Staphylococcus aureus* to methicillin PCR analysis showed the presence of the *mecA* gene in one isolate of *Staphylococcus aureus* originating from cows and isolates of group II (Fig. 1), which is 1 (7.69 %) of 13 isolates. *Staphylococcus aureus* isolates from group I, have not proved the presence of the *mecA* gene. The presence of genes *mecC* is not found in any of the 75 isolates examined.

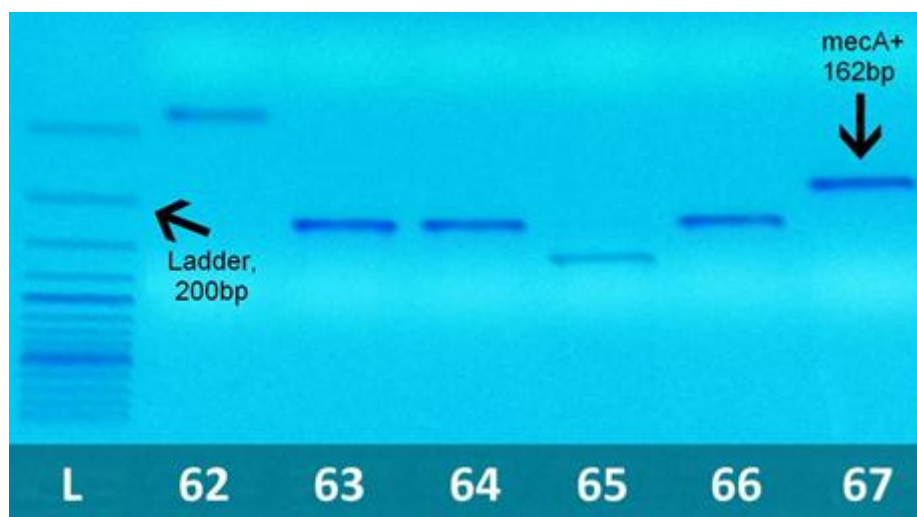


Figure 1. Agarose gel electrophoresis –determination of *mecA* gene in *Staphylococcus aureus* isolate (isolate Nr. 67) [Pajić, 2014]

Conclusion/Recommendation. It is known that the antimicrobial therapy efficiency during lactation is 45 %, but in dry cow period efficiency is over 70 %.

It can be concluded that:

- In acute mastitis we should also apply intracisternal and parenteral therapy
- In acute mastitis are recommend products with large volume and higher speed distribution in the parenchyma of the udder. The compositions with a longer retention time “long acting” in milk canal are designed for use in chronic therapy of galactophoromastitis and dried cows

- Milking therapy can be combined with antimicrobial drugs
- Treatment of certain quarters of the udder with antibiotics or sulfonamides has resulted in the secretion of certain concentrations of antimicrobial agents in the untreated udder quarter

Following recommendations arise from presented findings:

- Selection of substances which are effective in terms of certain pathogens. This process is carried out in the initial phase of therapy, according to the clinical experiences, after receiving the results of the test of antimicrobial susceptibility.

- Treatment must provide a high and sustainable finding of active drug concentration in tissue and blood, to the extent that, if possible, destroy or damage all causes, or will prevent their growth long enough for them bodily immunity is completely removed.

- Therapeutic options that support action of antimicrobial medications, for example, combinations of active ingredients and combinations with measures of antimicrobial and supportive therapy.

- Simultaneous application of chemotherapeutics adversely affect each other should be avoided.

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ЛІКУВАННЯ СТАФІЛОКОКОВОГО МАСТИТУ КОРІВ

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Лікування маститу проводиться з метою досягнення повного відновлення молочної паренхіми-*restitutio ad integrum* -та ліквідації збудника. Якщо пошкодження тканин більш явне, результат лікування менш якісний. Крім того парентеральна хіміотерапія, інтрамамарне застосування і ретельне доїння зараженої молочної долі також є ефективним. У випадках стафілококового маститу ефект лікування залежить від стадії лактації, пошкодження тканини та вибору антимікробного препарату. У даній роботі, протимікробну сприйнятливість *Staphylococcus aureus* випробовували диско-дифузійним методом Кірбі Бауера. Результати дослідження виявили, що найбільше число (89,33%) ізолятів *Staphylococcus aureus* мають резистентність до бацитрацину, та всі ізоляти були чутливі до амоксициліну/клавуланової кислоти, тетрацикліну, новобіоцину і триметоприму / сульфаметоксазолу. Резистентність *Staphylococcus aureus* до метициліну також визначали методом ПЛР в одному з 75 ізолятів *Staphylococcus aureus*.

Нуклеотидні послідовності, що кодують білок А ізолятів *Staphylococcus aureus* від корів в нашому дослідженні, викладені в GenBank під номерами доступу з KJ023978 до KJ024046 в Національному центрі Біотехнології у США. Наприкінці надані деякі рекомендації з метою підвищення ефективності лікування маститу.

Ключові слова: лікування маститу, корова, *Staphylococcus*, антимікробна резистентність.