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Some Biological Features of *Staphylococci* Isolated from Milk of Cows with Mastitis

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ABSTRACT

White *staphylococcus spp.* is the common name for *staphylococci* that do not form a golden pigment. It causes and complicates the course of many inflammatory processes in various organs and tissues of humans and animals. In particular, in dairy cattle breeding, white staphylococcus is the main cause of serous and serous-catarrhal mastitis. Low efficiency in the treatment of staphylococcal mastitis is due to the resistance of the pathogen to antibiotics. Accordingly, the use of chemotherapeutic drugs for mastitis without prior determination of the sensitivity of isolated strains of pathogenic microorganisms to antibiotics leads to double economic damage.

Introduction

Being conditionally pathogenic microorganisms, *staphylococci* are constantly found on the outer integuments and mucous membranes of animals. The reasons, contributing to the weakening of the immune system of animals, create favorable conditions for the active reproduction of *staphylococci*, complicating the course of the underlying disease. With mastitis, as with any pathological processes accompanied by inflammation, *staphylococci* play a key role in the pathogenesis of the disease, which must be taken into account when choosing therapy methods.

According to the International Dairy Federation, mastitis is registered annually in 25 % of cows in dairy farms around the world, and according to some researchers, this figure reaches 50 %, with 97 % of cases being subclinical mastitis (Altukhov and Afanasyev, 1990). According to American researchers, up to 51 % of cows with clinical forms of mastitis are detected annually in dairy farms in the USA, the treatment of which is not always effective: up to 25% of animals get sick again. Economic damage in the country annually reaches \$ 2 billion, which is equivalent to the loss of 11 % of the total volume of milk sold (Hogeveen, et al., 2019).

The non-infectious mastitis is mainly caused by the penetration of conditionally pathogenic microorganisms into the mammary gland, namely *Staphylococcus* – *Staphylococcus albus*, which includes *staphylococci* that do not form golden pigment (*Staphylococcus epidermidis*, *Staphylococcus saprophyticus*) and is widespread in nature characterized by relative resistance to physical and chemical environmental factors and antibacterial drugs (Mkrтчyan, et al., 2020, Steven, et al., 2015). White staphylococcus is found on the external integuments of animals, on the clothes and hands of service personnel, in dairy dishes and milking equipment, in litter, manure, feed and farm air and can penetrate into the mammary gland both through the nipple canal and through damage to the udder skin; therefore, prevention of mastitis is based on compliance with veterinary and sanitary requirements when milking cows (Veterinary and sanitary rules for dairy farms, 2011). According to numerous studies, a wet udder toilet before milking reduces the amount of conditionally pathogenic microflora in milk by up to 94 % (Pankratov, 1971, Isachenko, 1987).

Low therapeutic efficacy in the treatment of mastitis and frequent relapses are due to the resistance of white staphylococcus strains to certain antibiotics, therefore, the success of therapy largely depends on the preliminary determination of the sensitivity of isolated staphylococcus strains to them and the correct choice of antibacterial agents (Mkrтчyan, et al., 2021, Marami, et al., 2012).

The purpose of our research was to identify the specific composition of *staphylococci* in serous and serous-catarrhal mastitis of cows kept in some farms of the Kotayk region of Armenia and to determine the sensitivity of isolated strains of microorganisms to antibiotics, which would allow us to offer more effective therapy methods and prevention of this pathology.

Materials and methods

The first portions of milk from the contents of the nipple canal of cows with clinical signs of serous and serous-catarrhal mastitis served as research material. Milk samples were placed in sterile test tubes, which, after labeling, were delivered to the laboratory of the Department of Epizootology and Parasitology of the Armenian National Agrarian University. The test material was diluted with sterile saline solution in a ratio of 1:10 and 0.5 ml was sown on petri dishes with mannitol salt agar (Chapman Medium USP, Eur.Pharm), which is a selective nutrient medium for *staphylococci*. Petri dishes with a nutrient

medium after sowing were kept in a thermostat at 370 °C for 24 hours. The obtained colonies were counted by visual counting, the number of microorganisms in 1 ml of the studied material was calculated using the following formula:

$$M = N:m * C,$$

where M is the number of microorganisms in 1 ml (g) of the studied material, N is the degree of dilution of the material, m is the amount of seed (ml), C is the arithmetic mean number of colonies grown on the nutrient medium.

10 cows with signs of serous and serous-catarrhal mastitis were under observation, out of which 5 animals belonged to a private farm in the community of Nor Artamed in the Kotayk region of the Republic of Armenia, and 5 more animals belonged to the educational and experimental farm of the Armenian National Agrarian University in the community of Balahovit situated at the same region of the republic. The diagnosis of serous and serous-catarrhal mastitis was made on the basis of anamnesis and clinical signs during the examination of sick animals. The sensitivity of *staphylococci* obtained on the nutrient medium to antibiotics was determined by the disc-diffusion method (Mkrтчyan and Simonyan, 2020).

Results and discussions

As a result of the conducted studies, it was revealed that out of milk samples obtained from cows with serous and serous-catarrhal mastitis, colonies of white staphylococcus mainly grow on mannitol salt agar in the form of separate convex rounded disks with smooth edges or a solid white plaque with a smooth matte surface (Figure 1). Microscopy of smears prepared from the obtained colonies and stained by the Gram method reveals random clusters of spherical bacteria of purple color, resembling bunches of grapes (Pankratov, 1971). At the same time, as can be seen from Table 1, the number of colonies of *staphylococci* isolated on a selective nutrient medium, as well as the number of *staphylococci* in 1 ml milk obtained from cows with mastitis prevails in milk samples obtained from cows with mastitis belonging to a private farm in the community of Nor Artamet. As the data of Table 1 show, the relatively low content of bacteria in milk samples obtained from cows belonging to the educational and experimental farm of the Armenian National Agrarian University is obviously due to a higher level of compliance with veterinary and sanitary rules when keeping animals compared to private farming in the community of Nor Artamet.

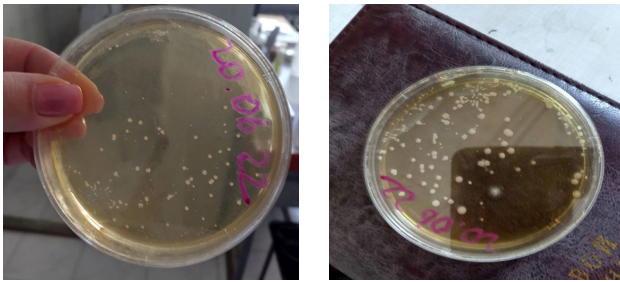


Figure 1. Colonies of *Staphylococcus albus* on mannitol salt agar.

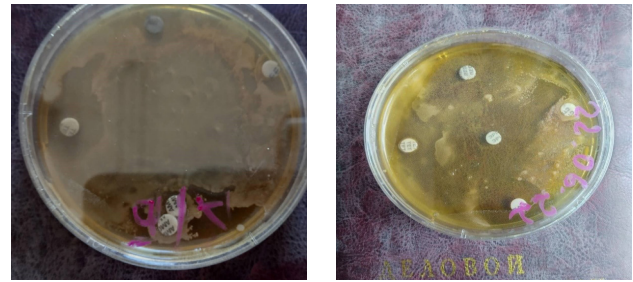


Figure 2. Determination of the sensitivity of *Staphylococcus albus* strains to antibiotics by the disc diffusion method.

Table 1. Results of bacteriological examination of milk samples from cows with mastitis*

Average number of staphylococcal colonies on mannitol salt agar		Number of staphylococci in 1 ml of milk	
Private farm in the community of Nor Artamet	Educational and experimental farm in Balahovit	Private farm in the community of Nor Artamet	Educational and experimental farm in Balahovit
46	19	920	380

Table 2. Sensitivity of *Staphylococcus albus* strains isolated from cows with mastitis to antibiotics*

Name of antibiotics	Colony growth delay zone (mm)	
	Private farm in the community of Nor Artamet	Educational and experimental farm in Balahovit
Cephasolin	0.5	2.0
Eritromicin	2.5	2.8
Levomicetin	1.0	2.0
Ampicillin	0.2	0.2
Streptomycin	0.1	0
Tetracilin	2.0	2.0

*Composed by the authors.

When determining the sensitivity of isolated staphylococcal strains to antibiotics by the disc diffusion method, it was revealed that staphylococcal strains isolated from milk samples from cows bred in the community of Nor Artamet are most sensitive to tetracycline and levomicetin (Figure 2) while staphylococcus strains isolated from milk samples of the cows kept at the experimental farm in the community of Balahovit are sensitive almost to all tested antibiotics except streptomycin. Data on the resistance

of isolated strains of *staphylococci* are given in Table 2.

The results obtained are consistent with the data of our previous studies, where almost similar sensitivity to antibiotics was observed in strains of staphylococcus isolated from the intestinal contents of dogs with gastroenteritis, as well as from the contents of the external auditory canal of dogs and cats with otodectosis (Mkrtchyan, et al., 2020, Mkrtchyan, et al., 2021).

Conclusion

Based on the results of the studies obtained, the following conclusions can be drawn:

1. In cows with serous and serous-catarrhal mastitis, the course of the disease is complicated by secondary staphylococcal infection.
2. There is a positive correlation between the degree of compliance with veterinary and sanitary rules on a dairy farm and the amount of contamination of milk with *staphylococci*.
3. Determination of antibiotic sensitivity isolated from milk samples of staphylococcus cows with mastitis will allow to achieve greater therapeutic effectiveness from chemotherapy drugs used for therapeutic purposes.

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