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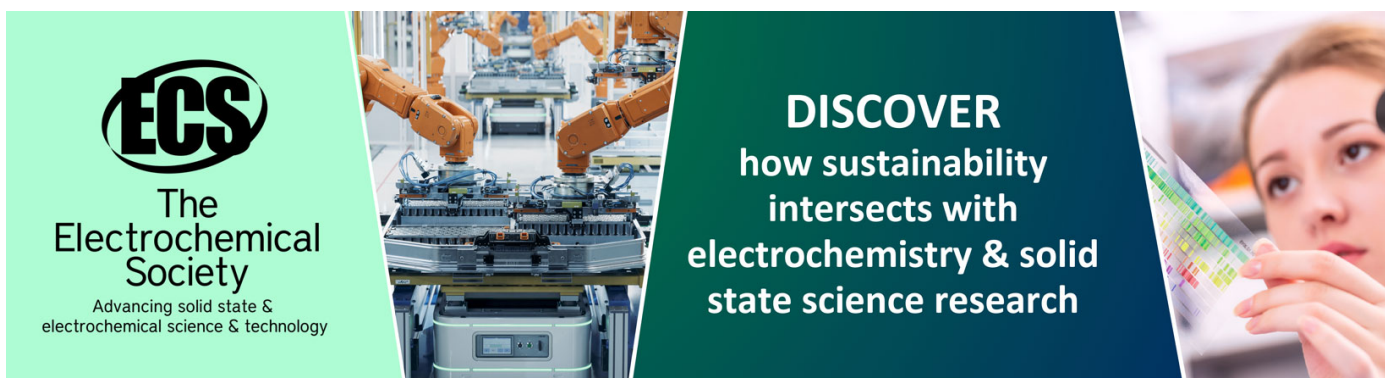
Problems and trends in the development of dairy livestock in Russia

To cite this article: L K Semina *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **848** 012090

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Problems and trends in the development of dairy livestock in Russia

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Abstract. Staphylococci are widespread in the environment, which is facilitated by the duration of their survival on various objects. In this regard, rapid and accurate identification of staphylococcal species is in demand, with the aim to assess their pathogenic properties and prescribe adequate drug therapy. This year, 48 staphylococcal cultures were isolated from the udder secretions of cows (sick with mastitis) from six regional agricultural enterprises and tested for a number of biochemical properties. By the minimum number of tests, including catalase activity, mannitol fermentation and plasma coagulation, isolated cultures were differentiated into coagulase-positive and coagulase-negative ones. According to the research results for *S. aureus*, the three above-mentioned tests are sufficiently informative to classify them as pathogenic staphylococci. *S. intermedius* strains are catalase- and coagulase-positive, and with respect to mannitol fermentation, the reaction can be either positive or negative, which requires additional research. In addition to the tests listed above, the presence of hemolytic and DNase activity was confirmed in strains of pathogenic staphylococci. The use of the reagents set of “Multimicrotests for biochemical identification of staphylococci (“MMT S”)” makes it possible to determine the species belonging of coagulase-negative staphylococci. Determination of biochemical properties of staphylococci isolated during bacteriological analysis makes it possible to identify them and assess their pathogenic properties.

1. Introduction

Staphylococci can affect any tissue, any organ, due to the presence of a large pathogenic factors complex in them. It is known that staphylococci have high biochemical activity: they ferment glycerin, glucose, maltose, lactose, sucrose, mannitol; form various enzymes: catalase, plasma coagulase, fibrinolysin, lecithinase, lysozyme, alkaline phosphatase, DNase, hyaluronidase and others [1]. These enzymes play an important role in the metabolism of staphylococci and largely determine their pathogenicity.

Catalase protects cocci from the action of O₂-dependent microbicidal mechanisms of phagocytes, b-lactamases destroy molecules of b-lactam antibiotics, lipases facilitate penetration into tissues, plasma coagulase activates prothrombin, which leads to increased blood clotting, prevents phagocytosis. Hyaluronidase promotes the spread of staphylococci in tissues. Lecithinase destroys lecithin, which is



part of the cell walls. Fibrinolysin, dissolving fibrin, promotes the generalization of the pathological process [2].

Along with the plasma coagulation reaction, another important ability of staphylococci that characterizes their potential pathogenicity, deoxyribonuclease (DNA) activity, has acquired great importance. Currently, this test can serve as a reliable differential indicator between pathogenic and non-pathogenic staphylococci [3].

Staphylococci are widespread in the environment, which is facilitated by the duration of their survival on various objects. In case of violations of zoohygienic conditions of keeping, high-protein feeding, risk of penetration of both pathogenic and opportunistic microorganisms into the body of animals increases, causing development of infectious and epizootic processes [4]. In this regard, rapid and accurate identification of staphylococcal species is in demand to assess their pathogenic properties and prescribe adequate drug therapy [5].

2. Materials and methods

Studies were carried out on the basis of the Vologda branch of All-Russian Research Institute of Experimental Veterinary Medicine, Russian Academy of Science, as well as in six livestock farms in the Vologda district of the Vologda region in accordance with “Methodological guidelines for bacteriological research of milk and udder secretions” (1983) [6].

Laboratory research included conducting microbiological studies according to generally accepted methods. To analyze biochemical properties of staphylococci, cultures in the amount of 48 strains isolated from cows with mastitis were used.

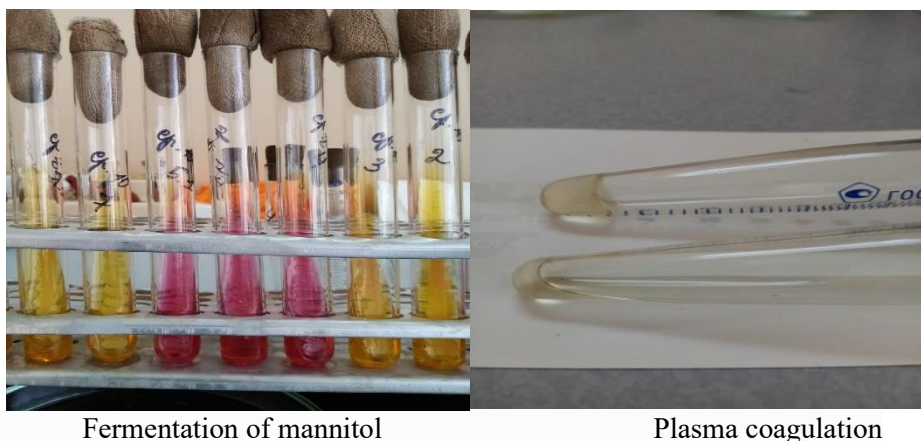
When identifying isolated microorganisms, we were guided by the “Guidelines for the microbiological examination of milk and udder secretions of cows for the diagnosis of mastitis” [7] and “Guidelines for the identification, determination of pathogenicity factors and antibiotic resistance genes in *Staphylococcus intermedius*” [7]. Determination of the species belonging of the isolated cultures of coagulase-positive and coagulase-negative staphylococci was carried out using a reagents set of “Multimicrotests for biochemical identification of staphylococci (“MMT S”)” by 12 indicators.

3. Results and discussion

According to the results of earlier studies of the udder secretions from cows with mastitis in agricultural enterprises of the Vologda region, staphylococci (pathogenic and opportunistic) were recognized as the main causative agents of infectious cow mastitis.

This year, 48 staphylococcal cultures were isolated from the udder secretions of cows sick with mastitis from six agricultural enterprises in the region and tested for a number of biochemical properties.

By the minimum number of tests, including catalase activity, mannitol fermentation and plasma coagulation, isolated cultures were differentiated into coagulase-positive and coagulase-negative ones. Figure 1 shows that four of the seven strains of staphylococci ferment mannitol, changing the colour of mannitol-salt agar (medium no. 10) from raspberry to golden yellow. A change in the colour of the medium indicates the release of pathogenic staphylococcus, which is capable of breaking down the carbohydrate mannitol according to a biochemical basis. The second part of the figure shows the ability of the coagulase enzyme in pathogenic staphylococci (upper test tube) to cause clotting of rabbit blood plasma to a clot held by tilting the test tube. Staphylococci with marked pathogenicity coagulate plasma within up to two hours.



Fermentation of mannitol

Plasma coagulation

Figure 1. Differentiation of coagulase-positive and coagulase-negative staphylococci.

It is known that coagulase-positive staphylococci (*S. aureus*, *S. intermedius*) are pathogenic for both animals and humans, can infect almost any organs and tissues of the body, and in our case, cause mastitis in cows [8].

In order to study additional properties of staphylococci isolated from the milk of cows with mastitis, we carried out a number of tests characterizing their pathogenicity for animals, including DNase and hemolytic activity (Figure 2).



DNase activity

Hemolytic activity

Figure 2. DNase and hemolytic activity.

It is shown in Figure 2 on the left, near the colonies of staphylococci producing transparent DNase, it forms a DNA cleavage zone (+), which ensures their pathogenicity for animals.

Figure 2 on the right shows positive hemolytic activity of staphylococci in three strains, expressed in the formation of a zone of complete and incomplete clearing of blood agar of a certain type around the microbes colonies (alpha and beta hemolysis), which also indicates their pathogenicity.

Based on the data in Table 1, in addition to the tests listed for *S. aureus* cultures, three tests are quite informative: catalase, coagulase activity, and mannitol fermentation. The *S. intermedius* strains are catalase- and coagulase-positive, and with respect to mannitol fermentation, the reaction can be either positive (colour change of medium no. 10) or negative. In this case, further identification of the culture was carried out using a set of reagents ("MMT S").

Table 1. Enzymatic activity of isolated staphylococci.

No.	Species affiliation	Exp. No.	Catalase	Coagulase	Mannitol frementation	Hemolysin	DNA se
Pathogenic staphylococci							
1.	<i>S. aureus</i>	1	3+	+	+	+ β	+++
2.	<i>S. aureus</i>	2	3+	+	+	+ λ	+
3.	<i>S. aureus</i>	3	3+	+	+	+ λ and β	+
4.	<i>S. aureus</i>	4	3+	+	+	+ λ and β	+
5.	<i>S. aureus</i>	5	3+	+	+	+ β	+
6.	<i>S. aureus</i>	6	3+	+	+	+ β	+++
7.	<i>S. aureus</i>	7	3+	+	+	+ λ	+
8.	<i>S. aureus</i>	8	3+	+	+	+ β	++
9.	<i>S. aureus</i>	9	3+	+	+	+ λ	+
10.	<i>S. intermedius</i>	10	3+	+	-	+ λ	+
11.	<i>S. intermedius</i>	14	3+	+	-	+ β and λ	++
12.	<i>S. intermedius</i>	17	3+	+	+	-	++
13.	<i>S. intermedius</i>	18	3+	+	-	+ β and λ	++
Conditionally pathogenic staphylococci							
14.	u/n staphylococci	11	3+	-	-	-	-
15.	-/-/-/-/-/-/-/-	12	3+	-	-	-	-
16.	-/-/-/-/-/-/-/-	13	3+	-	-	-	+
17.	-/-/-/-/-/-/-/-	15	3+	-	-	-	+
18.	-/-/-/-/-/-/-/-	16	3+	-	-	+ β	-

As a result of the study on the 5 above-mentioned tests, isolated staphylococci were classified as pathogenic (*S. aureus* and *S. intermedius*) and conditionally pathogenic species.

Unlike pathogenic coagulase-negative staphylococci, in most cases, they are not active against these enzymes. To differentiate them, it is not enough to carry out the above studies, with the exception of catalase activity, it is positive for all types of staphylococci and allows them to be differentiated from streptococci during the initial diagnosis. When determining their species, it is necessary to conduct biochemical identification of staphylococci, in our case, using the set “Multimicrotests for biochemical identification of staphylococci (“MMT S”)”.

Identification of staphylococci in this way is based on determination of enzyme systems in these microorganisms that act on the appropriate substrates. The kit allows you to determine the following biochemical properties of staphylococci: the presence of urease, arginine dihydrolase, β -galactosidase, utilization of maltose, sucrose, lactose, mannitol, trehalose, raffinose, fructose, the presence of phosphatase, nitroreductase. The type (subspecies) of staphylococcus was determined by the number of positive tests. The identification results are presented in Table 2 and Figure 3.

Table 2. Results of staphylococci identification.

Experiment no.	Short test names												Species affiliation	Hits number from 12 tests %
	urease	arginine dihydrolase	β -galactosidase	maltose	sucrose	lactose	mannitol	trehalose	raffinose	fructose	phosphatase	nitroreductase		
5	+	+	+	+	+	+	+	+	-	+	+	+		12/100.0
10	+	+	+	+	+	+	+	-	-	+	-	+		10/ 83.3

20	+	+	+	+	+	-	+	+	-	+	-	+	S. intermedius	10/83.3
24	+	+	+	+	+	+	+	+	-	+	+	+		12/100.0
26	-	+	+	+	+	+	+	+	-	+	+	+		11/91.7
29	+	+	+	+	+	+	+	+	-	+	+	+		12/100.0
30	+	+	+	-	+	+	+	+	-	+	+	+		12/100.0
19	+	+	+	+	+	+	+	+	-	+	+	+		12/100.0
1	+	-	+	+	+	-	+	-	-	+	-	+	S. xylosus	10/83.3
11	+	-	+	+	+	+	+	+	+	+	-	+		10/83.3
16	-	+	+	+	-	+	+	-	-	+	+	+		9/ 75.0
21	+	-	+	+	+	+	-	-	-	+	+	+		10/83.3
25	+	-	+	+	+	+	-	-	-	+	+	+		10/83.3
2	-	+	-	+	+	+	+	+	-	+	+	+	S. hyicuss (sp.chromog enes)	12/100.0
12	-	+	+	+	+	+	+	+	-	+	+	+		11/91.7
13	-	+	+	-	+	+	-	+	-	+	+	+		11/91.7
14	+	+	+	+	+	+	-	+	-	+	+	+		10/ 83.3
4	+	+	+	+	+	+	+	+	-	+	-	+	S. saprophytic us	10/83.3
17	+	-	+	+	+	+	+	+	-	+	-	-		12/100.0
27	+	+	+	+	+	-	-	-	-	-	+	+	S.epidemidi s	10/8.33
15	+	+	+	+	+	+	-	-	-	+	+	+		11/91.7
18	-	-	-	+	+	-	+	-	-	+	-	-	S. cohnii 1	9/75.0
6	+	+	+	+	+	+	+	+	+	+	-	+	S. simulans	11/ 91.7
7	+	+	+	+	+	+	-	+	-	-	-	+		10/ 83.3
28	-	+	+	+	+	+	-	+	-	+	-	+		10/ 83.3
No. 3, 8, 9, 22, 23													Not identified	5/27.8

From the data presented in Table 2, it can be seen that the largest number of identified crops is attributed to *S. intermedius* (32.0%), *S. xylosus* (20.0%), *S. hyicuss* sp. *chromogenes* (16%); significantly less – to *S. simulans* (12.0%), to *S. saprophyticus* and *S. epidermidis* (8.0% each) and the smallest amount – to *S. cohnii* 1 (4.0%). It should be noted that the number of coincidences of the results obtained for the types of staphylococci with test values (+) ranged from 9 (75.0%) to 12 (100.0%). At the same time, the largest number of cultures corresponding to 12 tests for species identification belongs to *S. intermedius* (five cultures out of eight studied), one culture - *S. hyicuss* and *S. saprophyticus*.



Figure 3. Identification of cultures using a set of reagents (“MMT S”).

Thus, the study of biochemical properties of the isolated staphylococci allows you to determine their species, assess pathogenic properties and prescribe adequate treatment for sick animals.

4. Conclusion

Due to the wide distribution of staphylococci in the environment, in the body of animals and humans, it is necessary to differentiate their types, since the degree of their pathogenic activity is different.

Determination of staphylococci biochemical properties isolated during bacteriological analysis makes it possible to identify them and assess their pathogenic properties.

Acknowledgements

The article was completed in accordance with the state assignment № 0578-2019-0003.

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