UDC 619:579.842.23:637.12
Chuzhebaeva G.D., Candidate of Veterinary Sciences, Associate Professor of the Department of Veterinary Sanitation, Head of Testing Laboratory of Food Production
Baymenov B.M., Ph.D student
Aliyeva G.K., Ph.D student
Aleshina Yu.E., Master
Kostanay State University named after. A. Baytursynov, Kostanay, Republic of Kazakhstan

STUDY OF PHENOTYPIC CHARACTERISTICS OF STAPHYLOCOCCAL STRAINS ISOLATED FROM VARIOUS BIOTOPES

Abstract

The article discusses the results of microbiological studies on the bacteriological and biochemical properties of 58 Staphylococcus strains; 34 of them belong to the species *Staphylococcus aureus,* 15 strains are identified as *Staphylococcus epidermidis* and 9 as *Staphylococcus saprophyticus*. As a result of the study, the most active cultures were isolated *Staphylococcus aureus*, which by their biochemical properties ferment mannitol, fructose, glucose, mannose, lactose, maltose, sucrose, trehalose, galactose, arginine, urease. Variability in relation to lactose was shown by *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. *Staphylococcus epidermidis*, in aerb conditions, does not decompose mannitol and does not ferment trehalose. Also, *Staphylococcus saprophyticus* showed variability in relation to mannitus. According to the biochemical properties of Staphylococcus aureus-coagulates the plasma of rabbits and oxidizes mannitol, shows resistance to novobiocin and polymyxin. 88.3 % of the isolated strains showed proteolytic activity regardless of the type of *Staphylococcus* infection. Lecithinatic activity was found in all the cultures studied, in addition to *Staphylococcus aureus*. The highest hemolytic activity was shown by a strain of *Staphylococcus* aureus isolated from the nasal cavity from cattle. The proportion of cultures with no hemolytic activity amounted to an average of 19.6%. This property was also typical for 8.3% of *Staphylococcus epidermidis* cultures. Most of the *Staphylococcus aureus* strains that were isolated from 58% of bird carcasses showed activity against DNA-se. *Staphylococci* isolated from nasal effusions from cattle showed the lowest percentage of DNA-se positive activity (16 %).

Keywords*: bacteriology, biochemistry, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus.*

Introduction. *Staphylococcus* are present in the air, dust, sewage, water, milk, food, on various surfaces in the environment, on the skin of people and animals. People and animals are the main reservoir of infection. Products that are most often the cause of staphylococcal food poisoning are meat and meat products, poultry, eggs, salads, milk and dairy products. *Staphylococcus* play an important role in the etiology of mastitis, which leads to the loss of a large amount of milk, its rejection and premature culling of cows. The presence of a significant number of toxigenic staphylococcus in milk (colostrum) leads to the fact that newborn calves are infected and suffer severe enteritis of staphylococcal etiology, which often kills them [1, P.66]. Studies by European scientists show that 4.7% of domestic birds are carriers of *S. aureus*, while finished products from these meat are contaminated in 36.4% of cases. It was also revealed that staphylococcus are widespread in poultry farms. The strains of staphylococcus are isolated in 22.5% of samples of pathological material of broilers [2, P. 127].

If possible, staphylococcus plasma coagulation is divided into 2 groups. *S. aureus, S. intermedius, S. hyicus*, and others belong to coagulase-positive staphylococcus (CPS). One of the most widely distributed food pathogens worldwide is *Staphylococcus aureus*, which produces several types of exogenous toxins. Other species are coagulase-negative (CBS) [3, P. 952]. Staphylococcus can grow in a wide range of temperatures from 7 to 48.5°C (optimum 30-37°C); pH 4.2 - 9.3 (optimum pH 7.0-7.5) and at high concentrations of sodium chloride (up to 15% NaCl). These properties allow bacteria to populate a wide variety of products. The pathogenic properties of a particular strain of staphylococcus are determined by the summing action of pathogenicity factors, toxins, and invasive properties of this strain. The pathogenicity of staphylococcus varies significantly [4, P. 21].

The aim of the research was to study the biological characteristics of staphylococcal strains isolated from various biotopes.

To achieve this goal, the following tasks were set:

- to identify strains of staphylococcus from veterinary and sanitary surveillance facilities;
- to identify and study the biological characteristics of strains isolated from various biotopes.

Material and methods. The research was carried out on the basis of the scientific innovation center of the KSU named after A. Baitursynov in the Department of microbiological research in the framework of the project «Monitoring of antibiotic resistance of enteropathogenic zooanthropozonous diseases of the Northern region of Kazakhstan» for 2018-2020.

The object of the study was Staphylococcus isolates (n = 58) isolated from animal flushes and biomaterials, as well as from animal and plant products. The study included 179 flushes from poultry carcasses and flushes of nasal and vaginal effusions of cattle, 75 samples of poultry eggs sold in the conditions of the Kostanay region markets, 54 samples of bovine biomaterial, as well as 50 samples of food products of animal and vegetable origin.

Research algorithm:

1. Identification:
- study of cultural and morphological properties;
- study of biochemical properties.

2.Study of virulence factors:
- study of coagulase activity (rabbit plasma coagulation);
- study of hemolytic activity;
- study of letitovitellase activity;
- study of DNA activity.

The biological properties of staphylococcus were determined by classical microbiological methods. Determination of the isolated strains was performed by seeding into a liquid selective medium, by re-seeding the culture liquid on the surface of the agarized selective diagnostic medium (MPA, HSA, KA). Test tubes with crops were incubated at a temperature of 37 °C for 24-48 hours.

Biochemical identification of cultures was carried out using GISS environment with sugars and «Stafi-test» test systems (ERBA Lachema s. r. o., Czech Republic).

In order to confirm that they belong to coagulase-positive staphylococcus, the ratio to gram staining and the ability to coagulate rabbit blood plasma were determined in grown microorganisms.

To do this, following the rules of asepsis, 0.1 cm^3 of each culture of staphylococcus and 0.3 cm^3 of rabbit plasma were added to sterile tubes and incubated at a temperature of 37^0C. Usually coagulation occurred after 4-6 hours. The test was considered positive when the contents of the test tube were coagulated.

The presumed presence of coagulase-positive staphylococcus in salt broth was determined by the turbidity of the medium. From presumably positive vials after 24 hours and from all remaining vials after 48 hours, inoculated on the surface of yolk-salt agar, Petri Dishes with crops were incubated at a temperature of 37 °C for 24-48 hours.

To determine the hemolytic activity of the studied cultures in the form of "plaques" were sown on blood agar (CA). The degree of hemolysin production was estimated by the radius of the hemolysis zone around the «plaques» (mm). To determine the lecithinase, the studied cultures in the form of «plaques» were sown on yolk-salt agar (ZHSA). Lecithinase activity was indicated by the presence of an iridescent Corolla around the «plaques». To determine the DNA activity, the studied cultures were seeded on the DNAse TEST AGAR (Manufactured by Hispanlab, Madrid).

The proteolytic properties of Staphylococcus are expressed in the ability to dissolve casein, dilute gelatin (slowly), and break down other protein substrates. To determine the gelatinase activity, the studied cultures were seeded with an injection into the frozen nutrient gelatin. With a positive reaction on the second day, the gelatin was liquefied.

The isolated strains were differentiated according to the following indicators:

1. The ability of S. aureus to produce coagulase is one of the main differential features of this species.
2. Resistance to the antibiotic novobiocin.
3. Resistance to polymyxin.

4. The Ability of staphylococcus to ferment glucose and mannitol under anaerobic conditions.

Research result. During the bacteriological study of the material, 58 strains of staphylococcus were isolated and identified; 34 of them were *Staphylococcus aureus*, 15 were *S. epidermidis*, and 9 were *S. saprophyticus*

Staphylococcus grow well on universal nutrient media at a temperature of 35-40 °C. Adding glucose or blood to the nutrient medium accelerated the growth of staphylococcus. A characteristic feature of most strains is the ability to grow in the presence of 15% sodium chloride or 40 % bile. The MPAS form round, slightly rising above the surface of the agar colonies with smooth edges with a diameter of 2-5 mm. The colonies can be colored: *S. aureus* synthesizes a golden or orange pigment; S. epidermidis synthesizes a white or yellow pigment; in most strains of *S. saprophyticus* pigment is absent.

When growing in BCH, staphylococcus initially cause diffuse turbidity with subsequent loss of a loose flake-like sediment.

Staphylococcus produce saccharolytic and proteolytic enzymes [5, P. 16].

All the studied strains hydrolyzed maltose, glucose, sucrose, urease to acid without gas, and did not hydrolyze xylose, arabinose, and salicin.

The most active biochemically are *S. aureus* strains: fermented glucose, fructose, mannose, mannitol, maltose, lactose, trehalose, arginine, galactose, sucrose, urease. In relation to lactose, *S. epidermidis* and S. *saprophyticus* showed variability. *S. epidermidis* did not ferment trehalose or decompose mannitol under aerobic conditions. *S. saprophyticus* also showed variable properties with respect to mannitol.

The isolated strains were differentiated according to the following characteristics (table 1):

Table 1 - Differential signs of isolated staphylococcus

Name of the attribute	Type		
	S.aureus	*S.epidermidis*	*S.saprophyticus*
Plasma-coagulase	+	-	-
Resistance to novobiocin	-	-	+
Resistance to polymyxin	+	+	-
The oxidation of mannitol	+	-	v
Number of detected microorganisms	34	15	9

As can be seen from the table, *S. aureus* coagulates rabbit plasma and oxidizes mannitol, which is resistant to novobiocin and polymyxin. Plasmocoagulating activity is the main species characteristic of *S. aureus*, which correlates perfectly with virulence and production of other pathogenicity factors (figure 1).

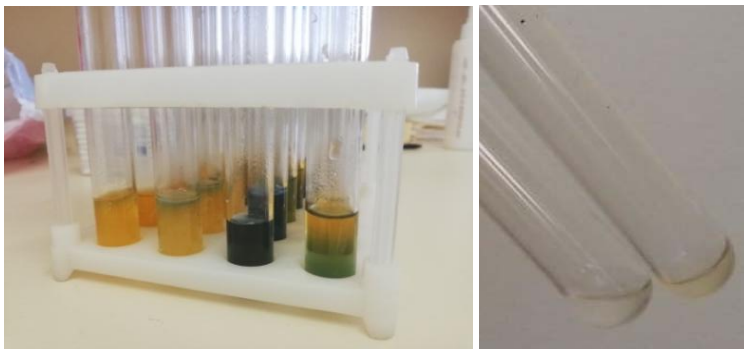

Figure 1-Mannitol fermentation and plasma coagulation reaction

Splitting carbohydrates and pigmentation cannot serve as a criterion for the pathogenicity of staphylococcus. The main factors determining the pathogenicity of these bacteria is the ability to produce exotoxins and enzymes coagulase, fibrinolysin and hyaluronidase. All these virulence factors are widely used to identify *S. aureus* and differentiate it from other staphylococcal species (table 2).

Table 2 – Selected virulence Factors of bacteria of the genus *Staphylococcus*

Type	Frequency of occurrence of the trait			
	Hemolytic activity	Lecithinase activity	DNA activity	Proteolytic activity
S. aureus (n=34)	+	+	+	+
S. saprophyticus (n=9)	-	-	-	+
S. epidermidis (n=15)	+	-	+	+
Total (n=58)				

Most cultures showed proteolytic activity regardless of the type of Staphylococcus and the site of discharge (83.6%). Lecithinase activity was found in all isolated cultures of *S. aureus*. The most pronounced lecithinase activity in staphylococcus isolated from milk and cattle biomaterial.

Various types of staphylococcus, especially *S. aureus*, are capable of producing a variety of hemolysins, among which the most active is α-hemolysin. When interacting with the cytoplasmic membrane, it causes the formation of pores, resulting in osmotic lysis of the cell (figure 2) [6].

Figure 2 – Hemolytic activity of *S. aureus* on blood agar

The highest hemolytic activity (hemolysis zone ≥ 2 mm) was shown by S. aureus cultures isolated from nasal effusions of cattle. The share of non-hemolytic crops averaged 19.6 %. Some cultures of *S. epidermidis* also had hemolytic activity (8.3%).

Among the isolated *S. aureus*, cultures with yellow pigment (88%) prevailed, compared with white (2%) and intermediate - cream (10%). According to scientific research, the yellow-orange pigment of most clinical isolates of *Staphylococcus aureus* is associated with increased bacterial survival in adverse conditions and increased pathogenicity of staphylococcus, since carotenoid-deficient cultures lose their resistance to the oxidative explosion of neutrophils [6].

Most *S. aureus* cultures isolated from bird carcasses (59%) showed DNA activity. The lowest percentage of DNA cultures was among those isolated from nasal effusions of cattle (14 %).

Conclusion. During the bacteriological study of the material, 58 strains of staphylococcus were isolated and identified; 34 of them were *Staphylococcus aureus*, 15 were *S. epidermidis*, and 9 were *S. saprophyticus*.

All the studied strains hydrolyzed maltose, glucose, sucrose, urease to acid without gas, and did not hydrolyze xylose, arabinose, and salicin.

The most active biochemically were *S. aureus* strains: they fermented glucose, fructose, mannose, mannitol, maltose, lactose, trehalose, arginine, galactose, sucrose, urease. In relation to lactose, *S. epidermidis* and *S. saprophyticus* showed variability. *S. epidermidis* did not ferment trehalose or decompose mannitol under aerobic conditions. *S. saprophyticus* also showed variable properties with respect to mannitol.

S. aureus coagulates rabbit plasma and oxidizes mannitol, resistant to novobiocin and polymyxin. Plasmo-coagulating activity is the main species characteristic of *S. aureus*, which correlates perfectly with virulence and the production of other pathogenicity factors.

Thus, the breakdown of carbohydrates and pigmentation can not serve as a criterion for the pathogenicity of staphylococcus. The main factors determining the pathogenicity of these bacteria is the ability to produce exotoxins and enzymes coagulase, fibrinolysin and hyaluronidase. All of these virulence factors are widely used to identify *S. aureus* and differentiate it from other staphylococcal species.

Most cultures showed proteolytic activity regardless of the type of Staphylococcus and the site of discharge (88.3%). Lecithinase activity was found in all isolated cultures of *S. aureus*. The most pronounced lecithinase activity in staphylococcus isolated from milk and cattle biomaterial.

Various types of staphylococcus, especially *S. aureus*, are capable of producing a variety of hemolysins, among which the most active is α-hemolysin.

The highest hemolytic activity (hemolysis zone ≥ 2 mm) was shown by *S. aureus* cultures isolated from nasal effusions of cattle. The share of non-hemolytic crops averaged 19.6 %. Some cultures of *S. epidermidis* also had hemolytic activity (8.3%).

The majority of S. aureus cultures isolated from bird carcasses (58%) showed DNA - activity. The lowest percentage of DNA + cultures was among those isolated from nasal effusions of cattle (16 %).

Thus, of all the studied species, S. aureus strains were statistically significantly more likely to have a set of virulence factors. Thus, only this species was recorded for lecithovitellase activity. Significant differences were obtained between the CBS isolates of all studied species and *S. aureus* in the presence of lecithovitellase activity. In addition, *S. aureus* strains were significantly more likely to have DNA activity compared to S. epidermidis strains.

REFERENCES

1. Pavlova I.Zh., Homich Yu.S. Biologicheskie svoystva *Staphylococcus aureus*, vyidelennyih iz razlichnyih lokusov bakterionositeley // Vestn. Chelyabinsk. gos. un-ta. Biologiya. – 2013. – Vyip. 2. – S. 66–67. *(in Russian)*

2. Granichnaya N.V., Zaytseva E.A., Bondar V.Yu. Fenotipicheskaya harakteristika biologicheskih svoystv koagulazonegativnyih stafilokokkov, vyidelennyih v kardiohirurgicheskom statsionare // Almanah klinicheskoy meditsinyi. – 2017. – № 45 (2). - S.127–132. *(in Russian)*

3. Kozlova Yu.N., Fomenko N.V., Morozova V.V., Saranina I.V. Geneticheskaya i biohimicheskaya harakterizatsiya stafilokokkov, vstrechayuschihsya v Novosibirske // Vavilovskiy zhurnal genetiki i selektsii. – 2017. – № 21(8). – S. 952-958. *(in Russian)*

4. Ryischanova R.M., Chuzhebaeva G.D., Mendyibaeva A.M. Vyidelenie i identifikatsiya shtammov salmonella spp. i staphylococcus spp. s opredeleniem profilya rezistentnosti k antibiotikam // 3 i: intellect, idea, innovation - intellekt, ideya, innovatsiya. - 2018. - № 3. - S. 21. *(in Russian)*

4 Kornienko A.M. Biohimicheskie i geneticheskie osobennosti realizatsii patogennosti gospitalnyimi shtammami Staphylococcus epidermidisi Staphylococcus haemolyticus. – M.: Federal'nyj nauchno-klinicheskij centr fiziko-himicheskoj mediciny federal'nogo mediko-biologicheskogo agenstva, 2016. – 127 s. *(in Russian)*

5. Gordina E.M. Vnutrividovyie osobennosti bakteriy roda Staphylococcus, izolirovannyih v usloviyah promyishlennogo ptitsevodstva. – M.: Permskij gosudarstvennyj medicinskij universitet imeni akademika Vagnera E.A. - 2015. – 139 s. *(in Russian)*

6. Rychsanova R. M., Chuzhebaeva G. D., Baymenov B. M., Alieva G. K. Biological features of staphylococcal strains isolated from various biotopes / / 3I: intellect, idea, inovation. – 2018. - № 3. - P.19-26. *(in Russian)*

ТҮЙІН

Мақалада стафилокктардың 58 штаммдарының бактериологиялық және биохимиялық қасиеттері бойынша микробиологиялық зерттеулердің нәтижелері қарастырылды; олардың 34 штамм Staphylococcus aureus түріне жатады, 15 штамм *Staphylococcus epidermidis* және 9 Staphylococcus saprophyticus ретінде анықталған. Жүргізілген зерттеу нәтижесінде ең белсенді Staphylococcus aureus бөлінген дақылдары болды, олар өзінің биохимиялық қасиеттері бойынша маннит, фруктоза, глюкоза, манноз, лактоза, мальтоза, сахароза, трегалоза, галактоза, аргинин, уреазаны ферменттейді. Лактозаға қатысты вариабельділік *Staphylococcus epidermidis* және *Staphylococcus saprophyticus* штаммаларын көрсетті. *Staphylococcus epidermidis*, аэробты емес жағдайда маннитт ыдыратады және трегалозаны ферменттейді. Сондай-ақ, *Staphylococcus saprophyticus* маннитке қатысты вариабельділікті көрсетті. *Staphylococcus aureus* биохимиялық қасиеттері бойынша-қоян плазмасын коагуляциялайды және маннитті тотықтырады, новобиоцин мен полимиксинге төзімділігін көрсетеді. Бөлінген штамдардың 88,3 % - ы

стафилокктың түрлік ерекшелігіне қарамастан протеолитикалық белсенділік танытты. Барлық зерттелген дақылдарда *Staphylococcus aureus*-тен басқа лецитинатикалық белсенділік табылды. Ең жоғары гемолитикалық белсенділік ірі қара малдан мұрын қуысынан бөлінген *Staphylococcus aureus* штаммы көрсетті. Гемолитикалық белсенділігі жоқ дақылдардың үлесі орта есеппен 19,6% құрады. Сондай-ақ, бұл сипат *Staphylococcus epidermidis* дақылдарының 8,3% - ына тән болды. Құс ұшасының 58%-дан бөлінген *Staphylococcus aureus* штаммдарының көп бөлігі ДНҚ-азға қатысты белсенділік танытты. Ірі қара малдан мұрын қуысынан бөлінген стафилококтар ДНҚ-аз оң белсенділіктің (16%) ең аз пайызын көрсетті

РЕЗЮМЕ

В статье рассматриваются результаты микробиологических исследований по бактериологическим и биохимическим свойствам 58 штаммов стафилокков; из них 34 штамма принадлежат к виду *Staphylococcus aureus*, 15 штаммов определены, как *Staphylococcus epidermidis* и 9 как *Staphylococcus saprophyticus*. В результате проведенного исследования наиболее активными были выделенные культуры *Staphylococcus aureus*, которые по своим биохимическим свойствам ферментируют маннит, фруктозу, глюкозу, маннозу, лактозу, мальтозу, сахарозу, трегалозу, галактозу, аргинин, уреазу. Вариабельность по отношению к лактозе показали штаммы *Staphylococcus epidermidis* и *Staphylococcus saprophyticus*. *Staphylococcus epidermidis*, в аэрбных условиях, не разлогает маннит и не ферментирует трегалозу. Также по отношению к манниту *Staphylococcus saprophyticus* продемонстрировал вариабельность. По биохимическим свойствам Staphylococcus aureus-коагулирует плазму кроликов и окисляет маннит, проявляет устойчивость к новобиоцину и полимиксину. 88,3 % выделенных штаммов проявили протеолитическую активность вне зависимости от видовой принадлжности стафилокка. Во всех исследованных культурах, помимо *Staphylococcus aureus*, была обнаружена лецитинатическая активность. Наиболее высокую гемолитическую активность показал штамм *Staphylococcus aureus* выделенный из полости носа от крупного рогатого скота. Доля культур с отсутствием гемолитической активности составила в среднем 19,6%. Также данное свойство была характерно для 8,3% культур *Staphylococcus epidermidis*. Большая часть штаммов *Staphylococcus aureus*, которые были выделенны от 58% тушек птиц, проявили активность в отношении ДНК-аз. Стафилококки, выделенные из носовых истечений от крупного рогатого скота показали наименьший процент ДНК-аз положительной активности (16 %).

UDC 615.28:636.5
Isabekov S.[1]**,** Ph.D student
Espembetov B.[2]**,** Candidate of Veterinary Sciences, Associate Professor
Alikhanov K.[1]**,** Ph.D, Associate Professor
Tagaev O.[3]**,** Doctor of Veterinary Sciences, Associate Professor
[1] NPJSC « Kazakh National Agrarian University», Almaty, Republic of Kazakhstan
[2]Scientific Research Institute for Biosafety Problems, Gvardeisky, Republic of Kazakhstan
[3]NPJSC «Zhangir Khan West Kazakhstan Agrarian-Technical University», Uralsk, Republic of Kazakhstan

THE RESULTS OF A STUDY ON THE PRODUCTION TEST OF THE POLYPHAGE PREPARATION IN THE MEAT PROCESSING PLANT OF «KARASU» LLP

Abstract

This article presents the results of a study on the production test of the Polyphage preparation in the slaughterhouse of Karasu LLP. The control was contaminated test objects, which were treated with sterile physical solution under similar conditions with experienced. The surface of test objects was seeded with 1 billion suspension of bacterial culture of *E. coli* 1257 at the rate of 1 cm^3 per 10 cm^2. Then the test objects were treated with 10% Polyphage disinfectant from a hand sprayer at the rate of 0.2-0.3 liters per 1 m^2. A 10% solution of the Polyphage disinfectant with a consumption rate of