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THE MAIN REGULARITIES OF RAW MILK CONTAMINATION WITH *STAPHYLOCOCCUS AUREUS*

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Summary. This publication represents results of the regularities of the milk contamination process in which golden staphylococci were studied, depending on the technologies of keeping and milking cows, the level of sanitary culture of the dairy farms, the spread of morbidity of cows to mastitis, the technology of primary milk processing and transfer to the processing enterprise.

S. aureus var. *bovis* is mainly isolated from raw cow milk. Its strains are characterized by the coagulation of blood plasma of cattle and horses, formation in the medium with crystal violet of yellow and orange colonies, phage-typing of Davidson, the lack of production of lecithinase on the Chystovych's medium with 10% sodium chloride solution. It is impossible to get the milk free from pathogens because of evolution caused environmental infection of *S. aureus* var. *bovis* to the skin of the udder of cows. As an objective reality there is a certain level of contamination of milk by *S. aureus* during the milking process. Under this level, you need to understand the number of *S. aureus,* which can come from healthy glands, skin of the udder of cows and milkmaids' hands.

Keywords: *Staphylococcus aureus,* raw milk, milking machines, mammary gland of cows

Introduction. Diseases caused by *Staphylococcus aureus*, have spread so rapidly that a staph infection is right to be called the modern 'plague' or *Staphylococcus* disaster. The epidemiological significance of *S. aureus* that infects food lies in its ability to produce enterotoxins and cause food toxicities of people.

The role of milk and dairy products in the emergence of staphylococcus-associated toxicoses is constantly growing and reaches more than 30% of all cases of mass outbreaks of toxicity in people (Ladanivskyi and Dnistrian, 2000; Ladanivskiy et al., 2007; Melnychuk et al., 2003). The mammary gland of the cow when inflamed is believed to be the main source of enterotoxigenic staphylococci (Kukhtyn, 2004, 2010; Kukhtyn et al., 2016, 2017; Malanyn et al., 1994; Slobodkin, Shelkova and Levytska, 2006). A separate group of scientists believes that the microflora of the environment is constantly penetrating into the udder of cows through the milking channel. Its main mass is being destroyed by factors of nonspecific protection of the mammary gland, but the strongest part of this microflora, in particular *S. aureus* can survive in these conditions, causing various forms of mastitis (Kartashova, 1980; Kravtsiv and Maslianko, 2009; Yakubchak, 2010). This was based on their approaches to improve the level of cows non-affected with mastitis, by isolating and treatment of animals, infected with pathogenic *Staphylococcus.*

There is no comprehensive description of microorganisms of the mammary gland of cows within a microecological approach in the literature. By this time, the biotope had not been included in the scope of interest of many authoritative experts in the field of milk hygiene.

The aim of this work was to study the regularities of the process of milk contamination by *S. aureus*, depending on the technology of animal husbandry and milking, the level of sanitation of dairy farms, the distribution of the incidence of mastitis of cows, and also on the technology of primary processing of milk and transferring to a recycling facility.

Materials and methods. More than 600 composite samples of raw milk were received from dairy factories of Ukraine. They were tested for the presence of *S. aureus* within the period from 2001 to 2016. More than 1,000 milk samples from separate quarter of udder of 350 cows (17 households), more than 2,000 rinses of milkmaids' hands, swabs of cows' skin, dairy utensils and milking machines swabs were also tested. Soil samples of walking grounds and fields that had been fertilized with manure from dairy farms were also collected and tested.

Microbiological examinations of milk, swabs and soil samples were performed according to conventional methods (Bergilevich et al., 2010). Studied material and its tenfold dilutions in the amount of 1 cm^3 were made on Baird-Parker agar ('BioMérieux', France) for identifying coagulase positive staphylococci. Typical

bacterial colonies were counted on the Petri dish after 24–48 hours of incubation at 37 °C. Then pure cultures were isolated and identified according to species using the test API ID32STAPF test ('BioMérieux', France). Coccal catalysation positive cultures that were fermentable to glucose in the Hugh-Leifson's medium were ranked to the species of *Staphylococcus*. The cultures that coagulated rabbit plasma were attributed to the species of *S. aureus*. If it was necessary, additional tests (fermentation of mannithol, the ability to produce phosphatase, lecithinase) were used. Type of hemolysins was determined by S. D. Elek, E. Levy and J. Marks, A. Vaugan, the presence of DNA-ase by C. D. Jeffris, D. F. Heltman, D. Guse. The phage typing was performed using phages of Davidson (Hájek and Marsálek, 1971).

Results. It was found, that 70% of clinically healthy cows are carriers of staphylococci in the udder, where *S. aureus* occurs in 6–15% of cases. Staphylococci were allocated from the nipple skin swabs in 100% of examined cows, but only 30% of cows were carriers of *S. aureus*. Staphylococci have constantly emanated from the skin of the udder and the inner surface of the skin of the thighs and pregnant heifers, but *S. aureus* was isolated from the secret of the mammary gland of heifers only 0.8% of the tested animals. Taking into account the results of examinations of different skin areas of cows, as well as the mucous membrane of the mouth, we got a conclusion that the ecological niche of *S. aureus* in cows is the skin of the udder. Number of animals-carriers of *S. aureus* decreased in summer up to 15%, and in winter it was increased up to 80%. Cows, which udder skin swabs contained *S. aureus*, were divided into permanent carriers (up to 20% of animals) and temporary, but there was a kind of turn, when carriage appeared in some of cows and disappeared in the others.

Sanitary treatment of the udder of cows-permanent carriers with 0.25% solution of desmolux or with the solution of chlorine bleach with 0.05% content of active chlorine did not liberate the skin of the udder from *S. aureus*. Antiseptic teat treatment after milking with emulsion for three months dramatically reduced the level of infection of the skin, but also did not eliminate it.

The number of *S. aureus* in 1 cm^3 of the aseptically milked out milk of healthy cows ranged from 10 to 150. In the case of subclinical staphylococcal mastitis the content of this microorganism increased to 6–8×10^3 CFU/cm^3, and only in the presence of clinical signs, this number increased at 5–6 times or more.

S. aureus was constantly emanated in un-disinfected surfaces of milking machines and dairy utensils. *S. aureus* emanated in the floor swabs and air of barns, but it was not detected in the soil of paddocks, as well as soils that are intensively fertilized with manure from dairy farms.

Therefore, we did not find any significant spread of pathogenic staphylococci in the environment. The distribution range of *S. aureus* was limited by objects that are in direct contact with cows and milk.

S. aureus allocated on average of 50–55% of cases in swabs off the hands of the milkmaids. Usually *S. aureus* var. *bovis* and var. *hominis* in equal amounts appeared in the swabs. The intensity of infection was within 100–200 microbial cells in 1 cm^3 of swab, but that the number of microorganisms increased to 5–10×10^4 CFU/cm^3 at the presence of cracks and erosions of the skin.

The contents of *S. aureus* in the milk delivered to recycling facilities was different from dairy farms without cooling. The milk delivered to the plant non-refrigerated 3–4 hours after milking in winter period contained 2.7±0.3×10^3 CFU/cm^3 of *S. aureus*, and in summer period — 57±5×10^4 CFU/cm^3, respectively.

The rate of *S. aureus* reproduction in bulk milk depended on both temperature 2–6 °C and the amount of the initial mesophilic microflora of milk. The containing of *S. aureus* among the mesophilic microorganisms (6.0–7.0 ×10^4 CFU/cm^3), allocated from the milk, increased in 6 hours at a temperature of 37 °C by 107 times, and whole mesophilic bacteria quantity — in 122 times. Under the same conditions but with the presence of mesophilic microorganisms in milk in rate 7.0–8.0×10^5 CFU/cm^3, the number of *S. aureus* increased in 50 times, and mesophilic microorganisms — in 880 times, respectively. When the temperature of the milk was 25 °C after 6 hours the contents of the *S. aureus* increased in only 5 times, but mesophilic microflora — in 62 times. And only at a temperature of 6±1 °C, the rate of reproduction of *S. aureus* and mesole microflora equaled, their number increased in 2–3 times within 24 hours.

The experiments revealed that in the absence of milking equipment disinfection with their surfaces dispatched in 1 cm^3 of milk on average 100 CFU of *S. aureus*. If the rules for sanitary processing of milking machines and dairy equipment, pre-milking disinfection of the udder of cows and isolation in a separate group of cows with subclinical mastitis are kept, the number of *S. aureus* in bulk milk immediately after milking does not exceed 300 CFU/cm^3.

The composition of the *S. aureus* isolated from combined milk was presented by *S. aureus* var. *bovis* — up to 90% of cultures (coagulation of blood plasma of horses and cattle, formation on the medium with crystal violet of yellow and orange colonies, phage-typing of Davidson, the lack of production of lecithinase on the

Chystovych's medium at 42 °C). Others isolates belonged to *S. aureus* var. *hominis* (no coagulation of the blood plasma of cows, produced mainly α-hemolysins, lecithinase, not sensitive to phages of Davidson). *S. aureus* var. *bovis* produced basically beta gemalten on agar with blood of cattle or sheep. No culture of *S. aureus* var. *bovis* and *S. aureus* var. *hominis*, which were capable of producing β-gemalten form the zone of β-hemolysis on agar with rabbit erythrocytes.

Such peculiarity that some part of coagulase negative staphylococci isolated from breast and skin of the udder of cows, according to some tests could be attributed to pathogenic variants must be taken into account (Table 1).

Table 1 — Pathogenicity tests of coagulase negative staphylococci of breast and udder skin of cows

Hemolytic formed	Cultures tested	Produced from them, %		
		DNA-ase	Phos-phatase	Pigment golden and orange
Alfa, Beta	74	97.3	87.8	93.2
Delta	80	8.7	43.7	40.0
Non-hemolytic	116	2.6	43.1	33.6

As can be seen from the table, the coagulase-negative *Staphylococcus* cultures that are able to produce α- and β-hemolysins, in 97.3% of cases DNA-ase was formed, in 87.8% — phosphatase, in 93.2% — golden or orange pigment.

Discussion and conclusions. The microorganisms of the species of *Staphylococcus* should be considered as a part of normal micro flora of cow udder skin. They are also a part of microbiocenosis of the breast. Therefore, the presence of *S. aureus* on the skin of the udder and in the breast is a natural phenomenon. This microbiocenosis has been forming for millions of years in the process of phylogenetic development of the cattle as a species. *S. aureus* is systematically allocated from the calve skin. The presence of staphylococci, including *S. aureus*, on the skin of the udder and mammary gland, do not always lead to the disease of its master. Only under certain conditions, *S. aureus*, and coagulase positive options that have other signs of pathogenicity, manifest themselves as pathogens, causing inflammation of the breast. On the other hand, the detection of staphylococci in udder secretions of cows sick with mastitis, suggests that these microorganisms are commensally microflora of the udder. At the same time, protection factors of the gland cannot distinguish *S. aureus* from other species of the species of *Staphylococcus*, therefore, *S. aureus* can colonize gland under the same laws of formation of microbiocenosis, and others as his representatives (*Corynebacterium*, *Streptococcus*, coagulase positive species of *Staphylococcus*).

Therefore, identifying of the critical points of risk of contamination of milk with *S. aureus* in the process of its formation it is necessary to associate it with the role of this microorganism as an integral part of autosecretary of skin of the udder and mammary gland. The colonization of the skin of the udder of not even functioning breast with aureus is observed long before the first calving. *S. aureus* actively inhabits the mammary gland after calving and the start of the milking. This settling occurs with certain regularities of formation of microbiocenosis of the breast, primarily in respect of the quantitative content and the ratio of staphylococcus to other members of autoflora. Therefore, a quantitative rate of *S. aureus* in milk of healthy mammary gland does not exceed 100 CFU/cm^3.

The desire to make a full recovery of the number of cows from carriage of *S. aureus* is almost unfulfilled and is not theoretically justified. As at carrying and at the subclinical form of mastitis the *S. aureus* is primary found in the external polysaccharides biofilm (Cucarela et al., 2004; Kukhtyn et al., 2016).

Only sharp decrease in the content of this microorganism in the milk could be the characteristic of introducing of appropriate sanitary mode: culling of cows with chronic untreatable staphylococcal mastitis, effective sanitation of milking and dairy equipment, udder of cows, hand hygiene of milkmaids, milk cooling to 3±1 °C not later than two hours after milking.

As an objective reality, there is a certain natural level of contamination of milk by *S. aureus*, which is impossible to eliminate. The number of *S. aureus* that is able to enter the milk from a healthy mammary gland, skin of the udder of cows and milkmaids' hands is considered under this level.

Specific content of *S. aureus* in fresh bulk milk can serve as the indicator of the effectiveness of sanitation on a dairy farm. Approximately no more than 500 CFU of *Staphylococcus aureus* per 1 cm^3 can be considered as this indicator.

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