

STAPHYLOCOCCAL CONTAMINATION OF RAW MILK AND HANDMADE DAIRY PRODUCTS, WHICH ARE REALIZED AT THE MARKETS OF UKRAINE

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Summary. Milk and dairy products are placed in the first group of risk because they can cause human diseases agent transmission. There is a risk of alimentary staphylococcal toxicosis, in the case of non-compliance with veterinary and sanitary requirements for the primary processing of raw materials, manufacture and realization of finished products. The aim of this study was to estimate a level of milk and dairy 'home-made' products contamination with coagulase positive staphylococci, to determine the biological origin of *Staphylococcus aureus*, isolated from dairy products, and its ability to produce enterotoxins.

The article presents the results of raw milk and dairy products study from private ('home-made') production, which are selling at agro-food markets in Ukraine. Bacteria of the genus *Staphylococcus* are isolated from raw milk and dairy products in 80.3–93.3% of cases. Coagulase positive *Staphylococcus* species were isolated from sour cream — in 57.8% of cases, which was 1.7 times more in comparison with the samples of raw milk and was 2.5 times more in comparison with curd. Two biotypes of *Staphylococcus aureus* (*S. aureus* var. *bovis* and *S. aureus* var. *hominis*) were isolated from samples of raw milk and dairy 'home-made' products, which were realized at the markets. In this case, $46.6 \pm 3.1\%$ of isolated cultures were classified as a biotype *S. aureus* var. *bovis*, and $53.4 \pm 3.7\%$ — to human ecovar. It means that the main source of contamination of 'home-made' cream by staphylococcus is people who do not follow proper hygiene and sanitation during the producing process, namely: milking of cows, primary processing of raw milk, production and realizing of dairy products. It was found that *S. aureus* var. *hominis* had produced type A of enterotoxin in raw milk and dairy products of private ('home-made') production, which, generally, is cause of food toxicosis in humans.

Keywords: milk, dairy products, staphylococci, biotypes, enterotoxins

Introduction. Prevention of alimentary origin diseases is the foreground task of the health system in the world. During the last decades the number of diseases, caused by food microorganisms, was significantly increased (Jaber, 2011; Normanno et al., 2007; Morris and Potter, 2013; Thaker, Brahmbhatt and Nayak, 2013; Sudhanthiramani, Swetha and Bharathy, 2015). According to N. R. Efimochkina (2010), 'emergent' bacterial infections with food transmitting path has a special importance (such as *Listeria*, *Salmonella*, enterohaemorrhagic *Escherichia coli*, *Staphylococcus aureus*, etc.).

Staphylococcus aureus is an opportunistic pathogenic microorganism, which often contaminates raw milk and dairy products and can cause food toxicosis (Kukhtyn, 2004; Thaker, Brahmbhatt and Nayak, 2013). People may become a source of contamination of food products, especially milk, produced at home, as those who carriers of staphylococci on the mucous membranes and the skin. Furthermore, the process of production and realization raw milk and dairy products at 'home' conditions is not always ensured compliance with sanitary and hygiene requirements.

The aim of this study was to estimate a level of contamination of milk and dairy 'home-made' products with coagulase positive staphylococci; to determine the biological origin of *Staphylococcus aureus*, isolated from dairy products, and its ability to produce enterotoxins.

Materials and methods. The research work was conducted in the Ternopil Research Station of the Institute of Veterinary Medicine of NAAS. Sampling and delivery those to the laboratory were carried out according to DSTU 7357:2013 (MEDTU, 2013). Isolation of staphylococci from raw milk, cream and curds was conducted on blood agar that consists of 5% of cattle blood and 5% of Sodium chloride.

The cooks' forms of bacteria are attributed to the genus *Staphylococcus*, which were painted by Gram positive, were produced catalase and were fermented glucose in the Hugh-Leyfson medium. We determined the ability of coagulation the rabbit plasma by the classical method.

Staphylococci was identified by their biochemical activity using commercial test systems: 'STAPHY-test 16', (Lachema, Czech Republic). Biotypes of

Staphylococcus aureus were determined by the Meer technique (Mayer, 1999).

In order to determine the staphylococcal enterotoxins we used a test system Ridascreen®Set A, B, C, D, E (R-Biofarm AG, Darmstadt, Germany) according to the Methodological guidelines 4.2.2429-08 'Method of determining staphylococcal enterotoxins in foods'.

58 samples of raw milk, 180 samples of sour cream and 47 samples of curd of 'home-made' production,

which were realized at the food-farm markets of Ukraine were tested. It was isolated and identified 322 coagulase positive *Staphylococcus* species.

Statistical estimation of the research results was conducted with Microsoft Excel 2007 software.

Results. We have studied the raw milk and dairy 'home-made' products' contamination, which were realized at the markets of Ukraine, with species of *Staphylococcus* of coagulase positive types (CPS). The research results are shown in Fig. 1.

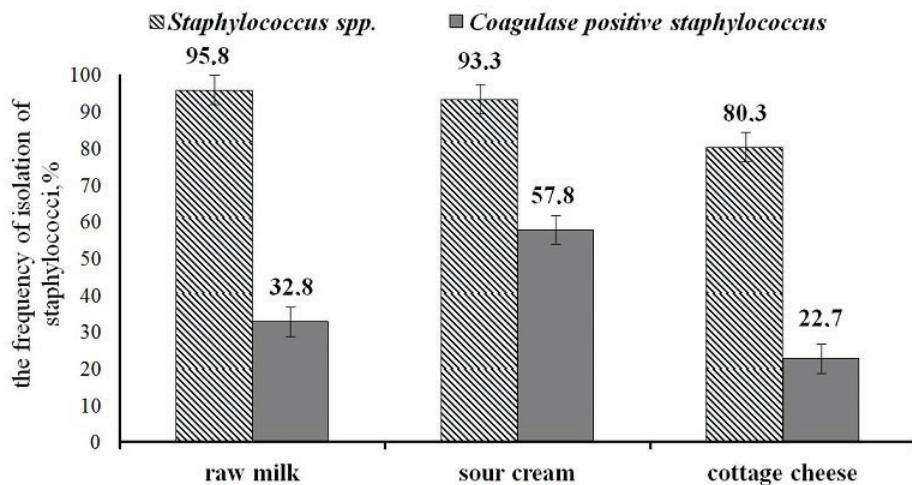


Figure 1. Staphylococcal contamination of raw milk and dairy products of 'home-made' production, which are realized at the food-farm markets of Ukraine

According to the diagram (Fig. 1), the frequency of isolation *Staphylococcus* bacteria were referred to normal microflora of raw milk and dairy products of 'home-made' production, as they isolated in 80.3–93.3% of cases of the investigated samples. Coagulase positive staphylococci species were isolated rarer. In most cases, they were isolated from sour cream — in 57.8% of cases that is 1.7 times more than the samples of raw milk and 2.5 times more than curds.

The results of quantitative estimation of coagulase positive *Staphylococcus* presence in milk and dairy products are demonstrated in the Table 1.

Samples of sour cream were mostly contaminated with coagulase-positive staphylococci — in 57.8% of cases. At the same time, the number in 37.9% of samples did not exceed 1,000 CFU/g, and in 19.94% of samples was more than 1,000 CFU/g. Only from 3.69 to 4.52% of samples of raw milk and curds were contaminated with coagulase-positive staphylococci in amount more than 1,000 CFU/g.

Moreover, we have determined the environmental origin of isolates of *Staphylococcus aureus* from raw milk and dairy products of domestic production, which were realized at the markets, to establish their true source of contamination (Fig. 2).

Two biotypes of *Staphylococcus aureus* (*S. aureus* var. *bovis* and *S. aureus* var. *hominis*) in the raw milk and dairy products of domestic production has been occurred. However, the proportion of their isolation is varied in different dairy products. Thus, it was isolated from raw milk 46.6 ± 3.1% cultures of *Staphylococcus aureus*, which were classified as biotype cattle (*S. aureus* var. *bovis*), and 53.4 ± 3.7% were classified as human ecospecies (*S. aureus* var. *hominis*).

It was obvious, that the isolation numerous *S. aureus* var. *hominis* was a result of manual milking cows and this biotype is ingress to raw milk from the people's hands. Apparently, the isolation a lot of *S. aureus* var. *bovis* indicated that raw milk from teats skin and cows' mammary gland was contaminated with this biotype.

At the same time, 64.3 ± 4.7% of *S. aureus* var. *hominis* was isolated from the sour cream of 'home' production. It was 1.8 times ($r \leq 0.005$) more than the amount of *S. aureus* var. *bovis*. We supposed, that amount of *S. aureus* var. *hominis* is much more than amount of *S. aureus* var. *bovis*, because of failure to comply with proper hygiene and sanitation during the manufacture and realization of sour cream.

Table 1 – The content of coagulase positive staphylococci in raw milk and dairy products of domestic production, %

Quantity of coagulase positive Staphylococcus, CFU/g	Type of product		
	Raw milk	Sour cream	Curds
Coagulase positive Staphylococcus are absent	67.2 ± 4.080	42.12 ± 2.247	77.33 ± 2.221
≤ 100	9.20 ± 0.822	3.41 ± 1.123	2.27 ± 0.172
101–300	7.74 ± 0.932	8.11 ± 0.445	4.54 ± 0.337
301–500	6.20 ± 0.654	8.90 ± 0.722	9.09 ± 0.753
501–1,000	5.14 ± 0.476	17.45 ± 1.141	3.11 ± 0.851
≥ 1,001	4.52 ± 0.361	19.94 ± 1.742	3.69 ± 0.886

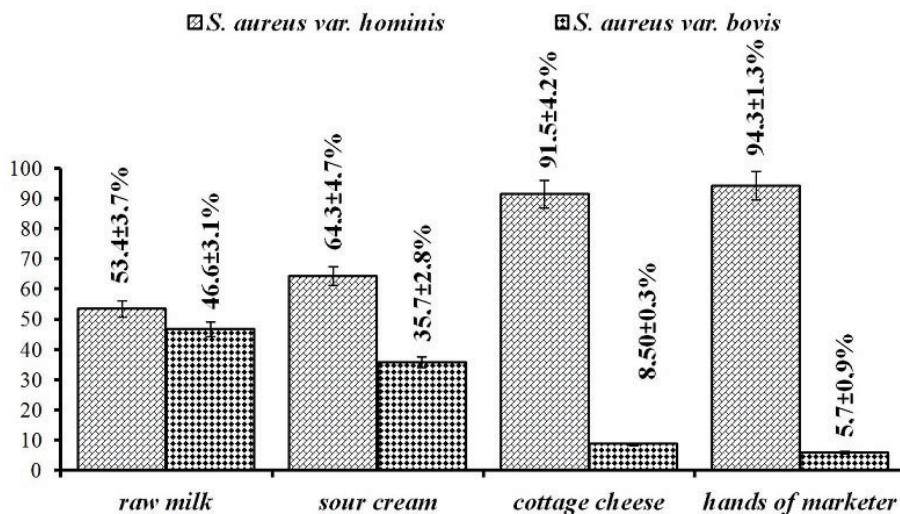


Figure 2. Biotypes of *Staphylococcus aureus* are isolated from raw milk and dairy products of 'home-made' production

Substantially, $91.5 \pm 4.2\%$ of *S. aureus* var. *hominis* was isolated from curds of 'home-made' production. Many of the human's ecospecies in curds are indicated by contamination it during manufacture, packaging and realization. In 'home' conditions specificity of making curds is long-time heating of sour milk and during this process almost all vegetative mesophilic microflora, including *Staphylococcus aureus*, were inactivated.

As shown on Fig. 2, *S. aureus* var. *hominis* ($94.5 \pm 1.5\%$) was dominated in the samples from hands of marketer, who selling dairy products, that is indicates that exactly people are a major source of staphylococcal contamination.

Thereby, hand-makers and sellers are constant source of contamination of dairy products by *S. aureus* var. *hominis* and research findings of biological typing of *Staphylococcus aureus* that is isolated from raw milk and dairy handmade products, that are sold in agricultural markets, confirming it. In order to prevent a food staphylococcal toxicosis, it continuous

microbiological survey of hand-makers of dairy products to identify people are require, who are the carriers of *S. aureus* var. *hominis*.

It is known, that *Staphylococcus* produces six antigenic variants of enterotoxin: A, B, C, D, E, F (Normanno et al., 2007). Generally, enterotoxin type A causes food intoxication, in rare cases — enterotoxin type D. We have studied types of produced enterotoxins concerning on *S. aureus* var. *bovis* and *S. aureus* var. *hominis* (Fig. 3).

43.5% of *S. aureus* var. *hominis* strains and 56.9% of *S. aureus* var. *bovis* strains produce no enterotoxines. Only 38.9% of strains of *S. aureus* var. *hominis* produce enterotoxin type A.

S. aureus var. *hominis* produce enterotoxin type C in 13.4% cases that is 1.6 times more ($p \leq 0.05$) than *S. aureus* var. *bovis*. At the same time, 4.2% of strains of *S. aureus* var. *hominis* produce enterotoxin type D, that is 2.3 times less ($p \leq 0.05$) than *S. aureus* var. *bovis*.

Furthermore, only 25.0% of strains of *S. aureus* var. *bovis* produce enterotoxins of types C and D.

Consequently, our scientific research indicates that in case of using raw milk and handmade dairy

products, exactly *Staphylococcus aureus* of human origin produced enterotoxin type A, which basically causes food intoxication.

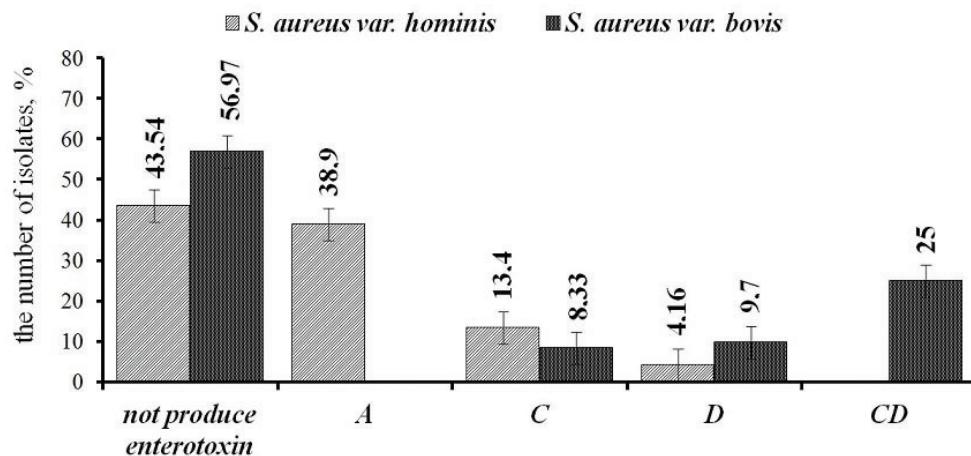


Figure 3. The types of enterotoxins, which produced by staphylococci that are isolated from raw milk and handmade dairy products

Discussion. The presence of the bacteria *Staphylococcus* genus (almost 100%) in samples of handmade dairy products can be explained by the fact dairy products are made from thermally untreated raw milk, and with nonobservance of sanitary norms and rules. Staphylococci are so-called resident (useful) microflora of the skin of the cows' udder. It is obviously, that in almost 100% of cases, they can be isolated from raw milk. According to the studies (Jaber, 2011; Thaker, Brahmbhatt and Nayak, 2013; Sudhanthiraman, Swetha and Bharathy, 2015) coagulase positive staphylococci are much less isolated from the surface skin of teats skin and mammary gland of healthy cows. In general, no more than 20% of cows are carriers of coagulase positive staphylococci on skin surface, and no more than 5% of clinically healthy cows are carriers of coagulase positive staphylococci in mammary gland. However, their quantity is much increased in raw milk upon mastitis condition or in cause of wounds, scratches and erosions on the skin's nipple.

So, it's an objective reality, that some contamination of dairy products of 'home-made' production with coagulase positive Staphylococci, particularly *S. aureus* var. *bovis*, so far as they are often present on the cows' teat skin of and in raw milk.

According to the EU Directive, quantity of coagulase positive staphylococci must be no more than 500 CFU/cm³ in raw milk. Taking it into account, we can conclude, that 10% samples of raw milk, 37.39% samples of sour cream and 6.8% samples of curds

were contaminated by excessive amount of coagulase positive staphylococci.

Among the most common four biotypes of *Staphylococcus aureus* (*S. aureus* var. *hominis*, *S. aureus* var. *bovis*, *S. aureus* var. *avium*, *S. aureus* var. *canis*) only two ecospecies (human and cattle) were isolated from raw milk and dairy products of 'home-made' production. *S. aureus* var. *hominis* was isolated more often compared to *S. aureus* var. *bovis*. It was suggested, that the main source of staphylococcal contamination of raw milk and 'home-made' dairy products are people, who do not follow proper hygiene and sanitation rules during the manufacturing process, such as: cows' milking, the primary milk handling, exactly production and realization of dairy products. Such significant contamination by coagulase positive staphylococci cannot be ignored, because of the possibility to propagate and produce enterotoxins, which can cause food toxicosis. It is believed, that dairy products mainly contaminated by staphylococci of cattle biotype (Kukhtyn, 2004; Wang et al., 2009), but the main of cases of staphylococcal dietary toxicosis were caused by enterotoxins types A and D, which were produced by staphylococci of human biotype (Normanno et al., 2007).

According to the results of research, enterotoxin type A was often produced by *S. aureus* var. *hominis* in 38.9% cases. Cultures of the cattle biotype of staphylococci mainly produce enterotoxins types C, D, and CD, which show clinical and subclinical cows' mastitis (Wang et al., 2009). Hence it seems that

the dairy products of 'home' production might be the cause of staphylococcal toxicity that causes a lot of *S. aureus* var. *hominis*.

Thus, knowledge and control of potential sources of contamination by *Staphylococcus aureus* of unprocessed food is the key to effective prevention of staphylococcal food toxicosis.

Conclusions. Coagulase positive *Staphylococcus* species were isolated from the following dairy products that are sold in agricultural markets: from the sour cream — in 57.8% of cases, from the raw milk — in 32.8% of cases, from cottage cheese — 22.7%. Two biotypes of *Staphylococcus aureus* (*S. aureus*

var. *bovis* and *S. aureus* var. *hominis*) were isolated from cow's milk and whole milk products. *S. aureus* var. *hominis* allocated to the cow's whole milk in $53.4 \pm 3.7\%$ cases, cream — $64.3 \pm 4.7\%$ cases, and cottage cheese — $91.5 \pm 4.2\%$ cases. It was found that *S. aureus* var. *hominis* had produced type A of enterotoxin in 38.9% cases, which, generally, can cause food toxicosis in humans. It was established that *S. aureus* var. *bovis* had produced types C and D of enterotoxin in 25.0% cases, but they show poor enterotoxic action. *S. aureus* var. *hominis* was 1.5–3.0 times more resistant to antibiotics compared to strains of *S. aureus* var. *bovis*.

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