

## ANNOTATION

**to the thesis of Kuzeubayeva Anar Sabyrbayevna**  
**«Development a method for molecular indication the contamination by**  
***Escherichia coli* and modeling the food safety assessment of cheeses produced**  
**in Kazakhstan» for the PhD degree of the Educational Program**  
**«8D09102-Sanitary and Ecological Safety of Animal Products»**

**Relevance.** Food safety guided by One Health concept ensures the sustainable existence of humanity at the global level through monitoring and organization of comprehensive research on the veterinary and sanitary quality of food products sold on the market. Ensuring sufficient food production and food safety are the main strategic directions of the agrarian policy of the Republic of Kazakhstan. Cheese production is included in list of priority sectors of the country's dairy industry.

Although cheeses are considered microbiologically safe products, foodborne infections are frequently reported in various regions of the world due to contamination of these dairy products with pathogenic bacteria.

Foodborne diseases are one of the most important public health problems. *E. coli* strains found in dairy products are not only a source of infections but also a reservoir of genetic determinants of antibiotic resistance. Antibiotic-resistant *E. coli* strains that contaminating food products can transmit resistance and virulence factors to other microorganisms and penetrate into the gut microbiota of humans and animals. Contamination of dairy products with pathogenic microorganisms causes toxic food infections that are especially dangerous for human health.

Studies the *E. coli* contamination of cheeses risk of in Kazakhstan has not been previously conducted at all, therefore, from the veterinary and sanitary point of view, the study of *E. coli* contamination's epidemiological characteristics in these products, determination the pathogenic strains and resistance of this species to antibiotics are an urgent scientific and practical problem of modern public health and veterinary medicine. In the dissertation work the significance of this problem is proved on the basis of analysis the diverse international, domestic scientific and statistical material, and the relevance of the research topic is justified.

According to the current food safety development, molecular genetic studies for bacteria identification, pathway identification, resistance and mutual phylogenetic relationships of pathogens, and mathematical modeling for food safety assessment of food products are considered important scientific tools.

**Purpose of the study** were an assessment the *E.coli* contamination and modeling of food safety of cheese produced in Kazakhstan and improvement of molecular indication of the bacterium.

**The study objectives**

1. To conduct a marketing study of the domestic cheeses' modern market.
2. To determine the *Escherichia coli* (*E. coli*) group bacteria contaminating cheeses.
3. To assess the quality of marketed cheeses by *E. coli* contamination.

4. To study molecular and genetic characterization of *E. coli* isolates from cheeses.
5. Modify the methodology for molecular indication of pathogenic *E. coli* strains.
6. Determine the phenotype and genotype profiles of antibiotic-resistant *E. coli* strains from cheese.
7. Develop a model for assessing the food safety of cheese contamination with *E. coli*.

### **Research methods**

Research was carried out at the «Veterinary Sanitation Department» and Kazakh-Chinese Laboratory of Biosafety of S. Seifullin Kazakh Agrotechnical Research University, Laboratory of «Food Safety and Molecular Diagnostics» of Istanbul Cerrahpaşa University, National Center of Biotechnology and Research Institute of Applied Biotechnology of Kostanay Regional University named after A. Baitursynov.

As objects of research there were used 207 samples of cheese produced at 22 large and 8 small enterprises of the central, eastern, southern and northern regions of Kazakhstan, data from official statistics, 94 isolates of coliform bacteria and 65 isolates of *E. coli* from cheese samples.

Conventional microbiological, mass spectrometric, molecular genetic methods, standard methods for determining antibiotic resistance such as disk diffusion testing and genotyping, bioinformatics methods, singular vector decomposition methodology, mathematical modeling and statistical programs were used in the course of the thesis work.

### **The main provisions submitted for defense.**

- Current situation on the cheese market of Kazakhstan
- Coliform microbiota contaminating domestic cheese
- Assessment the food safety of marketed cheeses by *E. coli* contamination
- Molecular genetic characterization of *E. coli* isolates by targeting *16S rRNA* and *dnaJ* genes.
- Detection of pathogenic *E. coli* strains by advanced multiplex PCR.
- Phenotypic and genotypic profiles of antibiotic-resistant *E. coli* strains isolated from cheeses.
- A model for assessing the food safety of cheese under *E. coli* contamination.

### **Description of the main results of the study**

Market analysis showed that Kazakhstan produced 43.2 thousand tons of cheese in 2023, which was 10.5% more than in 2022 and 16.8% more than in the first half of 2024. 48.1% of cheeses on the market were domestic products, including 68.4% semi-hard, 18.4% soft and 13.2% hard varieties.

According to microbiological studies, the total contamination of domestic cheese with four types of coliform bacteria was 45.4% [95%CI 39.40, 54.86], including hard type cheese 19.5% [95% CI 18.42, 30.27], semi-hard type 47.0% [95% CI 40.37, 71.51] and soft type was 59.3% [95%CI 45.25, 64.51]. The highest contamination level (72.2%) was found in cheeses produced in the north. The

distribution of *E. coli* in the product was 69.1%, *Enterobacter cloacae* – 10.6%, *Citrobacter freundii* – 11.7% and *Citrobacter braakii*-8.5%. The level of coliform contamination by CFU was 60±17.78.

*E. coli* was detected in 31.4% [95%CI 30.45, 38.88] the total cheese tested. According to the international standard, 92.3% of contaminated cheeses were rated as acceptable and satisfactory (CFU <20 or 20 <100, respectively) and 7.7% as unsatisfactory and unsuitable (>100 or ≥104). The last two categories were 4/5 in the soft type cheeses. *E. coli* contamination of cheeses from companies in the northern region reached 1/2 and was higher than in other regions. *E. coli* was detected in 100% of soft cheeses realized by slicing method. The level of contamination in small enterprises amounted to 65% and was more than twice high than in cheeses of large producers.

Molecular phylogenetic analysis of *E. coli* isolates showed 99-100% similarity to the reference strains *E. coli* NBRC 102203 AB681728 and *E. coli* NC\_000913.3:14168-15298, respectively, in the nucleotide sequences of the target genes *16S rRNA* and *dnaJ*. In addition, one isolate was found to be 100% analogic to the enteropathogenic *E. coli* O157:H7 strain NC\_002695.2:14185-15315 with the *dnaJ* gene.

The multiplex PCR method for identification of pathotypes of enteropathogenic *E. coli* (EPEC) was improved and tested during the study. For the first time in Central Asia, a pathogenic strain of *E. coli* O157:H7 was detected in domestic cheese, the level of contamination of the product with this strain was 0.5%.

According to the results of testing conducted according to standards of the Eurasian Customs Union and EUCAST 11.0, *E. coli* isolates showed resistance to 65% of 20 antibiotics. 44.6% were resistant to  $\beta$ -lactams, 29.2% to tetracyclines and nitrofurans, 20% to amphenicols, 13.9% to fluoroquinolones, 12.3% to sulfonamides, and 3.1% to quinolones. Genes encoding resistance to sulfanilamides were detected in 30.8% of the *E. coli* population, in 15.4% to  $\beta$ -lactams and in 9.3% - to quinolones. Polyresistant profile to antibiotics was shown in 24.6% of isolates.

The thesis used a singular value decomposition model, which showed that the eigenvalues of principal components in the dynamics of cheeses' Coliform contamination *E. coli* formed a separate cluster from other types. It contained the most extensive information on the variation of matrices of analyzed datasets by types of products, size of enterprises and packaging methods. The coordinates of *E. coli* eigenvectors were directed to soft types cheeses produced in small enterprises and sold in sliced form, and this product is the main factor to be taken into account when ensuring the food safety of cheeses.

### **Substantiation of the novelty and importance of the results obtained**

For the first time from the position of food safety there were studied epidemiological features of contamination with coliforms, including *E. coli*, cheeses of domestic production, presented in the Kazakhstan market. The features of contamination of cheese varieties by coliform species (*E. coli*, *Enterobacter cloacae*, *Citrobacter freundii* and *C. braakii*) were established. The food safety of cheese samples was evaluated by the *E. coli* CFU/g. As a result of molecular analysis using the *16S rRNA* and *dnaJ* target genes, the isolates showed 99-100% identity with two

reference *E. coli* strains and one isolate showed 100% concordance with enteropathogenic *E. coli* strains O157:H7 for the *dnaJ* gene. The multiplex PCR method for detection of *E. coli* pathotypes was improved during the study. Using this method, a pathogenic strain of *E. coli* O157:H7 was detected for the first time in Central Asia and Kazakhstan in 0.5% of domestic cheese samples in soft cheese of a small enterprise. When tested for antibiotic resistance, most of isolated *E. coli* strains showed genotypic resistance to sulfonamides,  $\beta$ -lactams and quinolones. A mathematical model of the dynamics of *E. coli* contamination of cheeses was created using the principal component method of spectral analysis. It showed that according to the values of dataset matrices, the most extensive information on the change in the coordinates of eigenvectors is available in soft type cheeses produced at small enterprises and sold in sliced form.

Based on research results, the author's copyright certificate «Modification of the method of molecular indication of pathogenic strains of *Escherichia coli* that contaminating food products» was obtained.

The provisions of the dissertation work are implemented in the educational process of the discipline «Health protection of animals in emerging infections» in S. Seifullin KATRU for «Veterinary Safety» specialty.

#### **Compliance with directions of scientific development or government programs**

The research was carried out within the framework of the scientific and technical program BR10764944 «Development of methods for analytical control and monitoring of food safety» target financing of the Ministry of Agriculture of the Republic of Kazakhstan in 2021-2023.

#### **Description the doctoral student's contribution to each publication**

Literature data analysis, practical research conducting, research results analyzing, writing all scientific articles and the thesis manuscript preparation were carried out with author's personal participation.

The main dissertation results have been published in 10 scientific papers, including two articles in journals included in Web of Science and Scopus databases; three articles in publications recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education; five articles in international conference proceedings.

Dissertation results were discussed in annual reports of S. Seifullin KATRU Academic Council (Astana, 2020-2023) and several international scientific conferences: «Achievements of modern veterinary science in the XXI century: innovations, problems and ways to their solution», Republican scientific and practical conference (Semey, 2021); International scientific-practical conference dedicated to the 70<sup>th</sup> anniversary of Semey zootechnical and veterinary institute «State and prospects of industrial-innovative development of agroindustrial complex of the Republic of Kazakhstan» (Semey, 2022); International scientific-practical conference «Seifullin Readings-18: Youth and science – a look at the future» (Astana, 2022); «Seifullin Readings-18(2): International scientific-practical conference Science of XXI century - the era of transformation» (Astana, 2022);

«Seyfullin Readings-19: International scientific-practical conference dedicated to Handelman's 110<sup>th</sup> anniversary» (Astana, 2023).

**The scope and structure of the dissertation.** The thesis is set out on 122 pages of computer text, includes normative references, definitions, designations and abbreviations, introduction, literature review, materials and methods of research, research results and their discussion, conclusion, practical recommendations, list of used literature, including 280 sources and four appendices. The dissertation is illustrated with 21 tables, 26 figures.