American Journal of Animal and Veterinary Sciences

Original Research Paper

Coliforms Diversity and Antibiotic Resistance in Kazakhstan Cheese

[1]Anar Kuzeubayeva, [1]Altay Ussenbayev, [2]Ali Aydin, [3]Zhannara Akanova, [1]Damegul Rakhimzhanova, [4]Raushan Rychshanova, [5]Alma Kairzhanova, [1]Dinara Seitkamzina and [1]Assylbek Zhanabayev

[1]Department of Veterinary Medicine, Veterinary and Husbandry Technology Faculty, S. Seifullin Kazakh Agro Technical Research University, Astana, Kazakhstan
[2]Department of Food Hygiene and Technology, Veterinary Medicine Faculty, Cerrahpaşa Istanbul University, Istanbul, Turkey
[3]Faculty of Veterinary Medicine and Livestock Technology, Kazakh-Chinese Laboratory for Biological Safety, S. Seifullin Kazakh Agro Technical Research University, Astana, Kazakhstan
[4]Applied Biotechnology Research Institute, Baitursynov Kostanay Regional University, Kostanay, Kazakhstan
[5]Applied Genetics Laboratory, National Biotechnology Center, Astana, Kazakhstan

Article history
Received: 26-10-2023
Revised: 22-11-2023
Accepted: 29-11-2023

Corresponding Author:
Altay Ussenbayev
1Department of Veterinary Medicine, Veterinary and Husbandry Technology Faculty, S. Seifullin Kazakh Agro Technical Research University, Astana, Kazakhstan
Email: altay.ussenbay@gmail.com

Abstract: Coliform bacteria contaminate dairy products and pose public health concerns including food poisoning and toxemia. We aimed to determine the species diversity of coliforms and to evaluate the resistance to antibiotics of *E. coli* in various types of cheese sold by local producers in Kazakhstan. We collected 197 samples from different cheeses sold by producers in the Kostanay (n = 89), East Kazakhstan (n = 70) and Akmola (n = 38) regions and studied by classical microbiological and mass spectrometric methods for contamination with coliforms in certificated for bacterial pathogens' research laboratories. Such investigation of cheese was experienced the first time in Central Asia. We isolated five coliform bacteria species (*Escherichia coli*, *Citrobacter freundii*, *Serratia liquefaciens*, *Enterobacter cloacae* and *Citrobacter braakii*) in 45.2% of the samples. *E. coli* and *C. freundii* were dominant. Antibacterial resistance of *E. coli* isolates (n = 65) to 19 antibiotics was investigated by the disc-diffusion and molecular genetic (PCR) methods. Isolates were sensitive to drugs of the aminoglycosides group and resistant to 60% of the β-lactams group drugs (ampicillin, cefpodoxime, cefoperazone) and to an agent out of four of the fluoroquinolones group (enrofloxacin). Some *E. coli* strains were multi-drug resistance to tetracyclines, beta-lactams and fluoroquinolones. PCR of *E. coli* isolates revealed genes that encoded the resistance to β-lactams in 15.4%, to sulfonamides in 30.8% and quinolones in 9.3% of cheese samples, providing sufficient biotic potential for the transfer of these genes to intestinal biocenosis bacteria of humans.

Keywords: Antibiotic Resistance Gens, Cheese, *Enterobacteriaceae*

Introduction

Natural cheese has been a historically valuable biotechnological dairy product of the human diet. Currently, there is a global trend of increasing popularity and consumption of various types of cheeses due to the number increasing and profitability of population over the world (Baranceli *et al*., 2014). In parallel, there is a growing market niche of consumers for artisanal cheeses that differ in organoleptic and nutritional properties (Fusté-Forné, 2020; Chourasia *et al*., 2021).

However, cheese, like other dairy products, can be a source of foodborne pathogens that can survive and multiply in the production environment under certain conditions. When foods are contaminated, they pose a public health concern and cause food poisoning and toxic infections (Kim *et al*., 2017; Ganz *et al*., 2020).

Epidemiologically, the most significant foodborne pathogens mostly belong to Gram-negative bacteria of the genera *Escherichia, Enterobacter, Citrobacter, Klebsiella* and *Cronobacte*r of the *Enterobacteriaceae* family, so-called coliforms (Martin *et al*., 2016), which emphasizes

the importance of this group from the microbiological food safety aspect. Thus, there have been reported cases when dairy products have proved to be a significant source the outbreaks of *Escherichia coli* infection in the US, France, Canada (Farrokh *et al*., 2013; Currie *et al*., 2018; Andretta *et al*., 2019). European Food Safety Authority has reported 14 outbreaks associated with coliforms in cheese and raw dairy products (EFSA Biohaz Panel *et al*., 2020).

So, regulation and control of coliforms in food are necessary for the following reasons. Firstly, these bacteria can cause spoilage of dairy products, which negatively affects the yield and quality of products and leads to significant economic losses (Blackburn, 2006). Secondly, their contamination of products above the required level leads to food poisoning, or in the presence of pathogenic strains of this group to food toxic infections (Bhunia, 2018).

Coliform bacteria are the normalized safety indicators for dairy products in countries of the Eurasian Economic Union. In same way the international safety and quality standards generally allow for dairy products to contain no more than 10 coliform cells in 1 cm^3/g (Shadrova *et al*., 2017). Official monitoring in 2017-2021 showed that coliform bacteria were present in dairy products sold through retailers in East Kazakhstan at levels exceeding the country's threshold standards for these microorganisms.

Contamination of dairy products with coliforms occurs post-pasteurization and has a secondary origin. The detection of coliform in prepared consumer products, including cheese, indicates unsanitary production conditions (Takci *et al*., 2020). Farm animals and the raw materials obtained from them determine the main way in which emerging pathogens are transmitted to humans through products. *E. coli* is an indicator of products' fecal contamination with thermotolerant coliform bacteria (Selover *et al*., 2021). Thus, the study of coliform microorganisms is necessary for assessment the dairy products' quality and safety.

The development of high-level resistance to antimicrobial drugs among bacteria is considered a world problem of modern human and veterinary medicine and agricultural production (Yang *et al*., 2019). Mostly, resistant microorganisms are transmitted between animals and humans by close contact or through infected food (Verraes *et al*., 2013). Therefore, monitoring of contamination of animal origin products with bacteria resistant to antimicrobial drugs and resistance coding genes in microbial populations is highly relevant (Sora *et al*., 2021). In addition, research of cheese coliforms from this approach was not provided in Central Asia.

The goal of the work was to determine the diversity of coliform species in various types of cheese sold by local producers in the Kostanay, East Kazakhstan and Akmola regions of Kazakhstan and to assess the antimicrobial resistance of isolated *E. coli*.

Materials and Methods

Sampling

The research was carried out at the Kazakh Chinese Biosafety Laboratory of Seifullin Kazakh Agrotechnical Research University, the Laboratory of Molecular Diagnostics and Food Safety of Istanbul University Cerrahpaşa, the Applied Biotechnology Research Institute of A. Baitursynov Kostanay Regional University and the National Biotechnology Centre.

A total of 197 samples of different types of cheese (hard, semi-hard, soft) from local producers of Kostanay (n = 89), East Kazakhstan (n = 70) and Akmola (n = 38) regions were randomly collected from retail outlets and farmers' markets in 2020-2023 (Figs. 1-2).

Samples were collected under aseptic conditions in an ice container and subjected to microbiological examination on the day of receipt. The remaining volume of samples for further studies was stored at -20°C.

Fig. 1: Preparation of cheese samples for investigation

Fig. 2: Sampling of national dairy

Bacteriological Studies

Microbiological studies included the identification of bacteria of the family *Enterobacteriaceae*, which were determined by seeding on generally accepted selective media for the studied microbes with re-seeding on appropriate differential diagnostic media, as well as using commercial compact dry EC/Coliform plates (Nissui PharmaCeutical CO., LTD, Tokyo, Japan) according to the instructions and isolation of pure cultures (Kuzeubaeva *et al*., 2022). Endo medium and CHROMagarTM *E. coli* (CHROMagarTM, Paris, France) were used to differentiate *Enterobacteriaceae*. The formation of dark red colonies with a metallic luster on the Endo medium testified to the affiliation of microorganisms that allowed the growth of lactose-positive enterobacteria on accumulative nutrient media.

For biochemical identification of lactose-positive *Enterobacteriaceae*, an oxidase test, gram staining, lactose fermentation and indole formation were performed.

After conventional microbiological analysis, bacterial strains were identified by mass spectrometry. For this purpose, cultures of coliform bacteria (single and pure colonies) were put into the 96-well MSP chip cells. Then, 1 µL of the α-cyano-4-hydroxycoric acid's matrix solution was applied with 50% acetonitrile and 2.5% trifluoroacetic acid and dried at room temperature (21±1°C). The chip was placed in the mass spectrometer MALDI-TOF Microflex LT (Bruker Daltonics GmbH and Co. KG., Germany). Then the chip was positioned in the ionization chamber and after achieving the required parameters, a calibration was performed using the applied standard. The spectra collection was conducted in automatic mode. There were used 40 laser pulses (frequency 60 Hz) for obtaining a single mass spectrum. The mass/charge range 2000-20,000 Da was analyzed.

Study of Antibiotic Resistance

Resistance to the most widely used antibiotics and Antimicrobial Drugs (AMD) was determined in isolated 65 *E. coli* colonies. Resistance testing of microorganisms conducted with the disc-diffusion method, using Muller-Hinton agar (HiMedia Laboratories, India, MV1084) and the results were interpreted according the European Committee on Antimicrobial Susceptibility Testing-(EUCAST, 2021) guideline (Yang *et al*., 2019).

There were used 19 antibiotic discs for testing: Beta-lactams (with 10 µg ampicillin, 25 µg amoxicillin, 75 µg cefoperazone , 30 µg cefoxitin , 10 µg cefpodoxime), aminoglycosides (with 10 µg streptomycin , 30 µg kanamycin, 120 µg gentamicin), amphenicols (with 30 µg levomycetin), tetracyclines (with 30 µg tetracycline, 30 µg doxycycline), fluoroquinolones (with 5 µg enrofloxacin, 5 µg ciprofloxacin, 10 µg norfloxacin, 5 µg ofloxacin), quinolones (with 30 µg nalidixic acid), sulfonamides (with 1.25/23.75 µg sulfamethoxazole and trimethoprim) and nitrofurans (with 300 µg furadonin, 300 µg furazolidone).

Molecular Identification

The 65 collected *E. coli* strains were studied for genotypic resistance to antibiotics by PCR. Bacterial DNA was isolated by lysis according to the recommendations of the European union reference laboratory of the antimicrobial resistance community. water without DNase (400 µL) was put into an Eppendorf tube, then several colonies were captured from a dense MPA medium with a disposable loop and dissolved in the tube and boiled for 10 min at 100°C on a thermostatically controlled orbital shaker (Biosan, Latvia). Then it was abruptly cooled by transferring the test tube to ice, centrifuged for 10 min/1000 g and the supernatant in the 100-150 µL volume was put into a new test tube. For amplification, 25 µL of the reaction mixture and 1 µL of the DNA sample were taken. The temperature regime of denaturation was 94°C/30 s and annealing 55-57°C, depending on the specific primer. Elongation was performed at 72°C/60 s. The amplification time was approximately 105 min. Products of amplification were detected in 1.5% agarose gel by electrophoresis. The following primers were used: For the group of β-lactams penicillin (blaTEM, blaSHV and OXA), aminoglycosides (aphA1 and aadB), tetracyclines (tetA and tetB), quinolones (qnrA and qepA) and sulfonamides (SUL3).

Statistical Analysis

Contamination level of cheese was analyzed using Microsoft Excel.

Results

89 isolates of Gram-negative bacteria were isolated (Table 1). According to the results of the primary screening of cultural-morphological and biochemical properties (growth on sugar agar), the isolates fermented lactose with the formation of gas.

As can be seen from Table 1, coliform isolates were more often found in soft cheese.

An average level of cheese contamination between regions has not widely variated. So, coliform bacteria were found in 42.7 (95% CI 29.11-56.29), 49.6 (95% CI 38.38-60.86) and 51.7% (95% CI 29.85-73.55) of samples in Kostanay, East Kazakhstan and Akmola regions respectively.

During a direct, automatic study of microorganism colonies grown in MPA, five species of bacteria belonging to the *Enterobacteriaceae* family were identified on a mass spectrometer: *E. coli, S. liquefaciens, E. cloacae, C. freundii* and *C. braakii*.

Table 1: Detection of coliform bacteria isolates in the studied cheese

№	Type of cheese	Number of samples	Contaminated isolates, %, mean	SD	95% CI
1	Semi-hard	32	30.23	4.37	(25.28-35.17)
2	Soft	89	34.90	0.37	(34.48-35.31)
3	Cream	58	68.56	8.27	(59.20-77.91)
4	Cottage	7	58.30	8.30	(46.79-69.80)
5	Curt	11	57.73	12.53	(43.55-71.90)
	Total	197	45.20	14.77	(36.95-62.84)

Table 2: Determination of antibiotic resistance genes in isolates with phenotypic antimicrobial resistance (n = 65)

Groups of antibacterial drugs	Anti-biotic resistance genes	Number of positive samples	Proportion, %
β-lactams	blaTEM	10	15.4%
Aminoglycosides	aadA, aphA1	0	0
Tetracyclines	tetA,		
tetB	0	0	
Sulfonamides	sul1,		
sul3	20	30.8%	
Quinolones	qnrA	6	9.3%

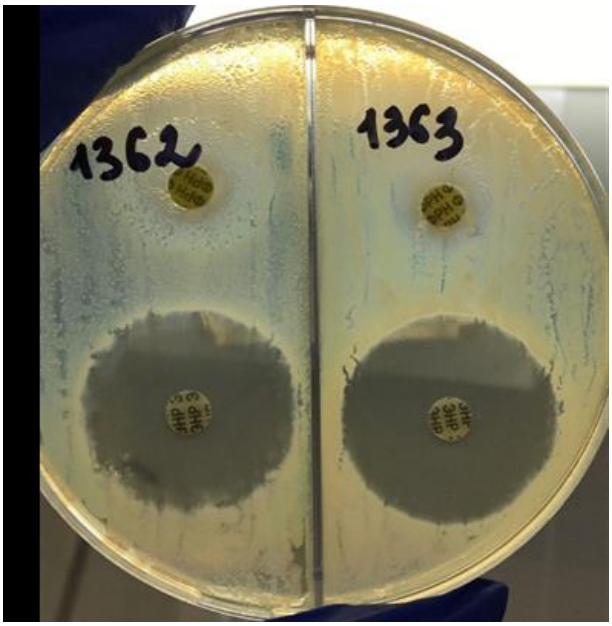

Fig. 3: Growth suppression zones of *E. coli* isolated from cheese

The most common among them were *E. coli* (73%), *C. freundii* (11.2%), *E. cloacae*, *Citrobacter braakii* and *S. liquefaciens* (8.9%).

When testing antibiotic resistance (Fig. 3) it was found, that the isolates were sensitive only to drugs of the aminoglycosides group and resistant to 60% of the β-lactams group drugs (ampicillin, cefpodoxime, cefoperazone) and to an agent out of four of the fluoroquinolones group (enrofloxacin).

The isolates showed Multi-Drug Resistance (MDR), i.e., 1.5% of them demonstrated resistance to 8 antibacterial drugs, 3.1%-to seven and six, 4.6%- to five, 6.2%-to four, three and two, 20%-to one drug.

Testing of *E. coli* isolates with phenotypic antimicrobial resistance by molecular methods showed the presence of drugs' resistance genotypes (Table 2).

Thus, genes encoding resistance to β-lactams were identified in 15.4%, to sulfonamides in 30.8% and quinolones in 9.3% of samples of isolated *E. coli*.

Discussion

Coliform species *E. coli, S. liquefaciens, E. cloacae, C. freundii* and *C. braakii* were found in 45.2% of the studied cheese samples from producers in Central, Northern and Eastern regions of Kazakhstan. According to our results, *E. coli* and *C. freundii* were the dominant species in populations of coliform bacteria contaminating cheese. The dominance of *E. coli* in bacterial metagenome profile of dairy products was noted recently (Rüstemoğlu *et al*., 2023).

Contamination with *E. coli* is quite often found in different cheese types. This species belongs to the foodborne zoonotic pathogens. Isolates of *E. coli* may be commensal or pathogenic to the consumer; their presence in food may indicate the presence of other enteropathogens (Ombarak *et al*., 2016). The *E. coli* detection frequency in cheese may be associated with the high resistance of this organism to adverse conditions during the entire processing period (Hammad *et al*., 2022).

Citrobacter spp. are also considered conditionally pathogenic microflora and are capable of causing gastroenteritis and toxic infection among humans. In the Czech Republic, *Citrobacter* spp. was detected in 26 of 59 samples of products tested, including cheese. In 2013, the Canadian ministry of health recalled three batches of the Vega one French Vanilla cheese product, after identifying *Citrobacter* spp. In 2016, 5.5% of 250 food samples, including cheese, examined in Turkey were

contaminated with *Citrobacter* spp. (MRA Series 6. Geneva: WHO, 2004).

So, the identification of dominant species, *E. coli* and *C. freundii*, among the coliform bacteria is essential for understanding the microbial composition of cheese samples.

The distribution and emergence of pathogenic bacteria strains, resistant to antibiotics, is a global public health problem and its resolution is considered a priority task of the modern world from the perspective of the one health concept. Bacterial isolates from Kazakhstani cheese samples had an increased level of resistance to the most prioritized and critically important antimicrobials used in veterinary and medical practice for control of *E. coli* infections and other contagious diseases. The phenotypic characteristics of *E. coli* isolates contaminating cheese showed they were resistant to tetracyclines, beta-lactams and fluoroquinolones. When studying the genotypic resistance of bacterial isolates, sulfonamides, β-lactams and quinolones resistance genes were revealed. The main transfer of antibiotic-resistance genes occurs when mobile genetic elements, encoding these genes, as plasmids or transposons are exchanged in microbiota of different environment. So, this study has shown the need for further work to identify genetic markers of resistance to bacteria that contaminate dairy products.

Understanding the problem of *E. coli* isolates' resistance to antibiotics will improve strategies for controlling and preventing the further spread of resistant forms of bacteria. The obtained results indicated that comprehensive measures should be taken to control and reduce contamination and the reproduction of *E. coli* and other *Enterobacteriaceae* in cheese produced in Kazakhstan.

Conclusion

Produced in Kazakhstan samples of different types of cheese were contaminated with five species of coliform bacteria (*E. coli*, *Citrobacter freundii*, *Serratia liquefaciens*, *Enterobacter cloacae* and *C. braakii*), with dominance of the first two bacteria. Isolated from cheese *E. coli* strains demonstrated the multi-drug resistance to tetracyclines, beta-lactams and fluoroquinolones. Genes of resistance to sulfonamides, β-lactams and quinolones have been identified in the genetic material of antibiotic-resistant bacteria (in 55.4% of samples of cheese). A significant level of antibiotic resistance genes in coliform populations provides sufficient biotic potential, which determines the conditions for the successful transfer of this genetic material to the bacterial community of intestinal biocenosis in humans and animals.

Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the resources and platform provided by the publisher, which have enabled us to share our findings with a wider audience. We appreciate the efforts of the editorial team in reviewing and editing our work, and we are thankful for the opportunity to contribute to the field of research through this publication.

Funding Information

The research was financially supported by the Agriculture Ministry of the Republic of Kazakhstan in the framework of the program BR10764944 "Development the methods of analytical control and monitoring the safety of food products" conducted in 2021-2023.

Author's Contributions

Anar Kuzeubayeva: Collected of samples and provision of all experiment, coordination the data analysis and drafted the article.

Altay Ussenbayev and Ali Aydin: Analyzed the experimental data, designed the study, critically reviewed for intellectual content.

Zhannara Akanova: Microbiological study of samples and drafted.

Damegul Rakhimzhanova: Antibacterial resistance studies and drafted.

Raushan Rychshanova: Genotypic research and drafted.

Alma Kairzhanova: Mass-spectrometric research and drafted.

Dinara Seitkamzina: Conducted collection and microbiological study of probes from the East Kazakhstan region.

Assylbek Zhanabayev: Provided statistical analysis of the study.

Ethics

This study was provided at the laboratories officially certificated for work with infection agents of II-IV pathogenicity groups.

References

Andretta, M., Almeida, T. T., Ferreira, L. R., Carvalho, A. F., Yamatogi, R. S., & Nero, L. A. (2019). Microbial safety status of Serro artisanal cheese produced in Brazil. *Journal of Dairy Science*, *102*(12), 10790-10798. https://doi.org/10.3168/jds.2019-16967

Baranceli, G. V., Oliveira, C. A. F., Corassin, C. H., Camargo, T. M., Santos, M. G., Novotny, L. C. M., & Porto, E. (2014). Occurrence of *Escherichia coli* and coliforms in minas cheese plants from São Paulo, Brazil. *J Adv Dairy Res*, *2*(120), 2. https://doi:10.4172/2329-888X.1000120

Bhunia, A. K. (2018). *Foodborne microbial pathogens: Mechanisms and Pathogenesis*. Springer. https://doi.org/10.1007/978-1-4939-7349-1

Blackburn, C. De W. (2006). Food Spoilage Microorganisms. 1st Ed. https://shop.elsevier.com/books/food-spoilage-microorganisms/blackburn/978-1-85573-966-6

Chourasia, R., Abedin, M. M., Chiring Phukon, L., Sahoo, D., Singh, S. P., & Rai, A. K. (2021). Biotechnological approaches for the production of designer cheese with improved functionality. *Comprehensive Reviews in Food Science and Food Safety*, *20*(1), 960-979. https://doi.org/10.1111/1541-4337.12680

Currie, A., Galanis, E., Chacon, P. A., Murray, R., Wilcott, L., Kirkby, P., ... & Flint, J. (2018). Outbreak of *Escherichia coli* O157: H_7 infections linked to aged raw milk Gouda cheese, Canada, 2013. *Journal of Food Protection*, *81*(2), 325-331. https://doi.org/10.4315/0362-028X.JFP-17-283

EFSA Biohaz Panel, Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bover-Cid, S., Chemaly, M., ... & Bolton, D. (2020). Pathogenicity assessment of Shiga Toxin-producing *Escherichia Coli* (STEC) and the public health risk posed by contamination of food with STEC. *Efsa Journal*, *18*(1), e05967. https://doi.org/10.2903/j.efsa.2020.5967

EUCAST. (2021): Breakpoint table v 11.0 (2021) posted for consultation 4 Dec. *European Committee on Antimicrobial Susceptibility Testing*. https://www.eucast.org/eucast_news/news_singleview?tx_ttnews%5Btt_news%5D=404&cHash=74a1f440876cb3eefb60e6ad39c622e7

Farrokh, C., Jordan, K., Auvray, F., Glass, K., Oppegaard, H., Raynaud, S., ... & Cerf, O. (2013). Review of Shiga-Toxin-producing *Escherichia Coli* (STEC) and their significance in dairy production. *International Journal of Food Microbiology*, *162*(2), 190-212. https://doi.org/10.1016/j.ijfoodmicro.2012.08.008

Fusté-Forné, F. (2020). Developing cheese tourism: A local-based perspective from Valle de Roncal (Navarra, Spain). *Journal of Ethnic Foods*, *7*(1), 26. https://doi.org/10.1186/s42779-020-00064-2

Ganz, K., Yamamoto, E., Hardie, K., Hum, C., Hussein, H., Locas, A., & Steele, M. (2020). Microbial safety of cheese in Canada. *International Journal of Food Microbiology*, *321*, 108521. https://doi.org/10.1016/j.ijfoodmicro.2020.108521

Hammad, A. M., Eltahan, A., Hassan, H. A., Abbas, N. H., Hussien, H., & Shimamoto, T. (2022). Loads of coliforms and fecal coliforms and characterization of thermotolerant *Escherichia coli* in fresh raw milk cheese. *Foods*, *11*(3), 332. https://doi.org/10.3390/foods11030332

Kim, K., Lee, H., Lee, S., Kim, S., Lee, J., Ha, J., & Yoon, Y. (2017). Microbial risk assessment of non-enterohemorrhagic *Escherichia coli* in natural and processed cheeses in Korea. *Korean Journal for Food Science of Animal Resources*, *37*(4), 579. 579. https://doi.org/10.5851/kosfa.2017.37.4.579

Kuzeubaeva, A. S., Ussenbayev, A. E., & Akanova, Z. Z. (2022). Microbiological assessment of cheese products of Kazakhstani producers. https://doi.org/10.51452/kazatu.2021.4(111).833

Martin, N. H., Trmčić, A., Hsieh, T. H., Boor, K. J., & Wiedmann, M. (2016). The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. *Frontiers in Microbiology*, *7*, 1549. https://doi.org/10.3389/fmicb.2016.01549

Ombarak, R. A., Hinenoya, A., Awasthi, S. P., Iguchi, A., Shima, A., Elbagory, A. R. M., & Yamasaki, S. (2016). Prevalence and pathogenic potential of *Escherichia coli* isolates from raw milk and raw milk cheese in Egypt. *International Journal of Food Microbiology*, *221*, 69-76. https://doi.org/10.1016/j.ijfoodmicro.2016.01.009

Rüstemoğlu, M., Erkan, M. E., Cengiz, G., & Hajyzadeh, M. (2023). Bacterial metagenome profiling of hand-made herby cheese samples utilizing high-throughput sequencing to detect geographical indication and marketing potential. *Heliyon*, *9*(2). https://doi.org/10.1016/j.heliyon.2023.e13334

Selover, B., Johnson, J., & Waite-Cusic, J. G. (2021). Population dynamics of coliforms in a commercial Cheddar cheese production facility. *Journal of Dairy Science*, *104*(7), 7480-7488. https://doi.org/10.3168/jds.2020-19808

Shadrova, N. B., Pruntova, O. V., Skitovich, G. S. (2017). Determination of Species Diversity of *E. coli* Bacteria in Ready Dairy Products. *Veterinay Medicine Today*. https://veterinary.arriah.ru/jour/article/view/299

Sora, V. M., Meroni, G., Martino, P. A., Soggiu, A., Bonizzi, L., & Zecconi, A. (2021). Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. *Pathogens*, *10*(11), 1355. https://doi.org/10.3390/pathogens10111355

Takci, H. A. M., Bakirhan, P., Ozdemir, E., & Yalcin, A. (2020). Antibiotic susceptibility patterns of biofilm producing gram negative bacilli isolated from Kilis local cheese (Food-related antibiotic resistance). *Banat's Journal of Biotechnology*, *11*(21), 58-63. https://doi.org/10.7904/2068-4738-XI(21)-58

Verraes, C., Van Boxstael, S., Van Meervenne, E., Van Coillie, E., Butaye, P., Catry, B., ... & Herman, L. (2013). Antimicrobial resistance in the food chain: A review. *International Journal of Environmental Research and Public Health*, *10*(7), 2643-2669. https://doi.org/10.3390/ijerph10072643

WHO. (2004). Enterobacter sakazakii and other microorganisms in powdered infant formula: Meeting report. *World Health Organization*. https://www.who.int/publications/i/item/9789241562775

Yang, X., Wang, D., Zhou, Q., Nie, F., Du, H., Pang, X., ... & Xu, Y. (2019). Antimicrobial susceptibility testing of *Enterobacteriaceae*: Determination of disk content and Kirby-Bauer breakpoint for ceftazidime avibactam. *BMC Microbiology*, *19*(1), 1-7. https://doi.org/10.1186/s12866-019-1613-5