Scifood
vol.. 19, 2025, p. 520-536
https://doi.org/10.5219/scifood.58
ISSN: 2989-4034online
https://scifood.eu

Received: 9.6.2025
Revised: 10.7.2025
Accepted: 29.8.2025
Published: 24.9.2025

Susceptibility of microflora to antibacterial drugs at different stages of hard rennet cheese production

Olha Iakubchak, Olha Martynenko, Tetiana Taran, Alina Menchynska, Mykola Nikolaenko, Oleksandr Savchenko, Yevheniia Marchyshyna, Alina Omelian

ABSTRACT

The research aimed to analyze the susceptibility of milk microflora isolated at different stages of hard rennet cheese production and its evaluation. The susceptibility to antibacterial drugs of isolated cultures of bacteria *Escherichia coli*, *Klebsiella pneumoniae, and Acinetobacter baumannii*, which were found in the finished product, was determined. For each study, 15 samples of bacterial cultures were selected, and the experiment was conducted in triplicate. A total of 180 bacterial culture samples were examined. The disk diffusion method was used to determine the susceptibility of bacteria to antibacterial drugs. Studies of the resistance of bacteria isolated at different stages of hard rennet cheese "Ukrainian" production showed that the final product contains Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii resistant to certain antibacterial drugs, which may pose a health threat to consumers. Among the microflora isolated from raw milk, *Escherichia coli* was resistant to fluoroquinolones, penicillins, cephalosporins, tetracyclines, aminoglycosides, trimethoprim, and fosfomycin. In the normalized mixture, *Escherichia coli* was resistant to gentamicin and amikacin and moderately resistant to doxycycline. After pasteurization, *Escherichia coli* was susceptible to ciprofloxacin and resistant to all other tested antibacterial drugs. Resistance to gentamicin and amikacin, as well as moderate resistance to doxycycline, was maintained at almost every stage of production and in the final product. After pasteurization, these bacteria isolated from the brine became resistant to most antibacterial drugs, except for ciprofloxacin, gentamicin, amikacin, and fosfomycin. *Klebsiella pneumoniae*, isolated from the brine, was resistant to ampicillin after its pasteurization. It was resistant to fosfomycin both before and after pasteurisation of the brine. The bacterium isolated from the finished cheese retained resistance to fosfomycin and ampicillin. *Acinetobacter baumannii*, isolated from both the raw milk and the final product, was resistant to cefotaxime. Moderate susceptibility to amikacin and fosfomycin was restored after cheese maturation.

Keywords: milk, cheese, bacteria, antibacterial drugs, antimicrobial resistance

INTRODUCTION

Antimicrobial resistance, characterised by the emergence of multidrug-resistant strains of bacteria, is a global problem and poses a serious threat to human and animal health **[1]**, and **[2]**.

Microorganisms can harbor antimicrobial resistance genes, thereby increasing the gene pool from which pathogenic bacteria can acquire resistance traits. These changes occur in so-called "hot spots," which may include, in particular, hospitals and livestock farms where antibiotics are actively used, as well as the surrounding environment. Yes, soil and water contain unique microorganisms and genes resistant to antibiotics. The uncontrolled use of antibiotics and the simultaneous failure to comply with sanitary standards for the disposal of antibiotics and their pollution of the environment affect microorganisms and their natural coexistence in water and soil. Thus, transferring antibiotic resistance genes into the environment is accelerated **[3]**, and **[4]**.

Antibiotics in animal husbandry can cause bacteria to mutate and adapt, rendering them resistant to antibiotics **[5]**.

Antimicrobial-resistant zoonotic microorganisms present in food products of animal origin pose a direct risk to public health **[6]**, **[7]**, and **[8]**.

Food products, particularly milk and dairy products, are among the key food products at risk of developing antibiotic-resistant strains of microorganisms.

The modern consumer has become more knowledgeable and discerning about the consumption of safe foods **[9]**, and **[10]**. For example, data from the Cornell University Research Institute (Ithaca, New York) indicate that consumers of cow's milk are willing to pay more for milk from farms that do not use antibiotics. The people surveyed, especially women, were aware of the health risks associated with milk contaminated by residual antibiotics **[11]**. Cows that require antibiotic treatment for production-related reasons must be permanently removed from the herd under organic milk production regulations **[12]**. In many countries, the uncontrolled use of antibiotics in both human and veterinary medicine is prohibited; however, scientists from various countries worldwide report the presence of antibiotic-resistant bacteria in finished products, particularly dairy products. The danger of uncontrolled antibiotic use in veterinary medicine is associated with the biotransformation of antibiotics from products of animal origin, which can enter the human body.

Microbial contamination of milk can cause several dangerous foodborne diseases. Thus, in raw and pasteurized milk, as well as in cheeses, scientists have detected *Campylobacter jejuni*, *Salmonella, Listeria monocytogenes* and toxic (STEC) strains of *Escherichia coli*, which can enter the milk from cows or the environment, in particular, due to insufficient hygiene of milk processing equipment **[13]**. In raw milk, yogurts, and on the surfaces of milk containers, drinking glasses, antibiotic-resistant S. aureus, E. coli, S. epidermidis, Klebsiella spp., and *Salmonella spp*. were detected **[14]**. Additionally, a frequent cause of food poisoning is raw milk contaminated with Staphylococcus aureus, which poses a double threat to human health due to the antibiotic resistance of these bacteria **[15]**.

Several scientists, having studied finished dairy products, have isolated various microorganisms with multiple antibiotic resistance, particularly antibiotic-resistant *E. faecalis*, *E. faecium*, *Enterococcus durans, Enterococcus gallinarum* **[16]**, and *Staphylococcus aureus* **[17]**.

Food products, particularly milk and dairy products, can become contaminated with antibiotic-resistant bacteria in several ways. The first way is the presence of antibiotic-resistant bacteria in raw milk from animals treated with antibiotics during their growth or use. For example, enterotoxigenic strains of *Escherichia coli* resistant to ampicillin and cephalexin have been isolated from raw milk found in milk tanks and filters, confirming their role as a source of antibiotic resistance **[18]**. Methicillin-resistant *Staphylococcus aureus* (MRSA) has also been detected in raw milk **[19]**.

Another way of contamination of dairy products is the possible presence of resistance genes in bacteria added to milk during processing for technological purposes (starter culture, probiotics, biopreservative microorganisms, etc.). Thus, in finished cheeses of commercial production, the genera *Lactococcus, Lactobacillus,* and *Streptococcus* were found to be insusceptible to streptomycin and sulfamethoxazole **[20]**. Additionally, studies on strains of *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*, which are commonly used in dairy product production, have found that many are resistant to gentamicin, kanamycin, chloramphenicol, and tetracycline. At the same time, the number of strains of lactic acid bacteria in raw milk has significantly decreased due to measures introduced in many countries to limit antibiotic use in livestock **[21]**, and **[22]**.

The last way of contamination of food products derived from milk is the cross-infection of antimicrobial-resistant bacteria during production. Due to their nutritional composition, milk and dairy products are a favorable environment for the growth of many microorganisms. Still, heat treatment and technological processes at dairy plants inactivate bacteria in raw milk, thereby reducing the risk of transmission of antimicrobial resistance. Failure to comply with pasteurization parameters or further processing of dairy products in violation of hygiene requirements and the resistance of some bacteria to disinfectants can lead to bacterial contamination of dairy products, in particular, *Salmonella enterica*, which has shown antibiotic resistance **[23]**, **[24]** and antibiotic-resistant staphylococci **[19]**.

Therefore, raw milk used to produce dairy products is often contaminated with various microorganisms, including pathogenic ones, and technological processes at the dairy processing plant do not always ensure their complete neutralization. This leads to the entry of microorganisms into the final product and, accordingly, onto the consumer's table.

Scientific Hypothesis

Dairy products and their production chains can serve as a repository for forming and transmitting antibiotic-resistant bacteria, which can circulate in processing facilities and persist in finished dairy products, posing a threat to human health.

Objectives

Analysis of the susceptibility to antibacterial drugs of milk microflora isolated at different stages of hard rennet cheese production (milk raw material for bactofugation, normalized mixture, pasteurization, finished hard rennet cheese "Ukrainian") and its evaluation.

MATERIALS AND METHODS

Samples

Samples description: For the research, bacterial cultures of *Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii* isolated at four stages of production of hard rennet cheese "Ukrainian" were used.

Sample collection: Bacterial cultures were collected from raw milk before centrifugation, a normalised mixture from the tank, a pasteurized mixture (74±2°C, 20 sec), and cheese after 30 days of maturation.

Sample preparation: At the beginning of the experiment, an inoculum of 0.5 McFarland standard (MF) was prepared (approximately 1.5 × 10^8 CFU/ml). To do this, select 3-5 identical, clearly isolated colonies of *E. coli*, *Klebsiella pneumoniae, Acinetobacter baumannii, Citrobacter braakii, Streptococcus gallolyticus*, which grew on nutrient media after 16-24 hours of incubation at 36±1°C. Then, the plates were inoculated. Before inoculation, the agar plates were brought to room temperature. A sterile cotton swab (commercially manufactured) was dipped into the prepared suspension of the microorganism, and the excess inoculum was pressed against the walls of the test tube. The inoculation was performed with streaking movements in 3 directions, rotating the Petri dish by 60°. Apply antibiotic disks to the surface of the culture medium with the inoculum using an automatic dispenser. The plates were incubated in a thermostat for 15 min after applying the disk at 36±1°C for 18-24 h. After 18-24 h of incubation, the plates were examined, and the diameters of the growth inhibition zones were measured with an accuracy of mm using a ruler; in this case, the zone of complete inhibition of visible growth was used as the reference. This method's study results in the assignment of the microorganism to one of the susceptibility categories: S (susceptible), M (moderately susceptible), or R (resistant). The diameters of the growth inhibition zones were interpreted according to the susceptibility categories outlined by EUCAST **[25]**.

Number of samples analysed: 180 samples of bacterial cultures were analysed.

Chemicals

Blood agar (BA) (BioMérieux, France).

Mueller-Hinton agar (Sanimed, Ukraine).

Antibiotic disks: Ciprofloxacin, 5 µg; Levofloxacin 5 µg; Ampicillin 10 µg; Amoxicillin/Clavulanate 20-10 µg; Cephalexin 30 µg; Cefotaxime 5 µg; Cefepime 30 µg; Doxycycline 30 µg; Eravacycline, 20 µg; Tigecycline 15 µg; Gentamicin, 10 µg; Amikacin 30 µg; Tobramycin 10 µg; Trimethoprim/Sulfamethoxazole 2.5 µg; Fosfomycin 200 µg. (Sanimed, Ukraine)

Animals, Plants, and Biological Materials

The raw milk samples were collected from the LLC "Gaisyn Dairy Plant" in the city of Gaisyn, Vinnytsia region, Ukraine. Raw milk comes from five dairy farms in the area. The dairy plant produces hard and soft rennet cheeses, butter, spreads, and dry dairy products. The facility operates under a food safety management system to meet the international standard ISO 22000 requirements.

Instruments

Incubator (thermostat), capable of maintaining temperatures of 30 and 37°C ±1°C with natural convection, BinderBD 115 (Binder, Germany), loop heater (SteriMaks, Germany), vortex (Biosan, Latvia), antibiotic disk dispenser (Sanimed, Ukraine), densitometer (Biosan, Latvia), loops made of platinum /iridium or nickel/, chromium, 3 mm in diameter (Ukraine), Pasteur pipette, plastic sterile (Labexpert, China), ruler, 20 cm (Ukraine), test tubes with cotton applicator (Voles, Ukraine).

Laboratory Methods

The standardised disk diffusion method EUCAST was used to determine the susceptibility of bacteria to antibacterial drugs **[25]**, **[26]**.

Description of the Experiment

Study flow: According to the results of our previous studies on microbiological screening using MaldiTOF technology from raw milk to the finished dairy product - hard rennet cheese "Ukrainian" **[27]**, 18 species of microorganisms were detected and identified in the final product, among which *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* are of particular concern for the safety of the cheese. The disk diffusion method was used to test the cultures of these microorganisms for resistance to antimicrobial drugs. Cultures isolated at four stages of production of hard rennet cheese "Ukrainian" were taken: before bactofugation (raw milk), normalized mixture, milk after pasteurization, and finished cheese. The resistance of these microorganisms to antimicrobial drugs at each stage of production and in the final product was compared. The results of the studies were subjected to statistical processing and analysis.

Quality Assurance

Number of repeated analyses: 15 bacterial culture samples were taken for each study.

Number of experiment replications: The study was conducted in triplicate

Reference materials: -

Calibration: Each device was calibrated annually according to the specified calibration interval. Temperature parameters were monitored daily.

Laboratory accreditation: Experiments were conducted in a laboratory accredited according to the international standard ISO 17025.

Data Access

The data supporting the results of this study are not publicly available.

Statistical Analysis

ANOVA statistical software for Microsoft Excel, version 3.1, ues.

Statistical processing of the research results was done using one-way analysis of variance. The dynamics of the indicators were analyzed using regression and correlation analyses. The table data are x ±SD (mean ± standard deviation). The group difference was considered significant at $p<0.05$ (considering the Bonferroni correction).

RESULTS AND DISCUSSION

Based on the results of our previous studies **[27]**, the most common *Enterobacteriaceae* species detected at all stages of harrowing rennet cheese production were *Escherichia coli* strains. Therefore, further studies were conducted to determine the susceptibility of *Escherichia coli* to antibacterial drugs isolated at different production stages (Figure 1).

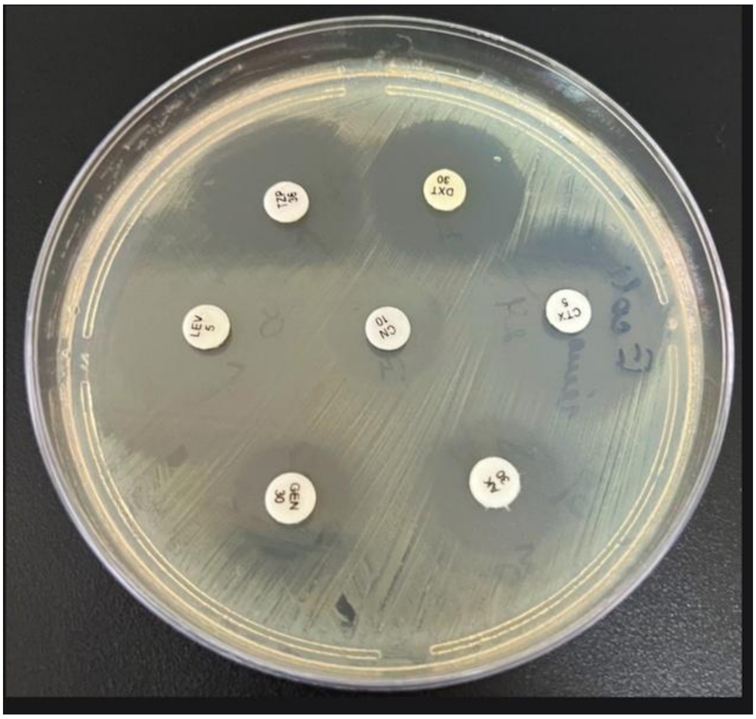

Figure 1 Determination of the susceptibility of Escherichia coli to antibacterial drugs by the disk method.

The susceptibility of these bacteria to antibacterial drugs was compared before bactofugation and the normalized mixture (Table 1).

Escherichia coli, which was isolated before bactofugation of milk, was resistant to all tested antibacterial drugs, namely ciprofloxacin, ampicillin, amoxicillin, cephalexin, cefotaxime, cefapim, doxycycline, gentamicin, amikacin, and trimethoprim, and had a growth inhibition zone from <6 to 16.23 ±0.18.

Similar studies of raw milk obtained from Romanian farms were performed using MALDI-TOF MS for bacterial identification, and it was found that the overall prevalence of *E. coli* was 22.45% among all isolated pathogens. Antibiogram analysis of 18 common antibiotics revealed that 27.51% of isolates were resistant to most of the drugs. The highest resistance was to penicillin (87.78%) and streptomycin (53.7%) **[28]**.

Table 1 Determination of susceptibility to antibacterial drugs of Escherichia coli isolated from milk before bactofugation and from normalised milk mixture.

Name of antibiotic	Norms, mm* diameter of growth retardation zone			Milk before bactofugation, mm, diameter of the growth inhibition zone	Normalised milk mixture, mm, diameter of growth retardation zone
	S	M	R		
Fluoroquinolones					
Ciprofloxacin	≥25	22-24	<22	<6**	30.11 ±0.49****
Penicillins					
Ampicillin	≥14	-	-	<6**	20.33 ±0.47****
Amoxicillin/ Clavulanate	≥19	-	-	14.36 ±0.21**	21.00 ±0.82****
Cephalosporins					
Cephalexin	≥14	-	<14	10.23 ±0.18**	23.33 ±0.58****
Cefotaxime	≥20	-	<17	10.57 ±0.12**	21.83 ±0.37****
Cefepime	≥27	-	<24	15.13 ±0.13**	29.5 ±0.50****
Tetracyclines					
Doxycycline	≥19	17-18	<17	8.23 ±0.38**	18.33 ±0.47***
Aminoglycosides					
Gentamicin	≥17	-	<17	8.61 ±0.62**	15.07 ±0.04**
Amikacin	≥18	-	<18	16.23 ±0.18**	16.11 ±0.15**
Others					
Trimethoprim/ Sulfamethoxazole	≥14	11-13	<11	<6**	17.17 ±0.56****
Fosfomycin	≥21	-	<21	<6**	29.52 ±0.19****

Note: S - Susceptible; M - Moderately susceptible; R - Resistant.
* According to EUCAST. ** - Resistant, *** - Moderately susceptible, **** - Susceptible. n=45, x±SD.

Other studies have also identified strains *of E. coli in raw milk from farms in Bangladesh, which were* found in 70% of cases, with 58.6% of these strains resistant to antimicrobials. In particular, strains were resistant to ampicillin, cefotaxime, gentamicin (100%), azithromycin (88%), oxytetracycline (27%), nalidixic acid, cotrimoxazole/trimethoprim (24%), and streptomycin (22%). In addition, 1 of the isolates showed resistance to a fourth-generation cephalosporin (cefepime) **[29]**. According to other authors, the prevalence of *E. coli* on farms in Indonesia was 70.4%, with 1.7% of the isolates resistant to antimicrobial agents **[30]**. In raw milk from farms in Zambia *E. coli* was isolated in 51.2% of cases, of which 21% of isolates were resistant to multiple antibacterial drugs: ampicillin (50%), tetracycline (40.1%), trimethoprim/sulfamethoxazole (28.5%), and amoxicillin/clavulanic acid (23.4%) **[31]**.

E. coli in raw cow's milk can be associated with unsatisfactory sanitary and hygienic conditions of milk production and with the ingress of milk impurities from cows with subclinical mastitis into raw materials for the production of dairy products. Thus, 59% of *E. coli* isolates were identified from the mammary gland secretion of cows affected by mastitis. Resistance to beta-lactam antibiotics is reported to be 84%, 72.7%, 52.27%, 50%, and 45.4% for cefotaxime, cefepime, cefuroxime, oxacillin, and cephalexin, respectively **[32]**.

Escherichia coli, which we isolated at the next stage of cheese production from the normalized mixture, was already susceptible to most antibacterial drugs according to the EUCAST interpretation. The exception was moderate susceptibility to doxycycline, and resistance to gentamicin and amikacin persisted. However, for gentamicin, the zone of growth inhibition changed from 8.61 ±0.62** to 15.07 ±0.04**mm, indicating an increase in the growth inhibition zone by 1.8 times. The obtained data indicate the influence of technological processes on the susceptibility of bacteria to antibacterial drugs. Perhaps such restoration of susceptibility is because

Escherichia coli, which remained after bactofugation, already had other properties or a set of bacteria that were next to them before bactofugation gave a cross-reaction, and, thus, *Escherichia coli* after bactofugation was already susceptible to most of the tested antibacterial drugs.

Next, we compared the susceptibility to antibacterial drugs of *Escherichia coli* isolated at the stage after the pasteurization of milk and from finished cheese (Table 2).

Table 2 Determination of susceptibility to antibacterial drugs of *Escherichia coli* isolated from milk after pasteurization of normalized milk mixture and from finished cheese.

Name of antibiotic	Norms, mm* diameter of growth retardation zone			Milk after pasteurization, mm, diameter of growth retardation zone	Ready-made cheese, mm, diameter of growth retardation zone
	S	M	R		
Fluoroquinolones					
Ciprofloxacin	≥25	22-24	<22	28.12 ±0.43****	31.36 ±0.23****
Penicillins					
Ampicillin	≥14	-	-	<6**	18.33 ±0.14****
Amoxicillin/ Clavulanate	≥19	-	-	<6**	21.15 ±0.09****
Cephalosporins					
Cephalexin	≥14	-	<14	<6**	23.33 ±0.58****
Cefotaxime	≥20	-	<17	<6**	25.16 ±0.23****
Cefepime	≥27	-	<24	<6**	30.13 ±0.17****
Tetracyclines					
Doxycycline	≥19	17-18	<17	<6**	17.66 ±0.58***
Aminoglycosides					
Gentamicin	≥17	-	<17	9.32 ±0.21**	16.09 ±0.21**
Amikacin	≥18	-	<18	12.13 ±0.23**	15.11 ±0.24**
Others					
Trimethoprim/ Sulfamethoxazole	≥14	11-13	<11	<6**	23.17 ±0.56****
Fosfomycin	≥21	-	<21	<6**	28.22 ±0.49****

Note: S - Susceptible; M - Moderately susceptible; R - Resistant.
* According to EUCAST, ** - Resistant, *** - Moderately susceptible, **** - Susceptible. n=45, x±SD.

According to the results of determining the susceptibility to antibacterial drugs of *Escherichia coli*, which was isolated from milk after pasteurization of the normalized mixture, it was found that it is resistant to ampicillin, amoxicillin, cephalexin, cefotaxime, cefapim, doxycycline, gentamicin, amikacin, trimethoprim, fosfomycin, as well as in the study of raw milk. However, *Escherichia coli* in this experiment was susceptible to ciprofloxacin, unlike *Escherichia coli* obtained from raw milk. Such a repeated change in the susceptibility of the bacteria at the next stage of production can be explained by the biofilm on the equipment used for pasteurizing milk. At this enterprise, milk used to produce hard rennet cheese "Ukrainian" is pasteurized at a temperature of 74.0±2°C for 20 seconds. The manufacturer considers several publications on the formation of specific taste characteristics in cheese, particularly those from unpasteurized milk **[33]**, and therefore employs the aforementioned pasteurization regime. However, one should also consider the possibility of biofilm formation on the equipment if temperatures are consistently insufficient.

The scientists note that pasteurization often does not ensure proper milk quality, especially if the raw material contains several pathogenic bacteria, including those of faecal origin. In addition, they identified bacterial isolates resistant to amoxicillin, cefoxitin, and/or erythromycin **[34]**.

Some scientists also describe cases of *Escherichia coli* presence after milk pasteurization, attributing this to heat-resistant bacteria that can withstand temperatures of 60°C for 6 minutes and form a biofilm in 97% of cases **[35]**.

In the literature, we could not find an explanation for changes in antibiotic susceptibility at different stages of dairy product production, as the data on this issue are contradictory. For example, studies conducted on thermoresistant *Escherichia coli* that form biofilms on pasteurization equipment have shown that they are susceptible to several antibacterial drugs **[35]**. Other studies, on the contrary, confirm the presence of resistant thermoresistant strains of *Escherichia coli* **[36]**. There have been reports of the isolation *of multi-resistant Escherichia coli after the pasteurization of milk* **[37]**. In China, 2.0% of *E. coli* isolates were isolated and identified from pasteurized milk, exhibiting antimicrobial resistance to amikacin (100%), streptomycin (50%), and tetracycline (50%) **[38]**. Scientists in Iran have reported the isolation of pathogenic *E. coli* strains from 5.55% of pasteurized milk and other dairy products, with 73.68% of these cases showing resistance to at least one antibiotic. The highest resistance was noted to streptomycin and tetracycline (50%). However, all strains were susceptible to amikacin **[39]**.

The data we obtained require further research to establish heat-resistant and multi-resistant strains of Escherichia coli and conduct studies on the antibiotic resistance of specific strains under different temperature regimes.

The change in the susceptibility of *Escherichia coli* to antibacterial drugs was also noted in the finished hard rennet cheese. After the cheese ripened, the bacterium regained susceptibility to most tested antibacterial drugs, except gentamicin and amikacin, and was moderately susceptible to doxycycline. The presence of *Escherichia coli* in the finished cheese is of particular concern, as it can produce Shiga toxin (STEC), which can cause intestinal diseases in humans **[40]**. Bacteria of the genus *Enterobacteriaceae* are the main microorganisms of food spoilage **[41]**. Moreover, scientists have isolated *Escherichia coli* from cheeses made from both pasteurized and unpasteurized milk. Studies of the microflora of cheeses made from unpasteurized milk, isolated from 20 types of cheeses made from cow's, goat's and sheep's milk, showed the presence of *Enterococcus, Staphylococcus, Enterobacteriaceae* in the finished cheeses*,* which were resistant to tetracycline, erythromycin and chloramphenicol 20% of the bacterial isolates were resistant to 1 antibiotic, 8% to two, 12% to all antibiotics **[42]**. *E. coli* has also been isolated from cheeses made from raw milk, with some strains demonstrating the ability to form biofilms. Moreover, these strains were resistant to tetracycline (25.6%), ampicillin (17.9%), cefoxin (7.7%), nalidixic acid (5.1%), and amoxicillin (2.6%) **[43]**. Other studies of raw milk cheese have shown the presence of thermotolerant *E. coli* strains (93.3%). Eleven strains (7.8%) have resisted third-generation cephalosporins **[44]**. *E. coli* was isolated from raw milk in northern China in 34.4% of cases, including strains producing shigatoxin and other toxins. The strains showed varying degrees of resistance to ampicillin (46.3%), amoxicillin-clavulanic acid (16.4%), trimethoprim-sulfamethoxazole (13.4%), tetracycline (13.4%), cefoxitin (11.9%), chloramphenicol (7.5%), kanamycin (7.5%), streptomycin (6.0%), tobramycin (4.5%), azithromycin (4.5%), and ciprofloxacin (1.5%). All *E. coli* isolates were susceptible to gentamicin **[45]**. In Brazil, 43% of heat-stable coliform *E. coli* and 4% of pathogenic strains (STEC) secreting Shiga toxin were detected in cheeses made from pasteurized milk. Two of the STEC strains were resistant to streptomycin **[46]**. Other authors have isolated uropathogenic strains of *E. coli* (UPEC) from 43% of cheeses made from pasteurized milk that formed biofilms **[47]**.

Since the brine used for salting the cheese before and after pasteurization also contained *Escherichia coli* and *Klebsiella pneumoniae*, the latter being the most significant regarding cheese safety among the identified bacteria **[27]**. These bacteria were also examined for their susceptibility to antimicrobial agents (Tables 3 and Table 4).

The data in Table 3 show that *Escherichia coli* in brine before pasteurization had only moderate resistance to doxycycline. It was susceptible to other antibacterial drugs. After pasteurization, *Escherichia coli* was susceptible to ciprofloxacin, gentamicin, amikacin, and fosfomycin in brine. It was resistant to most other antibacterial drugs, such as ampicillin, amoxicillin, cephalexin, cefatoxime, cefepime, doxycycline, and trimethoprim, which is probably due to the presence of biofilms on the pasteurization equipment **[33]**.

Determining susceptibility to antibacterial drugs for Klebsiella pneumoniae is presented in Figure 2 and Table 4.

Table 3 Determination of susceptibility to antibacterial drugs, *Escherichia coli,* isolated from brine used to produce hard rennet cheese before and after pasteurization.

Name of antibiotic	Norms, mm* diameter of growth retardation zone			Milk for bactofugation, mm, diameter of growth retardation zone	Normalized milk mixture, mm, diameter of growth retardation zone
	S	M	R		
		Fluoroquinolones			
Ciprofloxacin	≥25	22-24	<22	32.14 ±0.21****	28.25 ±0.11****
		Penicillins			
Ampicillin	≥14	-	-	24.63 ±0.41****	<6**
Amoxicillin/ Clavulanate	≥19	-	-	24.72 ±0.52****	<6**
		Cephalosporins			
Cephalexin	≥14	-	<14	23.77 ±0.56****	<6**
Cefotaxime	≥20	-	<17	30.32 ±0.47****	<6**
Cefepime	≥27	-	<24	30.93 ±0.83****	<6**
		Tetracyclines			
Doxycycline	≥19	17-18	<17	18.21 ±0.91***	<6**
		Aminoglycosides			
Gentamicin	≥17	-	<17	20.42 ±0.13**	25.07 ±0.23**
Amikacin	≥18	-	<18	22.31 ±0.17**	21.14 ±0.20**
		Others			
Trimethoprim/ Sulfamethoxazole	≥14	11-13	<11	22.18 ±0.21****	<6**
Fosfomycin	≥21	-	<21	32.11 ±0.25****	20.11 ±0.35****

Note: S - Susceptible; M - Moderately susceptible; R - Resistant.
* According to EUCAST. ** - Resistant, *** - Moderately susceptible, **** - Susceptible. n=45, x±SD.

Figure 2 Determination of susceptibility to antibacterial drugs of *Klebsiella pneumoniae* by the disk method.

Table 4 Determination of susceptibility to antibacterial drugs, *Klebsiella pneumoniae,* isolated from brine and finished cheese.

Name of antibiotic	Norms, mm* diameter of growth retardation zone			Milk for bactofugation, mm, diameter of growth retardation zone	Normalised milk mixture, mm, diameter of growth retardation zone
	S	M	R		
Fluoroquinolones					
Ciprofloxacin	≥25	22-24	<22	28.07 ±0.32****	30.23 ±0.19****/ 27.42 ±0.26****
Penicillins					
Ampicillin	≥14	-	-	19.85 ±0.41****	12.23 ±0.11**/ 10.23 ±0.25**
Amoxicillin/ Clavulanate	≥19	-	-	24.67 ±0.22****	24.13 ±0.03****/ 21.27 ±0.12****
Cephalosporins					
Cephalexin	≥14	-	<14	25.02 ±0.12****	23.81 ±0.27****/ 20.33 ±0.16****/
Cefotaxime	≥20	-	<17	28.21 ±0.34****	29.62 ±0.23****/ 27.32 ±0.19****/
Cefepime	≥27	-	<24	30.24 ±0.24****	30.12 ±0.22****/ 29.31 ±0.44****/
Tetracyclines					
Doxycycline	≥19	17-18	<17	24.14 ±0.75****	24.14 ±0.75****/ 22.26 ±0.82****
Aminoglycosides					
Gentamicin	≥17	-	<17	18.27 ±0.08****	19.22 ±0.16****/ 21.09 ±0.04****/
Amikacin	≥18	-	<18	18.86 ±0.33****	18.76 ±0.76**** 19.44 ±0.31****/
Others					
Trimethoprim/ Sulfamethoxazole	≥14	11-13	<11	25.28 ±0.15****	27.32 ±0.27****/ 19.22 ±0.16****/
Fosfomycin	≥21	-	<21	17.24 ±0.19**	18.35 ±0.48**/ 16.28 ±0.56**

Note: S - Susceptible; M - Moderately susceptible; R - Resistant.
* According to EUCAST. ** - Resistant, *** - Moderately susceptible, **** - Susceptible. n=45, x±SD.

Klebsiella pneumoniae isolated from brine before pasteurization was susceptible to all antibacterial drugs except fosfomycin. The bacterium isolated from brine after pasteurization was also resistant to fosfomycin and, in addition, to Ampicillin. It was susceptible to other antibacterial drugs tested. A similar pattern was observed in the finished product. Studies on the presence of *Klebsiella pneumoniae* at different stages of cheese production are not described in the literature; however, there is data on the isolation of the pathogen from finished cheeses **[48]**. It should also be remembered that *Klebsiella pneumoniae* is present in intensive care patients, and this bacterium may pose a health risk to the consumer, as it can cause dangerous nosocomial infections and, in intensive care units, even lead to the death of patients **[49]**.

According to our previous studies **[27]**, we paid special attention to Acinetobacter baumannii among the isolated microflora since it was isolated not only from raw milk but also from the final product - cheese, and in a larger number of samples. This bacterium, like *Klebsiella pneumoniae*, is often a causative factor in various infectious diseases in humans and animals **[50]** and is responsible for most nosocomial infections in different countries **[51]**. In addition, it often exhibits resistance to antimicrobial agents **[52]**.

Data on the susceptibility to antibacterial drugs of Acinetobacter baumannii isolated from raw milk and in the final product - hard rennet cheese- are presented in Table 5 and Figure 3.

Table 5 Determination of susceptibility to antibacterial drugs, Acinetobacter baumannii, isolated from milk before bactofugation and in finished cheese.

Name of antibiotic	Norms, mm* diameter of growth retardation zone			Milk for bactofugation, mm, diameter of growth retardation zone	Normalized milk mixture, mm, diameter of growth retardation zone
	S	M	R		
Fluoroquinolones					
Ciprofloxacin	≥25	22-24	<22	26.15 ±0.24****	26.23 ±0.21****
Penicillins					
Ampicillin	≥14	-	-	19.33 ±0.21****	18.56 ±0.32****
Amoxicillin/ Clavulanate	≥19	-	-	24.36 ±0.11****	25.16 ±0.18****
Cephalosporins					
Cephalexin	≥14	-	<14	24.32 ±0.42****	25.31 ±0.34****
Cefotaxime	≥20	-	<17	15.02 ±0.12**	16.21 ±0.34**
Cefepime	≥27	-	<24	21.32 ±0.12****	25.11 ±0.45****
Tetracyclines					
Doxycycline	≥19	17-18	<17	23.22 ±0.67****	26.31 ±0.56****
Aminoglycosides					
Gentamicin	≥17	-	<17	18.16 ±0.09****	20.13 ±0.18****
Amikacin	≥18	-	<18	18.71 ±0.36***	20.17 ±0.81****
Others					
Trimethoprim/ Sulfamethoxazole	≥14	13-11	<11	20.64 ±0.12****	25.22 ±0.38****
Fosfomycin	≥21	-	<21	21.78 ±0.64***	24.27 ±0.17****

Note: S - Susceptible; M - Moderately susceptible; R - Resistant.
* According to EUCAST. ** - Resistant, *** - Moderately susceptible, **** - Susceptible. n=45, x±SD.

Figure 3 Determination of the susceptibility of *Acinetobacter baumannii* to antibacterial drugs by the disk method.

Based on the data obtained, *Acinetobacter baumannii* was susceptible to the tested antibacterial drugs of the following groups: fluoroquinolones, tetracyclines, aminoglycosides, and trimethoprim. From the cephalosporin group, it was susceptible to cephalexin and cefepime, but resistant to cefotaxime. The bacterium was moderately susceptible to amikacin and fosfomycin.

Among the reports of other researchers, *Acinetobacter baumannii,* a multi-resistant bacterium to antibiotics, was found in both raw and pasteurized milk **[37]**.

The influence of technological processes on antibiotic susceptibility has been most noted for *E. coli.* Bactofugation is a process that utilizes centrifugal force to separate and remove microorganisms. Milk after bactofugation contains 90% fewer bacteria than before bactofugation **[53]**. However, during the experiment, we also observed an effect on antibiotic susceptibility, which can be attributed to the impact of physical factors on bacteria. The resistance of *E. coli* to penicillins and cephalosporins may indicate the presence of beta-lactamase resistance enzymes **[54]**. Since a factor such as temperature can affect the beta-lactamase enzyme produced by Gram-negative bacteria, including *E. coli*, we assume that bactofugation can also impact this enzyme and, accordingly, antibiotic susceptibility **[55]**. There is also evidence that bacteria can transfer resistance genes during their life cycle **[56]**, and therefore, antibiotic resistance may vary among bacteria circulating in a dairy plant. This is confirmed by the *Klebsiella pneumoniae* isolated from brine*,* which is resistant to fosfomycin and ampicillin. However, before pasteurization, this microorganism was susceptible only to fosfomycin in brine.

To explain the reasons for the differences in microbial susceptibility during cheese maturation, it is evident that additional studies are needed, focusing on the cultural properties of the identified microorganisms and the factors that may affect them during cheese maturation.

CONCLUSION

Studies of the resistance of bacteria isolated at different stages of hard rennet cheese “Ukrainian” production showed that the final product contains *Escherichia coli, Klebsiella pneumoniae,* and *Acinetobacter baumannii* resistant to some antibacterial drugs. This may pose a risk to the consumer's health. Among the microflora isolated from raw milk and tested for susceptibility to antibacterial drugs, *Escherichia coli* was resistant to fluoroquinolones, penicillins, cephalosporins, tetracyclines, aminoglycosides, trimethoprim and fosfomycin, which indicates the formation of its resistance to a significant number of antibacterial drugs on dairy farms that supply raw milk to the processing plant. In the normalized mixture, *Escherichia coli* was resistant to gentamicin and amikacin and moderately resistant to doxycycline. Those strains of bacteria that remained in the milk after bactofugation were probably susceptible to most antibacterial drugs. After pasteurization, *Escherichia coli* was susceptible to ciprofloxacin and resistant to all other tested antibacterial drugs. At this stage, the next appearance of resistant bacteria may indicate the formation of a biofilm on the equipment used for pasteurizing the milk mixture. The resistance of *Escherichia coli* was maintained in the final product - hard rennet cheese “Ukrainian”, to gentamicin, amikacin, and moderate resistance to doxycycline. The processes occurring in the cheese during the 30-day maturation restore the susceptibility of *Escherichia coli* to most antibacterial drugs. After pasteurization, these bacteria isolated from the brine became resistant to most antibacterial drugs, except for ciprofloxacin, gentamicin, amikacin, and fosfomycin. *Klebsiella pneumoniae* isolated from the brine became resistant to ampicillin after its pasteurization. It was resistant to fosfomycin both before and after the brine was pasteurized. The bacterium isolated from the finished cheese retained resistance to fosfomycin and ampicillin. The emergence of resistance in bacteria isolated from the brine after pasteurization may also indicate the presence of biofilms on the equipment used for pasteurization. *Acinetobacter baumannii* was isolated from both raw milk and the final product, and the isolate was resistant to cefotaxime. Moderate susceptibility to amikacin and fosfomycin was restored after cheese maturation. Considering the results of our experimental studies, recommendations for the manufacturer may include regular monitoring of the quality of washing and disinfection of production facilities and equipment, as well as monitoring the formation of biofilms on equipment and increasing the milk pasteurization temperature, monitoring the formation of biofilms on equipment, increasing the milk pasteurization temperature, and monitoring the presence of *Escherichia coli, Klebsiella pneumoniae*, and *Acinetobacter baumannii* at the final stage of cheese production.

REFERENCES

1. Myers, J., Hennessey, M., Arnold, J.-C., McCubbin, K. D., Lembo, T., Mateus, A., Kitutu, F. E., Samanta, I., Hutchinson, E., Davis, A., Mmbaga, B. T., Nasuwa, F., Gautham, M., & Clarke, S. E. (2022). Crossover-Use of Human Antibiotics in Livestock in Agricultural Communities: A Qualitative Cross-Country Comparison between Uganda, Tanzania, and India. In Antibiotics (Vol. 11, Issue 10, p. 1342). MDPI AG. https://doi.org/10.3390/antibiotics11101342
2. Galaburda, M., Yustyniuk, V., Kuzminska, O., Galat, M., & Correa, M. (2022). Awareness of antibiotic resistance for the environmental health and sustainable development: a cross-sectional study. In IOP Conference Series: Earth and Environmental Science (Vol. 1049, Issue 1, p. 012045). IOP Publishing. https://doi.org/10.1088/1755-1315/1049/1/012045
3. Shevchenko, L. V., Dobrozhan, Y. V., Mykhalska, V. M., Osipova, T. Y., & Solomon, V. V. (2019). Contamination of hen manure with nine antibiotics in poultry farms in Ukraine. In Regulatory Mechanisms in Biosystems (Vol. 10, Issue 4, pp. 532–537). OlesHonchar Dnipropetrovsk National University. https://doi.org/10.15421/021978
4. Kunhikannan, S., Thomas, C. J., Franks, A. E., Mahadevaiah, S., Kumar, S., &Petrovski, S. (2021). Environmental hotspots for antibiotic resistance genes. In MicrobiologyOpen (Vol. 10, Issue 3). Wiley. https://doi.org/10.1002/mbo3.1197
5. Hrustemović, E., Čaklovica, F., Đeđibegović, J., & Čaklovica, K. (2022). Antibiotic Resistance in Escherichia coli from Animals, Food, and Humans. In Indonesian Bulletin of Animal and Veterinary Sciences (Vol. 32, Issue 1). Indonesian Center for Animal Research and Development (ICARD). https://doi.org/10.14334/wartazoa.v32i1.2965
6. Ganan, M., Silván, J. M., Carrascosa, A. V., & Martínez-Rodríguez, A. J. (2012). Alternative strategies to use antibiotics or chemical products for controlling Campylobacter in the food chain. In Food Control (Vol. 24, Issues 1–2, pp. 6–14). Elsevier BV. https://doi.org/10.1016/j.foodcont.2011.09.027
7. Samy, A. A., Mansour, A. S., Khalaf, D. D., &Khairy, E. A. (2022). Development of multidrug-resistant Escherichia coli in some Egyptian veterinary farms. In Veterinary World (pp. 488–495). Veterinary World. https://doi.org/10.14202/vetworld.2022.488-495
8. Danchuk, V., Ushkalov, V., Midyk, S., Vigovska, L., Danchuk, O., &Korniyenko, V. (2021). Milk lipids and subclinical mastitis. In Food Science and Technology (Vol. 15, Issue 2). Odesa National University of Technology. https://doi.org/10.15673/fst.v15i2.2103
9. Sadvari, V. Y., Shevchenko, L. V., Slobodyanyuk, N. M., Tupitska, O. M., Gruntkovskyi, M. S., & Furman, S. V. (2024). Microbiome of craft hard cheeses from raw goat milk during ripening. In Regulatory Mechanisms in Biosystems (Vol. 15, Issue 3, pp. 483–489). OlesHonchar Dnipropetrovsk National University. https://doi.org/10.15421/022468
10. Sadvari, V. Y., Shevchenko, L. V., Slobodyanyuk, N. M., Furman, S. V., Lisohurska, D. V., &Lisohurska, O. V. (2024). Chemical composition of craft hard cheeses from raw goat milk during ripening. In Regulatory Mechanisms in Biosystems,(Vol. 15, Issue 4, pp. 666-673). OlesHonchar Dnipropetrovsk National University. https://doi.org/10.15421/022496.
11. Wemette, M., Greiner Safi, A., Wolverton, A. K., Beauvais, W., Shapiro, M., Moroni, P., Welcome, F. L., &Ivanek, R. (2021). Public perceptions of antibiotic use on dairy farms in the United States. In Journal of Dairy Science (Vol. 104, Issue 3, pp. 2807–2821). American Dairy Science Association. https://doi.org/10.3168/jds.2019-17673
12. Bulut, E., Stout, A., Wemette, M., Llanos-Soto, S., Schell, R. C., Greiner Safi, A., Shapiro, M. A., Moroni, P., &Ivanek, R. (2021). How does public perception of antibiotic use on dairy farms contribute to self-reported organic purchasing? In Journal of Food Science (Vol. 86, Issue 5, pp. 2045–2060). Wiley. https://doi.org/10.1111/1750-3841.15720
13. Krukowski, H., Bis-Wencel, H., &Prystupa, A. (2023). Milk and cheese are a source of human infection. In Medycyna Weterynaryjna (Vol. 79, Issue 07, pp. 6780–2023). MedycynaWeterynaryjna - Redakcja. https://doi.org/10.21521/mw.6780
14. Asfaw, T., Genetu, D., Shenkute, D., Shenkutie, T. T., Amare, Y. E., Habteweld, H. A., &Yitayew, B. (2023). Pathogenic Bacteria and Their Antibiotic Resistance Patterns in Milk, Yoghurt, and Milk Contact Surfaces in Debre Berhan Town, Ethiopia. In Infection and Drug Resistance: Vol. Volume 16 (pp. 4297–4309). Informa UK Limited. https://doi.org/10.2147/idr.s418793
15. Shalaby, M., Reboud, J., Forde, T., Zadoks, R. N., &Busin, V. (2024). Distribution and prevalence of enterotoxigenic Staphylococcus aureus and staphylococcal enterotoxins in raw ruminants' milk: A systematic review. In Food Microbiology (Vol. 118, p. 104405). Elsevier BV. https://doi.org/10.1016/j.fm.2023.104405

16. Amidi-Fazli, N., &Hanifian, S. (2022). Biodiversity, antibiotic resistance, and virulence traits of Enterococcus species in artisanal dairy products. In International Dairy Journal (Vol. 129, p. 105287). Elsevier BV. https://doi.org/10.1016/j.idairyj.2021.105287

17. Avila-Novoa, M. G., González-Gómez, J.-P., Guerrero-Medina, P. J., Cardona-López, M. A., Ibarra-Velazquez, L. M., Velazquez-Suarez, N. Y., Morales-del Río, J.-A., & Gutiérrez-Lomelí, M. (2021). Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) strains isolated from dairy products: Relationship of ica-dependent/independent and components of biofilms produced in vitro. In International Dairy Journal (Vol. 119, p. 105066). Elsevier BV. https://doi.org/10.1016/j.idairyj.2021.105066

18. Dell'Orco, F., Gusmara, C., Loiacono, M., Gugliotta, T., Albonico, F., Mortarino, M., &Zecconi, A. (2019). Evaluation of virulence factor profiles and antimicrobial resistance of Escherichia coli isolated from bulk tank milk and raw milk filters. In Research in Veterinary Science (Vol. 123, pp. 77–83). Elsevier BV. https://doi.org/10.1016/j.rvsc.2018.12.011

19. Dey, T. K., Shome, B. R., Bandyopadhyay, S., Goyal, N. K., Lundkvist, Å., Deka, R. P., Shome, R., Venugopal, N., Grace, D., Sharma, G., Rahman, H., & Lindahl, J. F. (2023). Molecular Characterization of Methicillin-Resistant Staphylococci from the Dairy Value Chain in Two Indian States. In Pathogens (Vol. 12, Issue 2, p. 344). MDPI AG. https://doi.org/10.3390/pathogens12020344

20. Yao, J., Gao, J., Guo, J., Wang, H., Zhang, E., Lin, Y., Chen, Z., Li, S., & Tao, S. (2022). Characterization of Bacteria and Antibiotic Resistance in Commercially Produced Cheeses Sold in China. In Journal of Food Protection (Vol. 85, Issue 3, pp. 484–493). Elsevier BV. https://doi.org/10.4315/jfp-21-198

21. Nunziata, L., Brasca, M., Morandi, S., &Silvetti, T. (2022). Antibiotic resistance in wild and commercial non-enterococcal Lactic Acid Bacteria and Bifidobacteria strains of dairy origin: An update. In Food Microbiology (Vol. 104, p. 103999). Elsevier BV. https://doi.org/10.1016/j.fm.2022.103999

22. Mylostyvyi, R., Midyk, S., Izhboldina, O., Cherniy, N., & Kornienko, V. (2023). Changes in the qualitative composition of the milk of Holstein cows during summer chronic heat stress. JurnalIlmuTernak dan Veteriner, (Vol 28, Issue 2, pp.112–121). https://doi.org/10.14334/jitv.v28.i2.3151

23. Sadvari, V. Y., Shevchenko, L. V., Midyk, S. V., Korniyenko, V. I., Slobodyanyuk, N. M., Pylypchuk, O. S., Naumenko, T. V., & Stetsiuk, I. M. (2025). Fatty acid profile of artisanal hard cheeses made from raw goat milk during the ripening process. Regulatory Mechanisms in Biosystems, 33(1), e25002. https://doi.org/10.15421/0225002

24. Garbaj, A. M., Gawella, T. B. B., Sherif, J. A., Naas, H. T., Eshamah, H. L., Azwai, S. M., Gammoudi, F. T., Abolghait, S. K., Moawad, A. A., Barbieri, I., &Eldaghayes, I. M. (2022). Occurrence and antibiogram of multidrug-resistant Salmonella enterica isolated from dairy products in Libya. In Veterinary World (pp. 1185–1190). Veterinary World. https://doi.org/10.14202/vetworld.2022.1185-1190

25. European Committee on Antimicrobial Susceptibility Testing EUCAST. Retrieved from: https://www.eucast.org/eucast_news/news_singleview?tx_ttnews%5Btt_news%5D=566&cHash=db55f3a8829726044512a1fe74cce41b#:~:text=EUCAST%20breakpoint%20table%2014.0

26. RI.BLS 7.2-09/05 Schemes for determining the sensitivity of microorganisms to antibacterial drugs by various methods.

27. Iakubchak, O., Martynenko, O., Taran, T., Pylypchuk, O., Naumenko, T., Tverezovska, N., Menchynska, A., &Stetsyuk, I. (2024). Analysis of the hard rennet cheese microbiota at different stages of the technological process. In Potravinarstvo, Slovak Journal of Food Sciences (Vol. 18, pp. 899–918). HACCP Consulting. https://doi.org/10.5219/2011

28. Drugea, R. I., Siteavu, M. I., Pitoiu, E., Delcaru, C., Sârbu, E. M., Postolache, C., & Bărăităreanu, S. (2025). Prevalence and Antibiotic Resistance of Escherichia coli Isolated from Raw Cow's Milk. Microorganisms (Vol. 13, Issue 1, p. 209). https://doi.org/10.3390/microorganisms13010209

29. Nahar, A., Islam, A. K. M. A., Islam, M. N., Khan, M. K., Khan, M. S., Rahman, A. K. M. A., & Alam, M. M. (2023). Molecular characterization and antibiotic resistance profile of ESBL-producing Escherichia coli isolated from healthy cow raw milk in smallholder dairy farms in Bangladesh. Veterinary world (Vol. 16, Issue 6, p. 1333–1339). https://doi.org/10.14202/vetworld.2023.1333-1339

30. Tyasningsih, W., Ramandinianto, S. C., Ansharieta, R., Witaningrum, A. M., Permatasari, D. A., Wardhana, D. K., Effendi, M. H., & Ugbo, E. N. (2022). Prevalence and antibiotic resistance of Staphylococcus aureus and Escherichia coli isolated from raw milk in East Java, Indonesia. Veterinary World (pp 2021–2028). https://doi.org/10.14202/vetworld.2022.2021-2028

31. Iakubchak, O. M., Vivych, A. Y., Hryb, J. V., Danylenko, S. H., & Taran, T. V. (2024). Production and meat quality of broiler chickens with the use of a probiotic complex of bifidobacteria and lactobacilli. Regulatory Mechanisms in Biosystems, 15(3), 477–482. https://doi.org/10.15421/022467

32. Deb, R., Chaudhary, P., & De, S. (2022). CRISPR/cas9 cassette targeting the Escherichia coli blaCTX-M specific gene of mastitis cow milk origin can alter the antibiotic resistant phenotype for cefotaxime. Animal Biotechnology (Vol. 34, Issue 5, pp. 1849–1854). https://doi.org/10.1080/10495398.2022.2053695

33. Terzić-Vidojević, A., Veljović, K., Popović, N., Tolinački, M., &Golić, N. (2021). Enterococci from Raw-Milk Cheeses: Current Knowledge on Safety, Technological, and Probiotic Concerns. In Foods (Vol. 10, Issue 11, p. 2753). MDPI AG. https://doi.org/10.3390/foods10112753

34. Didouh, N., Khadidja, M., Campos, C., Sampaio-Maia, B., Boumediene, M. B., & Araujo, R. (2023). Assessment of biofilm, enzyme production, and antibiotic susceptibility of bacteria from milk pre- and post-pasteurization pipelines in Algeria. In International Journal of Food Microbiology (Vol. 407, p. 110389). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2023.110389

35. Machado, M. A. M., Castro, V. S., da Cunha-Neto, A., Vallim, D. C., Pereira, R. de C. L., dos Reis, J. O., de Almeida, P. V., Galvan, D., Conte-Junior, C. A., &Figueiredo, E. E. de S. (2023). Heat-resistant and biofilm-forming Escherichia coli in pasteurized milk from Brazil. In Brazilian Journal of Microbiology (Vol. 54, Issue 2, pp. 1035–1046). Springer Science and Business Media LLC. https://doi.org/10.1007/s42770-023-00920-8

36. Vovkotrub, V., Kołacz, R., Iakubchak, O., Vovkotrub, N., & Shevchenko, L. (2024). Effect of lactic acid bacteria ferment cultures on pork freshness. Ukrainian Journal of Veterinary Sciences, 15(1), 48–65. https://doi.org/10.31548/veterinary1.2024.48

37. Statkevych, O. I., Kolomiiets, Y. V., Holembovska, N. V., Israelian, V. M., Babych, O. A., Slobodyanyuk, N. M., Babytskiy, A. I., & Statkevych, A. O. (2024). Effects of nutrient medium on various-age larvae of Hermetia illucens (Diptera, Stratiomyidae). Regulatory Mechanisms in Biosystems, 15(4), 907–911. https://doi.org/10.15421/0224131

38. Wang, S., Yu, Z., Wang, J., Ho, H., Yang, Y., Fan, R., Du, Q., Jiang, H., & Han, R. (2021). Prevalence, Drug Resistance, and Virulence Genes of Potential Pathogenic Bacteria in Pasteurized Chinese Fresh Milk Bar Milk. Journal of Food Protection (Vol. 84, Issue 11, pp. 1863-1867). https://doi.org/10.4315/JFP-21-094

39. Madani, A., Esfandiari, Z., Shoaei, P., & Ataei, B. (2022). Evaluation of Virulence Factors, Antibiotic Resistance, and Biofilm Formation of Escherichia coli Isolated from Milk and Dairy Products in Isfahan, Iran. Foods (Basel, Switzerland), (Vol. 11, Issue 7, pp. 960). https://doi.org/10.3390/foods11070960

40. Drugea, R.I., Siteavu, M.I., Bărăităreanu, S. (2023) Microbiological contamination of milk and the implications of bacterial strains in human pathology. RevistaRomâna de Medicina Veterinara (Vol. 33, Issue 2, pp. 55–68). https://agmv.ro/wp-content/uploads/2023/06/55_68_Drugea_14-c.pdf

41. Lokes, S., Shevchenko, L., Doronin, K., Mykhalska, V., Israelian, V., Holembovska, N., Tverezovska, N., & Savchenko, O. (2024). Influence of lactic acid bacteria starter cultures on microbiological parameters and shelf life of sausages. In Potravinarstvo, Slovak Journal of Food Sciences (Vol. 18, pp. 935–950). HACCP Consulting. https://doi.org/10.5219/2012

42. Nalepa, B., & Markiewicz, L. H. (2022). Microbiological Biodiversity of Regional Cow, Goat, and Ewe Milk Cheeses Produced in Poland and Antibiotic Resistance of Lactic Acid Bacteria Isolated from Them. In Animals (Vol. 13, Issue 1, p. 168). MDPI AG. https://doi.org/10.3390/ani13010168

43. de Campos, A. C. L. P., Puño-Sarmiento, J. J., Medeiros, L. P., Gazal, L. E. S., Maluta, R. P., Navarro, A., Kobayashi, R. K. T., Fagan, E. P., & Nakazato, G. (2018). Virulence Genes and Antimicrobial Resistance in Escherichia coli from Cheese Made from Unpasteurized Milk in Brazil. Foodborne Pathogens and Disease (Vol. 15, Issue 2, pp. 94–100. https://doi.org/10.1089/fpd.2017.2345

44. 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 (Basel, Switzerland) (Vol. 11, Issue 3, p. 332). https://doi.org/10.3390/foods110303322

45. Rogoskii, I., Mushtruk, M., Titova, L., Snezhko, O., Rogach, S., Blesnyuk, O., Rosamaha, Y., Zubok, T., Yeremenko, O., & Nadtochiy, O. (2020). Engineering management of starter cultures in study of temperature of fermentation of sour-milk drink with apiproducts. Potravinarstvo Slovak Journal of Food Sciences, 14, 1047–1054. https://doi.org/10.5219/1437

46. Rosario, A. I. L. S., Castro, V. S., Santos, L. F., Lisboa, R. C., Vallim, D. C., Silva, M. C. A., Figueiredo, E. E. S., Conte-Junior, C. A., & Costa, M. P. (2021). Shiga toxin-producing Escherichia coli isolated from pasteurized dairy products from Bahia, Brazil. Journal of Dairy Science (Vol. 104, Issue 6, pp. 6535–6547). https://doi.org/10.3168/jds.2020-19511

47. Vovkotrub, V., Iakubchak, O., Vovkotrub, N., Shevchenko, L., Lebedenko, T., Holembovska, N., Pylypchuk, O., & Omelian, A. (2024). Quality and safety of pork meat after cooling and treatment with lactic starters. Potravinarstvo Slovak Journal of Food Sciences, 18, 439–452. https://doi.org/10.5219/1954
48. Azwai, S. M., Lawila, A. F., Eshamah, H. L., Sherif, J. A., Farag, S. A., Naas, H. T., Garbaj, A. M., Salabi, A. A. E., Gammoudi, F. T., & Eldaghayes, I. M. (2024). Antimicrobial susceptibility profile of Klebsiella pneumoniae isolated from some dairy products in Libya as a foodborne pathogen. Veterinary world (Vol. 17, Issue 5, pp. 1168–1176). https://doi.org/10.14202/vetworld.2024.1168-1176
49. Garrouste-Orgeas, M., Marie, O., Rouveau, M., Villiers, S., Arlet, G., & Schlemmer, B. (1996). Secondary carriage with multi-resistant Acinetobacter baumannii and Klebsiella pneumoniae in an adult ICU population: relationship with nosocomial infections and mortality. The Journal of Hospital Infection (Vol. 34, Issue 4, pp. 279–289). https://doi.org/10.1016/s0195-6701(96)90108-5
50. Elbehiry, A., Marzouk, E., Moussa, I. M., Dawoud, T. M., Mubarak, A. S., Al-Sarar, D., Alsubki, R. A., Alhaji, J. H., Hamada, M., Abalkhail, A., A. Hemeg, H., & Zahran, R. N. (2021). Acinetobacter baumannii as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic analysis of biofilm formation, and phenotypic pattern of antimicrobial resistance. Saudi Journal of Biological Sciences (Vol. 28, Issue 1, pp. 1158–1166). https://doi.org/10.1016/j.sjbs.2020.11.052
51. Dahiru, M. & Enabulele, O. (2015). Acinetobacter baumannii in Birds' Feces: A Public Health Threat to Vegetables and Irrigation Farmers. Advances in Microbiology (Vol. 5, Issue 10, pp. 693–698). https://doi.org/10.4236/aim.2015.510072
52. Sadvari, V. Y., Shevchenko, L. V., Slobodyanyuk, N. M., Tupitska, O. M., Gruntkovskyi, M. S., & Furman, S. V. (2024). Microbiome of craft hard cheeses from raw goat milk during ripening. Regulatory Mechanisms in Biosystems, 15(3), 483–489. https://doi.org/10.15421/022468
53. Jovanovska, V. J., Sovreski, Z. S., Arapceska, M. A., Dimitrovska, G. D., Bojkovska, K. B., Joshevska, E. J., & Jovanovska, K. J. (2017). Devices for bactofugation in the function of providing quality long–life milk. Banat's Journal of Biotechnology, (Vol. 8, Issue 15, pp. 12–17). https://doi.org/10.7904/2068-4738-viii(15)-12
54. Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., & Jamil, K. (2014). Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. Revista do Instituto de Medicina Tropical de São Paulo (Vol. 56, Issue 4, pp. 341–346). https://doi.org/10.1590/s0036-46652014000400012
55. Cullmann, W., & Dick, W. (1990). Influence of Temperature on Beta-Lactamase Production and Outer Membrane Proteins in Gram-Negative Rods. Chemotherapy (Vol. 36, Issue 4, pp. 277–286). https://doi.org/10.1159/000238778
56. Liu, C., Sun, S., Sun, Y., Li, X., Gu, W., Luo, Y., Wang, N., & Wang, Q. (2024). Antibiotic resistance of Escherichia coli isolated from food and clinical environment in China from 2001 to 2020. Science of the Total Environment (Vol. 939, p. 173498). https://doi.org/10.1016/j.scitotenv.2024.173498

Funds:
This research received no external funding.
Acknowledgments: -
Competing Interests:
No potential conflict of interest was reported by the author(s).
Ethical Statement:
This article does not contain any studies that would require an ethical statement.
AI Statement:
Artificial intelligence was not used in the article.

Contact Address:

Olha Iakubchak
Affiliation: National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova Str., 16, 03041, Kyiv, Ukraine
Tel.: +380504408131
E-mail: olga.yakubchak@gmail.com
ORCID: https://orcid.org/0000-0002-9390-6578
Author contribution: writing – original draft

Olha Martynenko
Affiliation: "ECOLINE CORP" LIMITED LIABILITY COMPANY, Bandery Stepana avenue, building 21-A, 04073, Kyiv, Ukraine
Tel.: +380982143583
E-mail: olga.matynenko@gmail.com
ORCID: https://orcid.org/0009-0001-8239-2583
Author contribution: investigation

Tetiana Taran
Affiliation: National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova Str., 16, 03041 Kyiv, Ukraine
Tel.: +380961805067
E-mail: ttaran@ukr.net
ORCID: https://orcid.org/0000-0002-9370-8539
Author contribution: methodology, data curation

Alina Menchynska
Affiliation: National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Vystavkova Str., 16, 03041, Kyiv, Ukraine,
Tel.: +380976583888
E-mail: menchynska@ukr.net
ORCID: https://orcid.org/0000-0001-8593-3325
Author contribution: writing, review & editing

Mykola Nikolaenko
Affiliation: National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Public Health and Nutrition, Vystavkova Str.16, 03041 Kyiv, Ukraine
Tel.:+380984096549
E-mail: msnikolaienko@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-2213-4985
Author contribution: software

Oleksandr Savchenko
Affiliation: National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Vystavkova Str., 16, Kyiv, 03041, Ukraine
Tel.: +380503596271
E-mail: 63savchenko@gmail.com
ORCID: https://orcid.org/0000-0002-3940-6679
Authorcontribution: formalanalysis

Yevheniia Marchyshyna
Affiliation: National University of Life and Environmental Sciences of Ukraine, Mechanical and Technological Faculty, Department of Occupational Safety and Environmental Engineering, Heroyiv Oborony Str., 12B, Kyiv, 03041, Ukraine

Tel.: +380445278299
E-mail: marchyshyev@gmail.com
ORCID: https://orcid.org/0000-0001-8842-186X
Authorcontribution: conceptualisation

Alina Omelian
Affiliation: National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Heroiv Oborony Str., 15, 03041, Kyiv, Ukraine
Tel.: +380987846045
E-mail: alina_omelian@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-9004-5250
Author contribution: visualisation

Corresponding author: **Alina Menchynska**