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# GENOTYPIC RESISTANCE DRUGS OF ESCHERICHIA COLI STRAINS ISOLATED FROM KAZAKHSTANI PRODUCERS' CHEESES TO ANTIMICROBIAL

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**Abstract**

Global spread of antimicrobial resistance among pathogens is alarming for the modern society. The research aimed to study distribution of *Escherichia coli* in cheeses produced in Kazakhstan and to assess its resistance to antibacterial drugs. There were collected 101 samples of cheeses at retail outlets in different regions of Kazakhstan in 2021-2023, from which 55(54.4%) *E. coli* strains were isolated using conventional microbiological methods. The strains were phenotypically evaluated for antibiotic resistance, and the resistant isolates were tested for antibiotic resistance genes for the β-lactams group-penicillins (blaTEM, blaSHV, and OXA genes), aminoglycosides (aphA1, aadB), tetracyclines (tetA, tetB), quinolones (qnrA, qepA) and sulphonamides (sul1, sul2, and sul3 genes). Among studied *E. coli* strains 61% were resistant to at least one of the 21 antibiotics tested and were multi-resistant to the 15 antibacterial drugs. The greatest resistance was to sulfamethoxazole (43.6%), tetracycline (32%), followed by cefoxitin (29%). Isolates showed resistance to gentamicin (18.1%), ofloxacin (12.7%), furadonin (11%), amoxicillin (9.1%), doxycycline (7.2%). A gene encoding resistance to sulfonamides (sul3) was identified in eight *E. coli* strains. Thus, genotypic antibiotic resistance has been established in *E. coli* populations contaminating cheeses produced in the central, northern and eastern regions of Kazakhstan.

**Key words:** antimicrobials; cheeses; *Escherichia coli*; genotypic resistance; Kazakhstan; multi-resistance.

## Basic position and Introduction

*E. coli* species belongs to the Enterobacteriaceae family and considers the normal commensal bacteria of animals and humans [1]. However, *E. coli* turns into a comprehensive pathogen infecting humans and animals through mobile or horizontal transfer of pathogenic genetic material in bacterial colonies [2]. Currently, antimicrobial resistance has become one of the biggest global threats to public health [3]. Agricultural products can be carriers of antimicrobial-resistant bacteria with resistance genes to humans [4, 5, 6]. It is predicted that by 2050 antimicrobial resistance will lead to millions of deaths, a financial burden and a significant reduction in livestock production. Extensive studies related to the genetic characterization and antimicrobial control of resistant bacteria, which are often recorded in the environment, in humans and animals, show that extended-spectrum beta-lactamase-producing (ESBL) *E.coli* have negative consequences for humans and animals [7,8].

To control this pathogenic bacterium, a "One Health" approach was introduced, aimed at preventing the distribution of antimicrobial resistance among various local ecosystems. Intensive using of antimicrobials as a growth stimulants to increase cattle production in addition to therapeutic purposes in animal husbandry systems has allowed resistant bacteria to spread from animals to humans [8,9]. The rapid and global distribution of resistance mechanisms in gram-negative bacteria is the main reason for the increase in antimicrobial resistance. Data registered in the USA and the European Union on the spread of resistant bacteria confirmed that pathogenic bacteria isolated from food products have high antimicrobial resistance [10,11].

Recently epidemiological studies the genetic characteristics of ESBL *E. coli* in humans have shown that, in general, they were identified from animal products [12, 13]. Among the food goods offered to consumers the most dangerous are food products of animal origin. This is a case when the cheeses occupy a special place among a wide range of dairy products. The sanitary conditions of cheese industry enterprises, equipment, food products contaminated with bacteria through workers pose a potential danger to human health and life.

The purpose of these studies was to determine the distribution and genetic characteristics of antibiotic-resistant *E.coli* strains isolated from domestic cheeses in various geographical regions of Kazakhstan.

## Materials and Methods

Microbiological and molecular genetic studies were carried out in the period from May 2021 to September 2023 at the Kazakh-Chinese Biosafety Laboratory of the S. Seifullin Kazakh Agrotechnical Research University, the Laboratory of Molecular Diagnostics of Food Pathogens of the İstanbul

University-Cerrahpaşa, and the Research Institute of Applied Biotechnology of the A. Baitursynov Kostanay Regional University. In total, 101 samples of various cheeses from enterprises of the central, northern and eastern regions of Kazakhstan were examined, which were randomly sampled at retail outlets in six cities: Astana, Karaganda, Kostanay, Petropavlovsk, Semey and Uskaman (Figure 1).


Figure 1 - Geography of sampling by regions

Microbiological studies were carried out according to the methods of microbiological analysis of the GOST 32901-2014 "Milk and dairy products". Isolation and identification of *E.coli* strains were done by seeding to conventional nutrient selective media and re-culturing into appropriate differential diagnostic media. Isolated strains were Gram-stained, their lactose fermentation, catalase test, and indole formation were determined.

Antibiotic sensitivity testing was performed by Kirby-Bauer disco diffusion in accordance with EUCAST recommendations [14]. Discs containing the following 21 antibiotics were used for testing: beta-lactams (ampicillin-10 mcg, amoxicillin-25 mcg, cefoperazone-75 mcg, cefoxitin 30 mcg, cefpodoxime-10 mcg), aminoglycosides (streptomycin - 10 mcg, kanamycin-30 mcg, gentamicin-120 mcg), amphenicols (levomycetin-10 mcg 30 mcg), tetracyclines (tetracycline-30 mcg, doxycycline-30), fluoroquinolones (enrofloxacin-5 mcg, ciprofloxacin-5 mcg, norfloxacin-10 mcg, ofloxacin5 mcg), quinolones (nalidix acid-30 mcg), sulfonamides (sulfamethoxazole with trimethoprim-1.25/ 23.75), nitrofurans (furadonin-300 mcg, furazolidone-300 mcg).

To detect the genes encoding resistance to antibacterial drugs, the PCR method, represented by 1.5% agarose gel, was used. DNA isolation was carried out by thermal lysis. The reaction mixture contained water without DNAase, a Drean TaqGreen master mixture, direct and reverse primers, and DNA being tested. The primers listed in Table 1 were used to

determine the presence of genes by PCR.

Table 1 – Resistance genes' detection primers

| Primer | Length | $T^0$ | Sequence (5'–3') | A source |
|---|---|---|---|---|
| Beta-lactams group: penicillins | | | | |
| blaTEM | 857 | 56 | blaTEM-F-GAGTATTCAACATTTTCGT<br>blaTEM-R-ACCAATGCTTAATCAGTGAG | 15 |
| Beta-lactams group: carbapenems | | | | |
| OXA | 276 | 48 | OXAI-F-TCAACAAATCGCCAGAGAAG<br>OXAI-R-TCCCACACCAGAAAAACCAG | 15 |
| Tetracyclines | | | | |
| tetA | 210 | 60 | tetA F-GCTACATCCTGCTTGCCT<br>tetA R-CATAGATCGCCGTGAAGA | 16 |
| tetB | 930 | 64 | tetB F- CATTAATAGGCGCATCGCTG<br>tetB R -TGAAGGTCATCGATAGCAGG | 17 |
| Aminoglycosides | | | | |
| aadB | 634 | 68 | aadB-F -ATGGACACAACGCAGGTCGC<br>aadB-R-TTAGGCCGCATATCGCGACC | 15 |
| aphA1 | 500 | 55 | aphA1-F-AAACGTCTTGCTCGAGGC<br>aphA1-R-CAAACCGTTATTCATTCGTGA | |
| strA | 546 | 55 | strA-F-CCTGGTGATAACGGCAATTC<br>strA-R-CCAATCGCAGATAGAAGGC | |
| strB | 509 | 56 | strB-F-ATCGTCAAGGGATTGAAACC<br>strB-R-GGATCGTAGAACATATTGGC | |
| Sulfanilamides | | | | |
| SUL 1 | 547 | 65 | sul1-F-TTCGGCATTCTGAATCTCAC<br>sul1-R-ATGATCTAACCCTCGGTCTC | 15 |
| SUL 3 | 880 | 53 | sul3-F-GAGCAAGATTTTTGGAATCG<br>sul3-R-CATCTGCAGCTAACCTAGGGCTTTGA | |
| Fluoroquinolones | | | | |
| qepA | 218 | 60 | qepA F-GCAGGTCCAGCAGCGGGTAG<br>qepA R -CTTCCTGCCCGAGTATCGTG | 18 |
| qnrA | 516 | 53 | qnrA F- ATTTCTCACGCCAGGATTTG<br>qnrA R-GATCGGCAAAGGTTAGGTCA | 15 |

The amplification mode consisted the denaturation at $94^0$ C for 30 seconds, annealing temperature according to Table 1, elongation at $72^0$ C for 1 minute 30 seconds. The amplification time was 1 hour and 45 minutes. The determination of amplification products was carried out by electrophoresis in 105v on 1.5% agarose gel for 1 hour and 25 minutes. To carry out the reaction, specific markers, the TBE buffer solution of, the SYBR Safe DNA gel stain dye were used.

**Results**

There were isolated 55 *E. coli* strains from 101 cheese samples, which amounted to 54.4% (Table 2).

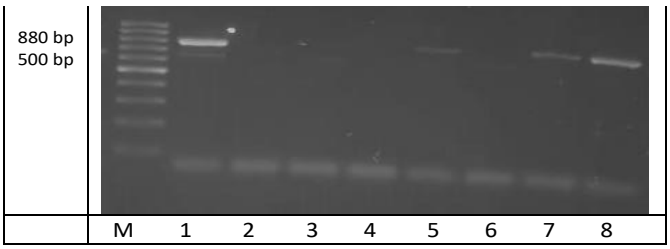
Table 2 - Contamination of Kazakhstani producers' cheeses by *E.coli* strains

| № | Country regions | Number of samples | Identified *E. coli* strains (%) |
|---|---|---|---|
| 1 | Northern | 33 | 18(32,6) |
| 2 | Eastern | 48 | 25(47,6) |
| 3 | Central | 20 | 20 (19,8) |
| | Total | 101 | 55 |

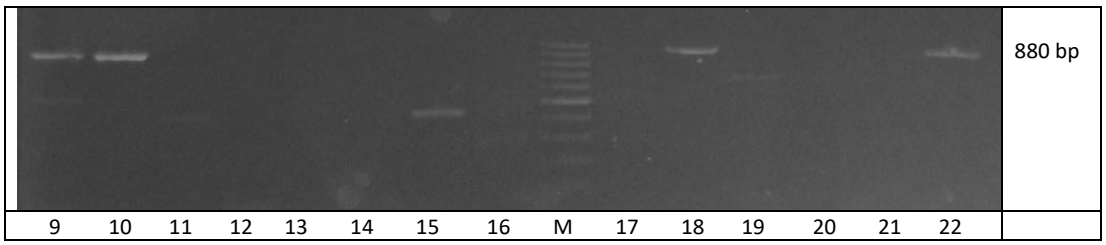
According to the phenotypic antimicrobial resistance's study of *E.coli* strains, a high level resistance was found in all sampling regions to most tested antibiotics. Among them, the greatest resistance was to sulfamethoxazole containing trimethoprim (43.6%), to cefoxitin (29%), followed by tetracycline (32%). Isolates showed also resistance to gentamicin (18.1%), ofloxacin (12.7%), furadonin (11%), amoxicillin (9.1%), doxycycline (7.2%).

In addition, resistance to three or more classes of different antibiotics was found in 15 of *E. coli* strains (27%). Similar results in Venezuela, as well as in Brazil and Egypt confirm that *E. coli* strains isolated from dairy products have multi resistance to many antimicrobial drugs [19, 20].

In the antibiotic resistance genotyping period, the sul3 resistance gene was detected in three samples of *E. coli* strains from the East Kazakhstan region, in two samples from the North Kazakhstan region and in three samples from Central Kazakhstan region. Thus, the molecular genetic study of strains having phenotypic resistance to six groups of antibacterial drugs shown that the sul3 resistance gene to sulfonilnamides (sulfamethoxazole with trimethoprim) is identified in eight samples (Figure 2).




M – marker, 1-8 samples (1, 5, 7, 8 – positive)




M – marker, 9-22 samples (9, 10, 18, 22 – positive)

Figure 2 - Determination of genotypic resistance

**Discussion**

One of the most alarming problems of this century is the continuous distribution of infections caused by resistant microorganisms, in particular bacterial pathogens with multidrug resistance [22].

The problem of antibiotic resistance, along with food safety, requires study in the veterinary, agricultural and environmental sectors. There is ample scientific evidence that drug-resistant bacteria can be transmitted through direct contact between animals and humans or through contaminated food. In 2015 the World Health Organization has adopted the Global action plan on antimicrobial resistance and called on each country to develop the National Action Plan to prevent global distribution of antimicrobial resistance.

*Escherichia coli* is recognized as a significant foodborne pathogen, so its identification is important for food hygiene management and rapid epidemiological studies. This studies are pilot research in terms of investigating cheeses of domestic producers for *E.coli* contamination. Strains of the bacterium were isolated in 54.4% of samples from the central, northern and eastern regions of Kazakhstan. Phenotypic analysis of resistance shown a high level of resistance of isolated *E.coli* strains to several antimicrobial drugs. Among *E. coli* isolates 61% were resistant to at least one of the 21 antibiotics tested and 27.3% were multi-resistant. At the same time, 43.6% of strains had intermediate sensitivity to cefoperazone and amoxicillin, 32% of strains shown resistance to sulfamethoxazole with trimethoprim. Similar studies conducted in Ethiopia confirm that *E. coli* strains isolated from dairy products have a similar level of resistance to the drugs sulfamethoxazole, tetracycline, doxycycline and cefoxitin [20].

Thus, the highest prevalence of *E. coli* strains' resistance was observed for β-lactams antibiotics cefotaxime (29%) and then for tetracyclines. It is known that beta-lactam antibiotics are widely used for therapeutic purposes in human and veterinary medicine. However, gram-negative ESBL *E. coli* is able to hydrolyze most β-lactams, which makes them resistant to β-lactam antibiotics. Resistance to these antibiotics has become a particular problem in recent years. Bacterial strains producing extended-spectrum beta-lactamases are highly resistant to many antibiotics, and infections caused by these strains are difficult to treat [8, 21].

As a result of these studies, it has been proved that in *E. coli* populations contaminated cheeses of Kazakhstani producers, there are strains that preserve the sul3 gene encoding resistance to sulfonilamides. Genes encoding resistance to trimethoprim are commonly found in many integral profiles. Integrons can exchange between species and play an important role in spreading antimicrobial resistance among bacteria [22].

At the next stage of the research, it is planned the computer modeling of the contamination risk of domestically produced cheeses by resistant to antibiotics *E. coli* strains, in order to develop measures ensured the sanitary safety of cheese products in the Kazakhstani dairy market.

**Conclusion**

The phenotypic and genotypic resistance of *E.coli* strains isolated from retail cheeses in the central, northern and eastern regions of Kazakhstan to 21 antimicrobial drugs was 61%. The highest prevalence of antibiotic resistance was observed for cefotaxime and tetracyclines, followed by β-lactams. There was established the genotypic resistance to sulfonilamides in the bacterial populations of this species.

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