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**Occurrence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* in bulk tank milk from dairy farms in Serbia**

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**Abstract:**

**Background/Objective:** Antimicrobial resistance (AMR) is a major concern in both human and veterinary medicine. *Escherichia coli,* as a ubiquitous bacteria, is often used as an indicator species for AMR monitoring. Extended-spectrum beta-lactamase (ESBL) producing *E. coli* has been identified in farm animals and foodstuffs of animal origin, with literature data emphasizing its occurrence on dairy farms. The bulk tank milk reflects the on-farm situation and can serve as a good indicator of the prevalence of AMR bacteria at the dairy farm level. The aim of this study was to evaluate the occurrence of ESBL-producing *E. coli* in bulk tank milk samples from dairy farms in Serbia.

**Methods:** Raw milk samples were collected from a total of 87 cooling tanks, originating from agricultural holdings (n=36, Northern Bačka district) and farms (n=16, Southern Banat district). Microbiological analyses included the enumeration of Enterobacteriaceae (ISO 21528-2:2017), *E. coli* (ISO 16649-2), and the isolation of ESBL-producing *E. coli*. Isolation of presumptive ESBL *E. coli* was done using the selective isolation method, according to the European Union Reference Laboratory – Antimicrobial Resistance (EURL-AR) protocol. Presumptive phenotypic identification of isolates included Gram staining, oxidase test, and IMViC test, which was later confirmed by Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry.

**Results:** Of 87 raw milk samples, seven samples (8.05%) tested positive. Positive samples originated from five farms, of which three farms had one positive sample (25%, 50%, and 12.5%), while two farms had two positive samples (25% and 20%).

**Conclusion:** The results of this study indicate that raw milk can be a potential reservoir of resistant bacteria. Despite the relatively low occurrence of ESBL-positive samples, the detection of these antibiotic-resistant bacteria in raw milk highlights the importance of monitoring AMR at the farm level.

**Keywords:** antimicrobial resistance, bulk tank milk, primary production of milk, *Escherichia coli*, extended-spectrum beta-lactamase (ESBL)

**Acknowledgment**: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-66/2024-03/200143).