

## Article

# Isolation and identification of antimicrobial-resistant strains of *Escherichia coli* from poultry meat

Valentina Crețu <sup>1,\*</sup>, Nicolae Starciuc <sup>1</sup>, Dragoș Aniță <sup>2</sup>, Cristina Mihaela Râmbu <sup>2</sup> and Adriana Elena Aniță <sup>2</sup>

<sup>1</sup>Technical University of Moldova, Faculty of Veterinary Medicine, MD-2004 bd. Stefan cel Mare, 168, Chisinau, Moldova

<sup>2</sup>Iași University of Life Sciences, Faculty of Veterinary Medicine, 7000489, Aleea Mihai Sadoveanu str. nr.8, Iasi, Romania

\* Correspondence: valentinaa.cretu@gmail.com

**Abstract:** The rising prevalence of antibiotic-resistant *Escherichia coli* (*E. coli*) serotypes in poultry meat represents a significant public health concern, complicating the treatment of infections in both humans and animals. This study aimed to isolate and identify various *E. coli* serotypes from poultry meat samples and evaluate their antibiotic resistance profiles. A total of 100 refrigerated poultry meat samples were collected from the Central Agricultural Market in Chisinau and analyzed using microbiological methods, including selective culturing on *E. coli*-specific media and typing using Bruker MALDI-TOF biotyper. Antibiotic susceptibility was assessed through disk diffusion method. The findings revealed a range of resistance levels to commonly used antibiotics in poultry farming. Most isolates exhibited typical *E. coli* characteristics, such as lactose fermentation on MacConkey agar and metallic green sheen on Eosin Methylene Blue agar. Notably, some serotypes demonstrated the ability to grow efficiently at temperatures exceeding 42°C. Resistance to at least one antibiotic was observed in over 40% of the serotypes, while 17% showed multidrug resistance (resistant to three or more antibiotics). The highest resistance rates were found for chloramphenicol, aztreonam, and enrofloxacin. These results highlight the need to revise treatment protocols in poultry production and emphasize cautious use of antibiotics—whether for treatment or prophylaxis—to curb the spread of resistant *E. coli* strains.

**Keywords:** *Escherichia coli*; poultry meat, MALDI-TOF, antibiotic resistance.

## 1. Introduction

Poultry meat remains one of the most in-demand and accessible sources of animal protein worldwide. Poultry farming, whether under intensive or extensive systems, requires adherence to appropriate technological and hygiene standards tailored to the production purpose, as well as feeding and biosecurity protocols that vary according to the birds' age, breed, and productivity level [EFSA and ECDC, 2015]. It is important to note that achieving optimal poultry productivity depends on a range of intrinsic and extrinsic factors, among which veterinary-sanitary measures play a decisive role. These measures have a direct impact on both animal health and public health. In this context, the control and surveillance of zoonotic diseases—particularly foodborne pathogens—are of critical importance [Bintsis et al., 2017].

In recent years, growing attention has been directed toward the incidence and role of zoonotic bacteria in poultry products, particularly in relation to antimicrobial resistance (AMR). Researchers are increasingly investigating the correlation between AMR in pathogens isolated from poultry and the reduced efficacy of antimicrobial treatments in animals, birds, and humans [Botelho and Schulenburg, 2021; Nasrollahian et al., 2024]. Various studies have highlighted the significance of bacterial dissemination—especially of *Escherichia coli*—and the global implications of its antimicrobial resistance, emphasizing the urgent need for comprehensive research to support public health strategies [Nji et al., 2021]. *E. coli*, a member of the order *Enterobacterales* and family *Enterobacteriaceae*, is recognized for its exceptional adaptability and complex phylogenetic structure, which contribute to its ability to acquire and disseminate resistance genes across diverse environments and hosts. Over 160 serotypes of *Escherichia coli* have been classified based on their major surface antigens: H (flagellar proteins), O (oligosaccharide components of the lipopolysaccharide), and K (capsular polysaccharides) [Tuttle et al., 2021]. In January 2024, the European Food Safety Authority (EFSA) released a scientific opinion addressing the persistence of microbiological hazards in food and feed production and processing environments [EFSA Panel on Biological Hazards (BIOHAZ); Koutsoumanis K et al., 2024]. The report underscored the critical need for robust monitoring

and control strategies to mitigate the risks posed by persistent pathogens, which are capable of surviving and proliferating in production and processing settings, ultimately leading to food contamination [Mather et al., 2024; Abo-Elmagd et al., 2023]. In light of these findings, the objective of our investigation was to determine the presence and prevalence of *E. coli* strains in poultry meat, with particular emphasis on their antimicrobial resistance profiles.

## 2. Materials and Methods

The research was conducted on poultry meat samples collected from three supermarkets in the Republic of Moldova, as well as from specialized poultry meat stores located within the Central Agricultural Market in Chişinău. Each sample was collected following standardized procedures to avoid contamination during handling, using sterile instruments and sealed in sterile plastic bags and containers. Samples were stored and transported under refrigerated conditions at 2–8°C until microbiological analysis. Microbiological investigations were carried out at the Regional Center for Advanced Research in Emerging Diseases, Zoonoses, and Food Safety (ROVETEMERG) at Iaşi University of Life Sciences. Bacterial isolation from the meat samples was performed by enriching step in Buffered Peptone Water, followed by culturing on selective media for *E. coli*, including Eosin Methylene Blue Agar (Levine) and MacConkey Agar No. 3, and isolation of pure colonies on Muller-Hinton agar (Figure 1).

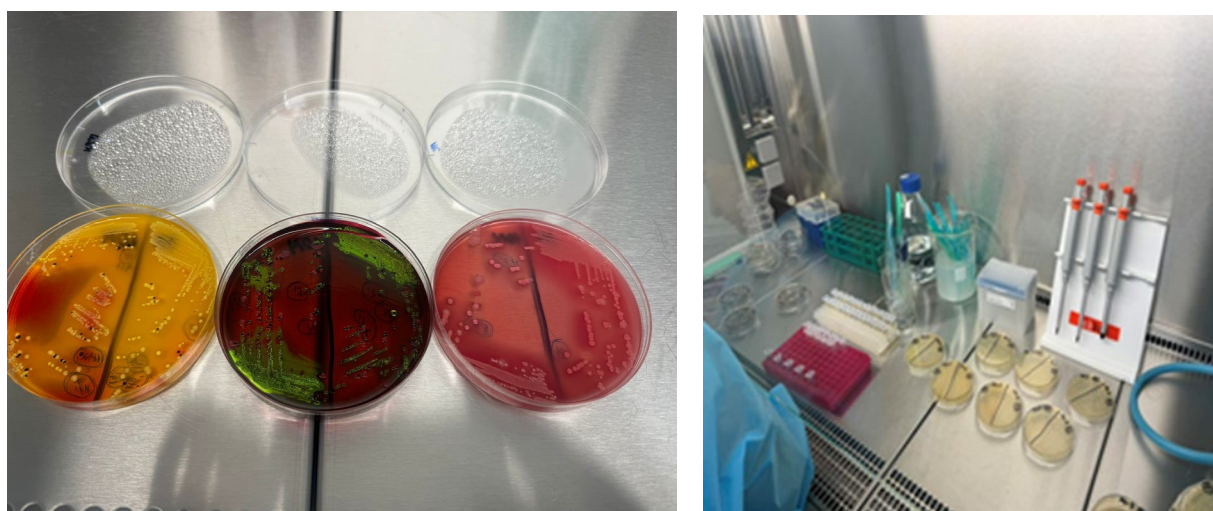


**Figure 1.** Isolation of *E. coli* on selective agar plates.

Following incubation at 37°C for 24 hours, isolated bacterial colonies were confirmed using MALDI-TOF MS. Additional phenotypic screening media were also used for the identification of carbapenemase-producing and extended-spectrum beta-lactamase (ESBL) *E. coli* strains. Simultaneously, the isolated *E. coli* serotypes were tested to assess the presence and degree of antimicrobial resistance. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method on the isolated *E. coli* strains, employing a panel of 15 antibiotics, including ampicillin, ciprofloxacin, tetracycline, chloramphenicol, and gentamicin. The diameters of the inhibition zones were measured and interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

## 3. Results

The bacteriological analysis of refrigerated poultry meat samples revealed that all specimens harbored a mixed microflora. Notably, out of the 100 poultry meat samples collected and examined, *E. coli* strains were isolated and identified in 55% of the samples. The isolated bacterial colonies exhibited morphological and biochemical characteristics typical of the *E. coli* genus, including lactose fermentation on MacConkey agar and the formation of colonies with a distinctive greenish metallic sheen, particularly evident on Eosin Methylene Blue agar. Additionally, the *E. coli* isolates demonstrated enhanced growth at elevated temperatures up to 42°C. Identification of the isolated colonies was performed using MALDI-TOF mass spectrometry (Bruker Biotyper) (Figure 2).

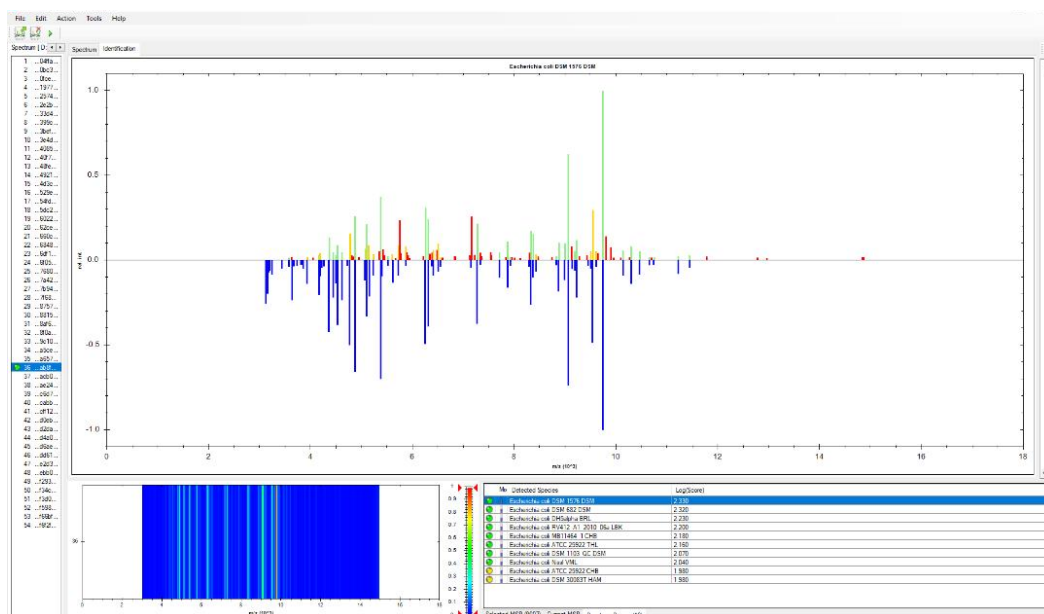


**Figure 2.** Analysis of MALDI-TOF mass spectra for the identification of *Escherichia coli*.

Carbapenem resistance among *E. coli* is primarily driven by two mechanisms. The first involves reduced membrane permeability due to porin loss, often in conjunction with the production of extended-spectrum beta-lactamases (ESBLs). The second mechanism is the production of carbapenemase enzymes, which can hydrolyze and inactivate carbapenems. The isolated *E. coli* strains were first cultivated on phenotypic screening media (Figure 3) and then tested for susceptibility to commonly used antimicrobials, reflecting usage patterns in both intensive and extensive poultry farming systems.

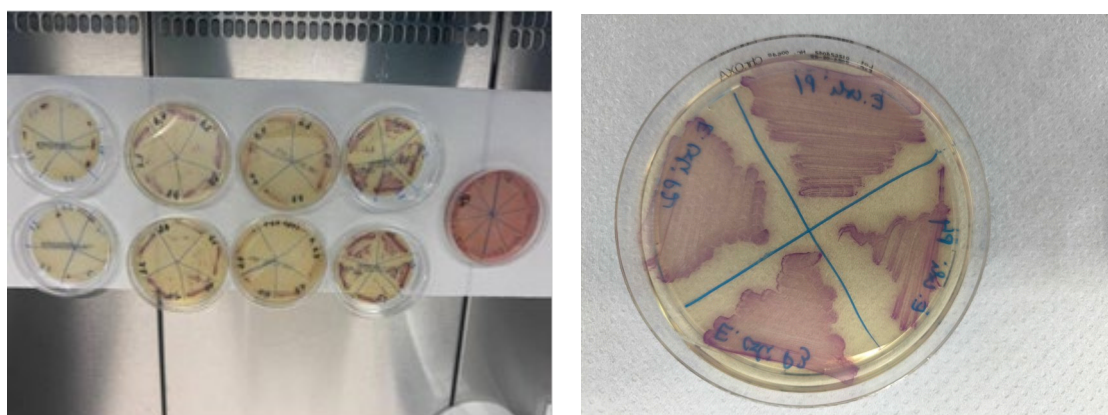


**Figure 3.** Growth of phenotypically ESBL/KPC-positive *E. coli* on phenotypic screening media.

The results showed that over 40% of the isolated *E. coli* strains exhibited resistance to at least one antibiotic, while 17% were resistant to three or more classes of antimicrobials. The highest resistance rates were observed against chloramphenicol, aztreonam and enrofloxacin (Figure 4).

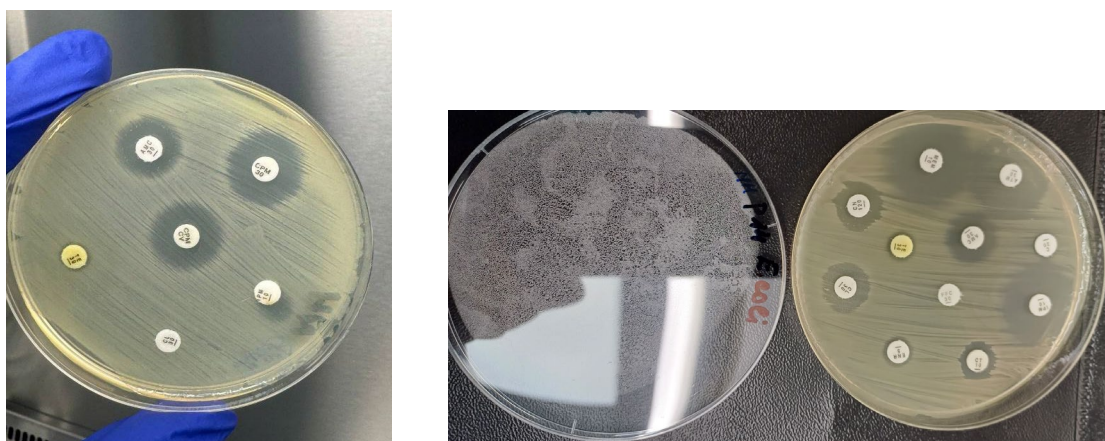


**Figure 4.** The results of the antibiogram highlighted the presence of moderately resistant *E. coli* strains, indicating partial susceptibility to several antimicrobials and suggesting emerging resistance trends.

#### 4. Discussion

Food quality is a multifaceted concept, encompassing nutritional value, sensory (organoleptic) attributes, sanitary conditions, and environmental sustainability. Among these dimensions, sanitary and sensory qualities are especially susceptible to microbial influences. While microorganisms can contribute positively to food characteristics—particularly in the context of fermentation—they also pose significant risks when unintentionally introduced, especially through contamination during processing, handling, or storage. These microbial communities, collectively known as the food microbiota, often originate from raw materials, processing environments, or intentional inoculation. Their presence is especially critical in perishable products such as poultry meat, where microbial contamination can directly compromise food safety and public health.

Enterobacteria, a diverse family of Gram-negative bacteria, play a central role in this microbial landscape. Although many enterobacterial species are natural commensals within the gastrointestinal tracts of humans and animals, their widespread distribution in the environment—facilitated by fecal contamination and inadequate sanitation—renders them frequent contaminants in food products. *Escherichia coli*, the predominant species within this group, is often used as an indicator organism for fecal contamination. While many strains of *E. coli* are harmless, certain serotypes are pathogenic and can lead to serious foodborne infections [Fodor et al., 2012]. The risk is particularly high in poultry meat, which serves as a potential reservoir for zoonotic *E. coli* strains that exhibit virulence factors and antimicrobial resistance [Iorgoni et al., 2025].

This study focused on assessing the microbiological safety of refrigerated poultry meat marketed through major retail outlets in the Republic of Moldova. The detection and characterization of *E. coli* strains revealed the presence of thermotolerant and antimicrobial-resistant variants. A particularly concerning finding was the ability of several *E. coli* isolates to grow robustly at elevated temperatures (42°C), indicating potential adaptation to thermal stress and raising concerns about the reliability of standard incubation protocols (typically 37°C) for identifying these strains.

Moreover, antimicrobial susceptibility testing revealed an alarming level of resistance among the isolated *E. coli* strains. Over 40% exhibited resistance to at least one antibiotic, while 17% were classified as multidrug-resistant, showing resistance to three or more classes of antimicrobials. High resistance rates were observed for antibiotics commonly used in veterinary practice, including chloramphenicol, aztreonam, and enrofloxacin. Resistance to colistin and gentamicin—often considered antibiotics of last resort—was also detected, along with moderate sensitivity to cephalexin and meropenem. These findings are consistent with global trends indicating a growing threat of antimicrobial resistance in foodborne pathogens, often linked to the overuse and misuse of antibiotics in animal husbandry.

The emergence of antibiotic-resistant *E. coli* strains in poultry meat poses a serious public health challenge. Contaminated meat can serve as a vector for transferring resistant bacteria to consumers, potentially leading to infections that are more difficult and costly to treat. This underscores the critical need for integrated surveillance systems, stricter hygiene controls throughout the food production chain, and the responsible use of antimicrobials in agriculture.

Additionally, exploring alternative therapeutic strategies—such as bacteriophage therapy, probiotics, or plant-based antimicrobials—may offer promising avenues to combat resistant strains.

In conclusion, the findings of this study reinforce the importance of continuous microbiological monitoring of poultry meat and the need for robust interventions at multiple points in the production and distribution chain. The detection of thermotolerant and multidrug-resistant *E. coli* strains highlights both an immediate food safety concern and a broader implication for antimicrobial resistance management within the food system.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, A.A. and S.N.; methodology, R.C.; software, A.D.; validation, A.D., R.C. and Z.Z.; formal analysis, A.D.; investigation, C.V.; resources, X.X.; data curation, C.V.; writing—original draft preparation, C.V., S.N.; writing—review and editing, A.D.; visualization, R.C.; supervision, A.D.; project administration, A.A. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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