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Research of the Prevalence of Some Commensal *E. coli* Strains and ESBL/*Amp*C in Healthy Animals

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Abstract: *E. coli is the first bacterium to colonize the gastrointestinal tract, immediately after birth, in both animals and humans, living as a commensal and synthesizing a series of essential substances for the body. In cases of immunodepression, or when the intestinal barrier is overcome, this bacterium can cause a whole series of diseases. In Romania, a major cause of economic losses, in all livestock sectors, is represented by infections with various pathogens, among which E. coli pathotypes occupy an important place, especially in pigs, causing post-weaning diarrhea or neonatal diarrhea. On the other hand, colibacillosis in birds has a worldwide distribution, recording an increased morbidity and mortality in this livestock sector. Also, the increased antibiotic resistance of E. coli strains potentiates the pathogenic effect of these strains. In this context, the main objective of this study, following the isolation and identification of E. coli strains from samples taken from broilers, fattening turkeys, pigs and cattle, was to differentiate between commensal E. coli strains and strains producing extended-spectrum beta lactamases (ESBL)/AmpC-type beta-lactamases.*

• Introduction

Antimicrobial resistance (AMR) is a complex, multifactorial phenomenon reflecting bacterial adaptation to ongoing antibiotic pressure. In *Escherichia coli*, resistance often occurs via β-lactamase enzymes—such as extended-spectrum β-lactamases (ESBLs), AmpC cephalosporinases, and carbapenemases—which inactivate penicillins, cephalosporins, and carbapenems. As β-lactams are among the most widely used antibiotic classes for both Gram-positive and Gram-negative infections, the emergence of resistant strains severely limits treatment options. The World Health Organization has raised concerns over carbapenem-resistant *E. coli*, with carbapenems now widely used as first-line agents against ESBL-producing strains, showing high in vitro efficacy (~98%). However, the spread of multidrug-resistant (MDR), zoonotic *E. coli* strains is a growing One Health concern. Studies show that both pathogenic and non-pathogenic *E. coli* strains are widespread in the environment and play diverse roles in host microbiomes, human and animal diseases, and as research models. In Romania, *E. coli* remains a major cause of economic loss in livestock, especially in pigs (post-weaning and neonatal diarrhea) and poultry (colibacillosis), with resistance trends amplifying their impact.

• Materials and methods

The research was carried out between 2020 and 2023 at the Sanitary Veterinary Laboratory of Satu Mare, as part of Romania's National Program for Monitoring and Reporting Antimicrobial Resistance (AMR) in Zoonotic and Commensal Bacteria. This activity aligns with EU Implementing Decision No. 2020/1729, which mandates all Member States to monitor AMR in *Escherichia coli* isolates from animals under the One Health framework. The study aimed to isolate and identify both commensal and ESBL/AmpC-producing *E. coli* strains and evaluate their antimicrobial susceptibility profiles.

A total of 281 samples of cecal contents were collected from healthy animals at slaughterhouses, including: 31 samples (11%) from fattening turkeys, 71 (25%) from broiler chickens, 19 (7%) from cattle under one year of age, and 160 (57%) from pigs. These samples originated from 11 counties: Alba, Arad, Bistrița-Năsăud, Cluj, Hunedoara, Maramureș, Mehedinți, Satu Mare, Sălaj, Sibiu, and Vâlcea. All samples were transported under refrigerated conditions (2–4°C) and processed within 24 hours.

To isolate commensal *E. coli*, cecal contents were directly streaked onto MacConkey agar using a microbiological loop. For the detection of ESBL/AmpC-producing strains, a pre-enrichment step was performed by mixing 1 gram of cecal content with 9 mL of buffered peptone water, followed by incubation at 44°C for 18 hours. A 10 µL aliquot of this enrichment was then inoculated onto MacConkey agar supplemented with cefotaxime (1 mg/L) and incubated under the same conditions.

Bacterial colonies were evaluated macroscopically based on growth characteristics and microscopically through Gram-stained smears using oil immersion (100x objective). Presumptive *E. coli* colonies were confirmed biochemically using differential media such as TSI (Triple Sugar Iron), MIU (Motility Indole Urea), and ONPG (Ortho-Nitrophenyl-β-galactoside). All confirmed *E. coli* isolates were subjected to antimicrobial susceptibility testing against a panel of substances to assess resistance profiles.

• Results and discussions

Commensal *E. coli* strains, when inoculated onto plain MacConkey agar, developed round, convex colonies that were pink to light red, non-mucoid, and occasionally surrounded by a precipitated bile salt zone. These strains fermented lactose, producing acid that lowered the pH below 6.8, turning the colonies pink. The surrounding bile salts precipitated, causing turbidity in the medium.

In pre-enrichment buffered peptone water, presumptive ESBL/AmpC-producing *E. coli* strains exhibited uniform turbidity, sometimes with a surface ring and gradually developing loose, powdery sediment, which was easily resuspended. On MacConkey agar supplemented with cefotaxime, these strains grew similarly to commensal strains, suggesting resistance to cefotaxime.

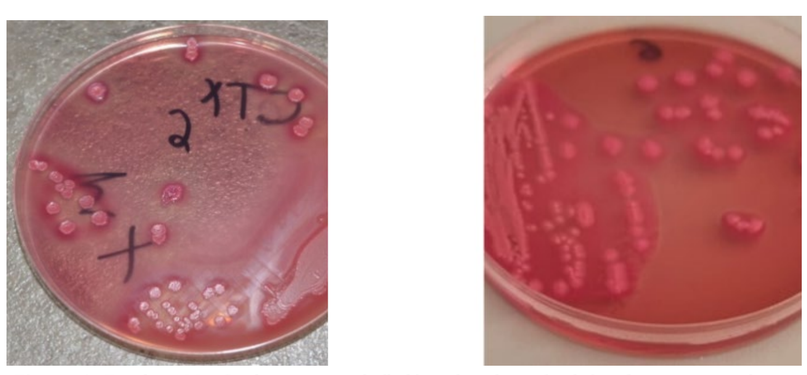

Figure 1. *E. coli* on MacConkey agar with (left) and without (right) cefotaxime supplement.

On TSI (Triple Sugar Iron) agar, *E. coli* caused the medium to shift from red to yellow, accompanied by gas production, but no black precipitate formed, indicating no hydrogen sulfide production. The slant turned yellow, suggesting fermentation of lactose and sucrose. Most strains exhibited motility, with growth beyond the inoculation line in MIU medium, and no color change, indicating urease-negative results. All strains tested positive for indole production, confirmed by a red ring after adding Kovac's reagent.

API 20E identification results for the strains: ONPG+, ADH−, LDC+, ODC−, CIT+, H_2S−, URE−, TDA−, IND+, VP+, GEL−, GLU+, MAN+, INO−, SOR−, SAC+, MEL+, AMY−, ARA+—consistent with *E. coli*, confirmed by the Apilab Plus software.

Over the four-year study, a total of 281 *E. coli* strains were isolated: 102 from poultry (36.3%), of which 31 were from turkey samples and 71 from broiler chickens; 160 strains (56.94%) from pigs; and 19 strains (6.76%) from cattle. The strains were preserved by freezing in glycerol for future antimicrobial susceptibility testing using the microdilution method in microplates.

Table 1. Categories of *E. coli* strains isolated from the four species

No.	Species	Commensal Strains no (%)	ESBL/AmpC Strains no (%)	Total no (%)
1.	Turkeys	14 (45)	17 (55)	**31 (11.03)**
2.	Broilers	28 (39)	43 (61)	**71 (25.27)**
3.	Cattle	14 (74)	5 (26)	**19 (6.76)**
4.	Swine	74 (46)	86 (54)	**160 (56.94)**
Total		**130 (46)**	**151 (54)**	**281 (100)**

• Conclusions

A total of 281 *E. coli* strains were isolated: 102 from poultry, 160 from swine, and 19 from cattle. In turkeys, 55% were ESBL/AmpC producers, while 45% were commensal. In broiler chickens, 61% were ESBL/AmpC producers, and 39% were commensal. Cattle showed a lower prevalence of ESBL/AmpC strains (26%), with 74% being commensal. Swine had a similar prevalence to broilers, with 54% ESBL/AmpC producers. The highest prevalence of ESBL/AmpC strains was in broiler chickens (61%), followed by turkeys (55%) and swine (54%).
All isolates were from healthy animals fit for slaughter, with a higher number of ESBL/AmpC producers than commensals. These findings align with other studies. Antimicrobial resistance (AMR) remains complex, with limited data on resistance transmission from non-food animal sources.