EVALUATION OF THE ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM EXHIBITION PIGEONS

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Abstract. *Antimicrobial resistance represents one of the major current challenges for both human and veterinary medicine, with antibiotic use exerting selective pressure that favors the emergence and dissemination of resistant bacteria. Pigeons (Columba livia domestica), through their frequent contact with humans, other animal species, and various sources of contamination in urban and peri-urban environments, may contribute to the circulation of antimicrobial-resistant microorganisms. Escherichia coli, a commensal bacterium of the avian gastrointestinal tract, is also an important indicator for antimicrobial resistance surveillance and may include strains with pathogenic and zoonotic potential. The aim of this study was to evaluate the antimicrobial susceptibility profiles of Escherichia coli strains isolated from pigeons in Romania. The identified resistance profiles were analyzed to highlight co-resistance patterns and phenotypic associations among the antimicrobial agents investigated and were descriptively contextualized against available data on human E. coli isolates. This study contributes to a better understanding of the role of pigeons as potential reservoirs of antimicrobial-resistant bacteria and provides relevant data for antimicrobial resistance surveillance at the animal–human–environment interface within a One Health framework.*

Keywords: *Escherichia coli*; antimicrobial resistance; pigeons; co-resistance; One Health.

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1. Introduction

Antimicrobial resistance (AMR) is a major challenge for human and veterinary medicine. Antibiotic exposure creates selective pressure that favors resistant bacteria, and both human medicine and animal production contribute to their emergence and dissemination [1–5].

The historical use of antimicrobials as growth promoters and their continued inappropriate use in some regions have reinforced the need for prudent antimicrobial use and alternative preventive approaches [3–7].

Pigeons (Columba livia domestica) are of particular interest in AMR surveillance because they live in close contact with humans, domestic animals, wild birds, contaminated water, animal feces, and urban waste [8–11]. These ecological interfaces may facilitate acquisition, carriage, and environmental dissemination of resistant microorganisms.

Escherichia coli is a common intestinal commensal of birds and mammals and an important indicator organism for AMR surveillance [12-16]. Avian pathogenic E. *coli* (APEC), enteropathogenic E. coli (EPEC), and Shiga toxin-producing *E. coli* (STEC) are relevant because avian strains may share virulence and resistance traits with human extraintestinal pathogenic *E. coli*, raising One Health concerns [17-25].

Previous studies from Romania have also documented antimicrobial-resistant *E. coli* in avian and other animal hosts, further supporting the relevance of integrated AMR surveillance [26–29].

In Romania, domestic pigeons are maintained for racing, exhibitions, ornamental breeding, and recreational purposes, often in urban or peri-urban settings. Despite their close interaction with humans and other birds, systematic information on antimicrobial susceptibility in these populations remains limited. Studies conducted in other European countries have demonstrated that domestic pigeons may carry *E. coli* isolates displaying resistance to several antimicrobial classes [30,31].

The aim of the present study was to evaluate antimicrobial susceptibility profiles of *E. coli* strains isolated from Romanian pigeons during 2024–2025. Co-resistance patterns, clustering, MDR phenotypes, and MIC distributions were assessed, and the results were descriptively contextualized against available human *E. coli* resistance data within a One Health framework.

2. Materials and methods

2.1 Study Design

The present study was designed as a cross-sectional observational investigation conducted during 2024–2025. The study population consisted of domestic pigeons

(*Columba livia domestica*) raised for racing, recreational purposes, or breeding in several regions of Romania.

Samples were collected during the National Exhibition organized by the Fauna Banatului Association in Timișoara. The pigeons included in the study originated from lofts located in the counties of Timiș, Arad, Bihor, Hunedoara, Caraș-Severin, Gorj, and Alba and were presented at the exhibition. The flocks were selected based on breeder availability, previous collaboration with the official veterinarian, and geographical distribution, with the aim of ensuring the broadest possible representation of different regions of the country.

The inclusion criteria comprised clinically healthy birds over six months of age that had not received antimicrobial treatment during the two weeks preceding sample collection. Birds showing clinical signs of disease or for which the antimicrobial treatment history was unknown were excluded from the study.

Oropharyngeal and cloacal samples were collected using sterile swabs placed in Deltalab transport systems containing Amies medium. Bacteriological identification was performed by inoculating the samples onto ChromoBio Coliform agar, and the resulting pure cultures were preserved using Microbank systems and stored at −80 °C until further analyses were performed.

A total of 134 *Escherichia coli* strains were included in the final analysis.

The sample size was determined pragmatically based on field accessibility and the logistical feasibility of the study and was considered sufficient for the planned statistical analyses. Strains were selected based on colony morphology, sample type, and flock of origin, with the aim of reducing the overrepresentation of particular isolates and maximizing epidemiological diversity within the practical constraints of bacterial isolation and characterization.

2.2. Determination of the Minimum Inhibitory Concentration (MIC)

The antibiotics included in the susceptibility testing were selected based on their documented and routine use in veterinary medicine, particularly for the treatment of bacterial infections in domestic pigeons in Romania. All pigeons included in the present study were domestic birds raised for breeding, racing competitions, or ornamental purposes. They were maintained under controlled conditions and housed in pigeon lofts or aviaries. None of the birds included in the study originated from wild populations or were free-ranging. The antibiotic panel included antimicrobial agents relevant both to avian clinical practice and from a public health perspective. The phenotypic expression of resistance was evaluated by determining minimum inhibitory concentration (MIC) values with reference to the Clinical and Laboratory Standards Institute (CLSI) framework for dilution

susceptibility testing [38]. Breakpoints were established according to CLSI recommendations [32] and were compared, where applicable, with epidemiological cutoff values (ECOFFs) defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [33]. For antimicrobial agents for which species-specific interpretive criteria were unavailable, additional criteria were derived from relevant scientific literature [30,31,34].

Prior to testing, bacterial strains stored at −80 °C were resuspended in 3 mL of cation-adjusted Mueller–Hinton broth (CAMHB) and incubated for 18–24 h at 37 °C. Antimicrobial susceptibility testing was performed using 96-well microtiter plates. All wells, except those in the first column, were loaded with 90 µL of CAMHB. Stock solutions of the tested antibiotics (Merck KGaA, Darmstadt, Germany) were prepared at a concentration of 1,024 µg/mL in accordance with CLSI recommendations [32].

Amoxicillin and the amoxicillin–clavulanic acid combination were prepared at a 2:1 ratio (pH 7.2; 0.01 mol/L), while imipenem was dissolved in phosphate buffer (pH 6; 0.1 mol/L). Doxycycline, neomycin, tylosin, and vancomycin were dissolved in distilled water. For preparation of the trimethoprim–sulfamethoxazole mixture (1:19 ratio), sulfamethoxazole was dissolved in hot water with a few drops of 2.5 mol/L NaOH, while trimethoprim was dissolved in distilled water containing 0.05 mol/L HCl. Enrofloxacin was prepared by dissolving the compound in distilled water with the addition of 100 µL of 1 mol/L NaOH. Florfenicol was dissolved in distilled water with the addition of 100 µL of 95% ethanol to improve solubility.

A dilution with a concentration of 512 µg/mL was prepared from these solutions, and 180 µL was dispensed into the first column of the microtiter plates. Two-fold serial dilutions were subsequently performed across the plate, with 90 µL removed from the final well so that each well contained a final volume of 90 µL. Bacterial suspensions were adjusted to a 0.5 McFarland standard using a nephelometer, and 10 µL of bacterial suspension was inoculated into 90 µL per well, as documented in the laboratory record. The final inoculum concentration was not recorded in the available documentation and is therefore acknowledged as a methodological limitation [38].

MIC values were determined using the automated Sensititre™ SWIN™ MIC Reader together with VIZION System software, version 3.4. Escherichia coli ATCC 25922 was used as the quality-control strain. The resulting MIC values were interpreted against the specified international criteria.

MIC_{50} and MIC_{90} values were calculated as the 50th and 90th percentiles, respectively, of the MIC value distribution for all tested isolates. These values represent the minimum inhibitory concentrations at which 50% and 90%,

respectively, of the investigated bacterial isolates were inhibited. MIC distributions were obtained from the complete dataset of 134 *Escherichia coli* isolates tested for each antibiotic, and the percentiles were calculated using the base functions of R software. These parameters provide an overall assessment of antimicrobial susceptibility trends within the bacterial population analyzed.

2.3. Statistical Analysis

Statistical analyses were performed in R version 4.2.2 [35]. The analyses reported here focused on descriptive susceptibility summaries, binary resistance associations, clustering, and exploratory modeling.

Resistance data were binarized (resistant = 1; susceptible = 0). Pairwise associations were evaluated using the phi coefficient (φ), and heatmaps were generated with corrplot and pheatmap. Antimicrobial variables with zero variance were excluded from phi-coefficient calculations because associations cannot be estimated when all isolates have the same binary outcome. Accordingly, trimethoprim–sulfamethoxazole (potentiated sulfonamide), for which no resistant isolates were detected, was excluded from the phi correlation matrix. Agglomerative hierarchical clustering was explored using Jaccard dissimilarities. PCA visualization was performed with factoextra.

Co-resistance networks were constructed with igraph and ggraph, with edge weights representing the frequency of simultaneous resistance. Decision-tree models (rpart) were used exploratorily to identify predictors of MDR phenotypes.

Uncertainty around MDR prevalence was evaluated using a Monte Carlo simulation with 10,000 iterations, in which the initial resistance rates were randomly varied by ±20%. The resulting distribution was used to estimate the mean, median, standard deviation, and 95% percentile interval of the simulated distribution (not a confidence interval for observed prevalence).

2.4. Definitions of Resistance Categories

The definitions proposed by Magiorakos et al. [36] were used as a conceptual framework. MDR denotes acquired non-susceptibility in at least three relevant antimicrobial categories. Intrinsically inactive agents, including tylosin and vancomycin for E. coli, were not considered informative for acquired-resistance classification. XDR and PDR were not reported as confirmed phenotypes because the antimicrobial panel did not provide sufficiently complete category coverage for robust classification according to the Magiorakos framework.

3. Results and Discussions

3.1. Characteristics of the Study

A total of 660 samples (330 cloacal and 330 oropharyngeal samples) were collected from 330 domestic pigeons. E. coli was identified in 328/660 samples (49.7%). From these positive samples, 134 isolates representing 20 flocks were selected for antimicrobial susceptibility testing according to the predefined selection criteria.

The majority of isolates originated from Timiș County (52.2%). Most strains (50.0%) were isolated from racing pigeons, while the majority of the birds investigated were young adults (32.8%) and adult birds used for breeding (28.4%). Most pigeon breeders (47.8%) maintained medium-sized flocks comprising between 51 and 100 birds.

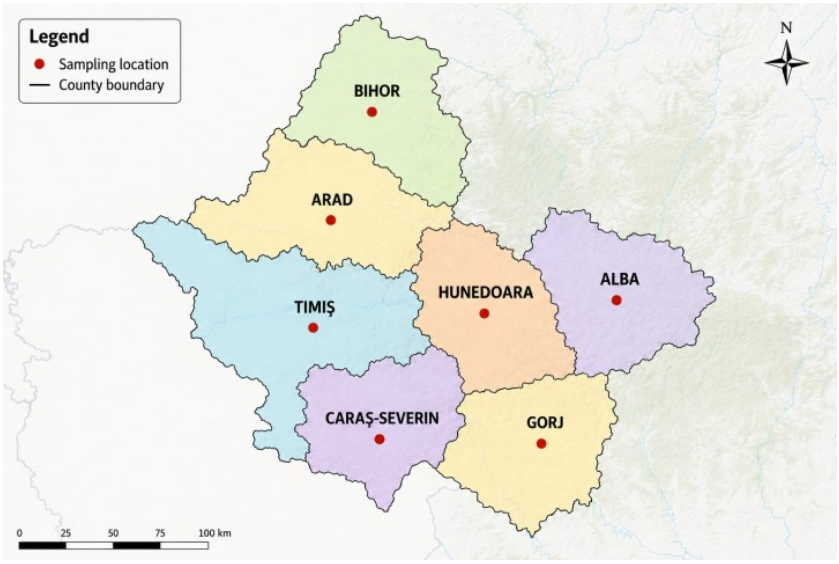


Figure 1. Geographical distribution of the sampling locations included in the study. The map highlights the seven counties in western Romania where biological samples were collected: Timiș, Arad, Bihor, Hunedoara, Caraș-Severin, Gorj, and Alba.

A total of 134 *E. coli* isolates representing 20 flocks from seven western Romanian counties were included in susceptibility testing.

Birds were used mainly for racing, ornamental, meat-production, or breeding purposes, with ages ranging from 6 months to over 4 years. Table 1 summarizes their distribution by county and primary purpose of rearing.

Table 1. Distribution of E. coli isolates according to county and primary purpose of pigeon rearing.

County	**Racing pigeons n (%)**	**Meat production n (%)**	**Ornamental n (%)**
Timiș	26 (19.4%)	21 (15.7%)	23 (17.2%)
Arad	11 (8.2%)	0 (0.0%)	13 (9.7%)
Bihor	3 (2.2%)	0 (0.0%)	5 (3.7%)
Hunedoara	0 (0.0%)	5 (3.7%)	0 (0.0%)
Caraș-Severin	4 (3.0%)	0 (0.0%)	0 (0.0%)
Gorj	10 (7.5%)	0 (0.0%)	0 (0.0%)
Alba	13 (9.7%)	0 (0.0%)	0 (0.0%)

3.2. Resistance Correlations and Cluster Analysis

Phi coefficient analysis revealed varying degrees of association among antibiotic resistance profiles. Using the heatmap representation (Figure 2), stronger correlations were identified that may indicate the phenotypic co-occurrence of resistance traits. Although these correlations do not demonstrate a causal relationship or the presence of underlying genetic mechanisms, they may reflect phenomena such as co-selection, co-resistance, or cross-resistance, for example, as a result of resistance genes being located on the same mobile genetic element or the concurrent use of multiple antimicrobial agents.

The strongest positive correlation was observed between ceftriaxone and imipenem ($\varphi = 0.70$). Other positive associations included florfenicol and neomycin ($\varphi = 0.36$) and amoxicillin–clavulanic acid and enrofloxacin ($\varphi = 0.31$). The most pronounced negative correlation was observed between amoxicillin and colistin ($\varphi = -0.13$). Trimethoprim–sulfamethoxazole was not included in the phi analysis because no resistant isolates were detected, resulting in a zero-variance binary variable for which φ is undefined.

Cluster analysis identified three main groups, within which different antimicrobial agents were predominant in terms of resistance.

In Cluster 1, neomycin showed the highest frequency of resistance (95.2%), whereas Cluster 2 was characterized by a predominance of doxycycline resistance (75.8%). In Cluster 3, neomycin was again the predominant antibiotic, with a resistance frequency of 92.3%.

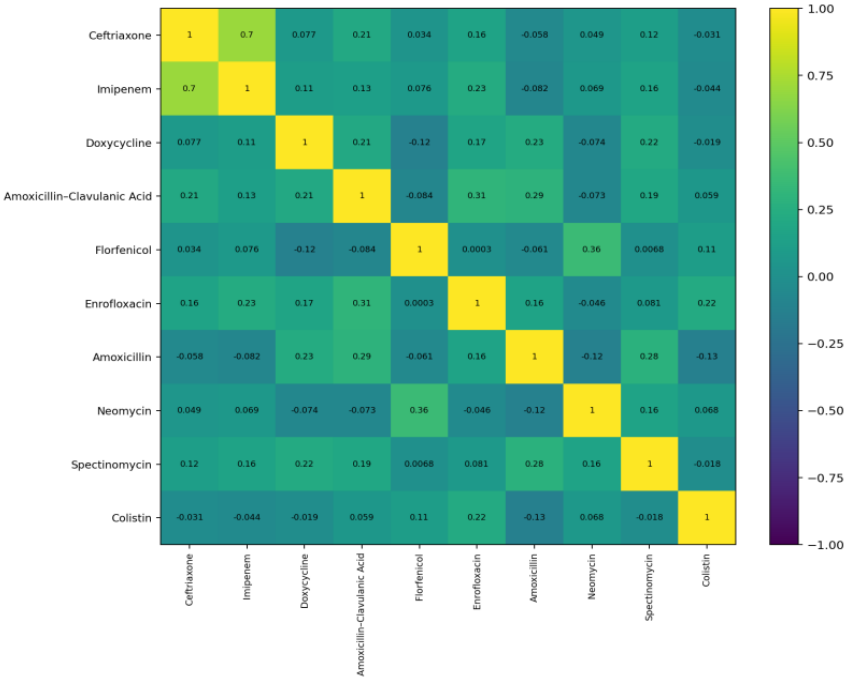


Figure 2. Heatmap of phi coefficients for pairwise antibiotic resistance profiles among Escherichia coli strains (n = 134) isolated from pigeons. Trimethoprim–sulfamethoxazole was excluded because the resistance variable had zero variance (0 resistant isolates).

3.3. Co-Resistance Network Analysis

Network Graph Analysis revealed the relationships and frequency of connections among antibiotic resistance profiles. Within the network, line thickness represents the frequency of co-resistance between pairs of antibiotics. Antibiotics are represented by light blue circles, with their sizes proportional to the number of resistant isolates associated with each antimicrobial agent.

The most frequent associations were observed among neomycin, doxycycline, and florfenicol, indicating frequent phenotypic co-occurrence of resistance to these antimicrobial agents.

3.4. Predictive Modeling and Simulation

The decision tree-based model enabled the prediction of multidrug-resistant (MDR) Escherichia coli strains. The model results demonstrated that resistance to certain antibiotics represents an important predictor of whether a strain belongs to the MDR category. In particular, resistance to neomycin, doxycycline, and

amoxicillin emerged as important predictors of the MDR phenotype in this exploratory model.

To assess the potential variability in the prevalence of MDR phenotypes, a Monte Carlo simulation was performed, in which the initial resistance rates were randomly varied by ±20%. The estimated mean prevalence of MDR strains was 62.1%, while the median was 61.9%, indicating an approximately symmetrical distribution of the results.

The standard deviation was 7.2%, suggesting that the occurrence rate of MDR strains generally ranged between 54.9% and 69.3%. The 95% simulation percentile interval ranged from 47.7% to 75.3%, reflecting the variability generated under the specified simulation assumptions; this interval should not be interpreted as a confidence interval for the observed prevalence.

3.5. Prevalence of Antimicrobial Resistance and MIC Values

Based on the determined minimum inhibitory concentration (MIC) values, a frequency table (Figure 3) was generated to summarize the results obtained, including the clinical breakpoint for each antibiotic. MIC_{50} and MIC_{90} values were also calculated for the bacterial population investigated.

		MIC (µg/mL)																					
Antibiotic	**n**	**≤0.002**	**0.004**	**0.008**	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**≥512**	**MIC_{50}**	**MIC_{90}**	**ECOFF**
Amoxicillin	134							2 (1.5%)	3 (2.2%)	5 (3.7%)	9 (6.7%)	15 (11.2%)	21 (15.7%)	24 (17.9%)	20 (14.9%)	15 (11.2%)	10 (7.5%)	6 (4.5%)	4 (3.0%)		4	64	8
Amoxicillin/ clavulanic acid	134							1 (0.7%)	4 (3.0%)	8 (6.0%)	14 (10.4%)	22 (16.4%)	26 (19.4%)	22 (16.4%)	16 (11.9%)	10 (7.5%)	6 (4.5%)	3 (2.2%)	2 (1.5%)		2	32	8
Ceftriaxone	134				18 (13.4%)	26 (19.4%)	32 (23.9%)	28 (20.9%)	16 (11.9%)	8 (6.0%)	4 (3.0%)	2 (1.5%)									0.06	0.25	0.25
Colistin	134						20 (14.9%)	30 (22.4%)	32 (23.9%)	26 (19.4%)	16 (11.9%)	8 (6.0%)	2								0.125	0.5	2
Doxycycline	134						4 (3.0%)	8 (6.0%)	14 (10.4%)	22 (16.4%)	32 (23.9%)	28 (20.9%)	16 (11.9%)	10 (7.5%)							1	2	4
Enrofloxacin	134						2 (1.5%)	6 (4.5%)	10 (7.5%)	18 (13.4%)	28 (20.9%)	32 (23.9%)	24 (17.9%)	10 (7.5%)	4 (3.0%)						1	2	0.5
Florfenicol	134							8 (6.0%)	14 (10.4%)	28 (20.9%)	36 (26.9%)	28 (20.9%)	16 (11.9%)	4 (3.0%)							0.5	1	4
Imipenem	134								28 (20.9%)	36 (26.9%)	32 (23.9%)	24 (17.9%)	10 (7.5%)	4 (3.0%)							0.5	1	1
Neomycin	134							4 (3.0%)	8 (6.0%)	16 (11.9%)	26 (19.4%)	32 (23.9%)	28 (20.9%)	16 (11.9%)	4 (3.0%)						1	2	8
Trimethoprim/ sulfamethoxazole	134						2 (1.5%)	6 (4.5%)	12 (9.0%)	24 (17.9%)	32 (23.9%)	28 (20.9%)	20 (14.9%)	8 (6.0%)	1 (1.5%)						0.5	1	2
Spectinomycin	134								12 (9.0%)	20 (14.9%)	28 (20.9%)	32 (23.9%)	26 (19.4%)	12 (9.0%)	4 (3.0%)						1	2	8
Tetracycline	134							2 (1.5%)	6 (4.5%)	12 (9.0%)	24 (17.9%)	32 (23.9%)	28 (20.9%)	20 (14.9%)	8 (6.0%)	2 (1.5%)					1	2	4

Figure 3.. Frequency distribution of minimum inhibitory concentration (MIC) values for *Escherichia coli* strains (n = 134) isolated from pigeons and tested against antibiotics for which clinical breakpoints have been established.

Among the antimicrobial agents tested, ceftriaxone and imipenem exhibited MIC_{90} values below the established clinical breakpoints, indicating that at least 90% of the tested isolates had MIC values below the corresponding clinical

breakpoints. Analysis of the MIC_{50} values showed that amoxicillin, amoxicillin–clavulanic acid, ceftriaxone, colistin, enrofloxacin, and imipenem had values below the established clinical breakpoints. These findings indicate that at least half of the isolates retained susceptibility to these antimicrobial agents.

When the results were compared with the epidemiological cutoff values (ECOFFs) established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), it was observed that, for ceftriaxone, colistin, and imipenem, at least half of the investigated isolates were most likely classified as wild-type strains. This comparison was performed to distinguish acquired resistance (non-wild-type strains) from intrinsic susceptibility, independently of the clinical breakpoints used in medical practice. By concurrently evaluating phenotypic resistance and ECOFF-based wild-type status, a more detailed assessment of emerging antimicrobial resistance trends was sought, including among isolates that are not yet clinically classified as resistant. Such information may support the early detection of emerging resistance trends and epidemiological surveillance within a One Health framework.

Detailed MIC values, together with the individual data corresponding to each bacterial isolate, are provided in the Supplementary Material.

The highest resistance levels were recorded for neomycin (76.1%), an aminoglycoside antibiotic, and florfenicol (72.4%), a member of the phenicol class. In contrast, no phenotypically resistant isolates were identified for the trimethoprim–sulfamethoxazole combination, representing a potentiated sulfonamide. The lowest resistance levels were observed for ceftriaxone (0.7%), a third-generation cephalosporin, and imipenem (1.5%), a carbapenem antibiotic.

3.6. Multidrug Resistance Patterns

The occurrence of multidrug-resistant (MDR) phenotypes was evaluated, while the applicability of XDR and PDR classifications was assessed according to the Magiorakos framework.

Based on the operational MDR classification applied in the present analysis, 85 of 134 isolates (63.4%) were assigned an MDR phenotype. XDR and PDR frequencies were not reported as confirmed findings because the antimicrobial panel did not provide sufficiently complete category coverage for robust classification according to the Magiorakos framework. The absence of resistance to trimethoprim–sulfamethoxazole further precluded a confirmed PDR designation within the tested panel.

3.7. Comparison with Human Antibiotic Resistance Data

The resistance profiles of *Escherichia coli* isolates obtained from pigeons were compared with antibiotic resistance data from human clinical isolates provided by the Timișoara Public Health Directorate, in accordance with the study objective of investigating potential overlaps in resistance patterns within the One Health framework (Figure 4).

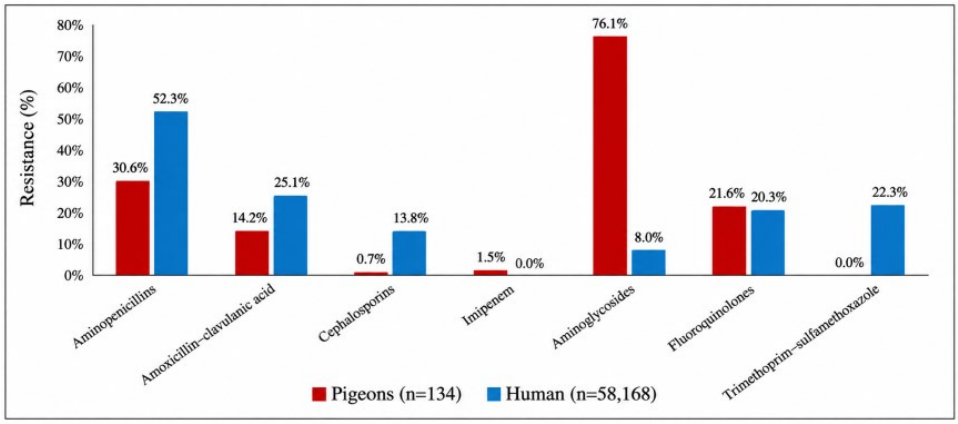


Figure 4. Comparison of the resistance profiles of *Escherichia coli* strains isolated from pigeons with antibiotic resistance data from the human public health system.

Regarding β-lactam antibiotics, lower resistance levels were observed among pigeon isolates compared with human clinical strains; these differences may reflect distinct antimicrobial-exposure patterns and the differing sampling frameworks of veterinary and human surveillance. An exception was imipenem, for which a resistance rate of 1.5% was identified among pigeon isolates, whereas no resistance was recorded in the corresponding human medicine data. The detection of imipenem resistance in a small proportion of pigeon isolates warrants further investigation, particularly through molecular characterization of the underlying resistance determinants. Resistance to fluoroquinolones showed a relatively similar distribution between isolates of veterinary origin and those from human medicine. For the trimethoprim–sulfamethoxazole combination (potentiated sulfonamide), no resistant strains were identified among the pigeon isolates. In contrast, a high resistance frequency was observed for neomycin, the aminoglycoside included in the pigeon antimicrobial panel.

3.8. Discussion of Antimicrobial Resistance in *Escherichia coli* Strains Isolated from Pigeons

The present study identified a high burden of antimicrobial resistance among 134 E. coli isolates from domestic pigeons. Based on the operational classification applied in the present analysis, 63.4% (85/134) of isolates exhibited an MDR phenotype. XDR and PDR frequencies were not reported because the antimicrobial panel did not provide sufficiently complete category coverage for robust classification according to the Magiorakos framework.

Karim et al. reported a lower MDR prevalence (23.8%) in pigeon *E. coli* from Bangladesh, with frequent resistance to amoxicillin, ampicillin, erythromycin, and tetracycline [37]. Differences from the present study may reflect regional antimicrobial-use practices, sampling design, husbandry, and veterinary oversight. The absence of reliable treatment records in the investigated flocks prevents direct attribution of the observed phenotypes to previous antimicrobial exposure.

The MIC results further characterized the susceptibility profile. MIC90 values for ceftriaxone (0.25 μg/mL) and imipenem (1 μg/mL) remained below clinical breakpoints, whereas MIC50 values indicated retained susceptibility in at least half of isolates for several agents. A Belgian study reported enrofloxacin MIC_{50} <0.25 μg/mL and MIC_{90} 32 μg/mL [34], compared with 0.125 and 8 μg/mL, respectively, in the present dataset. Recent data from Hungary have also demonstrated heterogeneous antimicrobial susceptibility profiles among *E. coli* isolates from domestic pigeons, emphasizing the importance of geographical differences in antimicrobial exposure and resistance epidemiology [30].

Resistance frequencies differed substantially among agents. Neomycin and florfenicol showed the highest resistance levels, whereas ceftriaxone and imipenem remained low. No resistance was detected to trimethoprim–sulfamethoxazole. Comparable international studies have reported heterogeneous profiles, emphasizing geographical variation in antimicrobial selection pressure and management practices [18,31].

Correlation and clustering analyses identified structured co-resistance patterns. The strongest positive association occurred between ceftriaxone and imipenem ($\varphi \approx 0.7$), with additional positive associations observed between florfenicol and neomycin and between amoxicillin–clavulanic acid and enrofloxacin. Trimethoprim–sulfamethoxazole was excluded from the phi analysis because all isolates were susceptible, making the binary resistance variable invariant and the phi coefficient undefined. Such phenotypic associations do not demonstrate shared genetic mechanisms but may be compatible with co-selection, linked resistance determinants, altered permeability, or multidrug efflux systems.

Cluster and network analyses consistently highlighted neomycin, doxycycline, and florfenicol. High doxycycline resistance may reflect established tetracycline-resistance mechanisms, including efflux and ribosomal protection, while the broad distribution of aminoglycoside resistance reduced the discriminatory value of neomycin across clusters. Molecular studies are required to determine whether mobile genetic elements contribute to these patterns.

Comparison with human surveillance data showed lower resistance among pigeon isolates for several β-lactams, while some resistance trends were similar across sectors. These descriptive parallels should not be interpreted as evidence of transmission because the veterinary and human datasets differ in sampling design, surveillance methodology, and antimicrobial panels. Nevertheless, they support integrated One Health surveillance.

The study has several limitations. Sampling was pragmatic rather than strictly randomized; antimicrobial-use histories were incomplete; the cross-sectional design does not support temporal or causal inference; and resistance mechanisms were inferred from phenotypic data without PCR or whole-genome sequencing. These factors limit generalizability and mechanistic interpretation. The available laboratory documentation did not record the final broth-microdilution inoculum concentration. In addition, incomplete antimicrobial-category coverage precluded robust classification of XDR and PDR phenotypes.

Future studies should combine longitudinal sampling with standardized antimicrobial-use records and genomic characterization of resistance determinants. Harmonized veterinary and human surveillance would also improve comparison across sectors and help clarify the epidemiological role of domestic pigeons.

Overall, the findings support continued AMR monitoring in pigeon populations and antimicrobial stewardship among breeders. The substantial MDR proportion observed under the operational classification, together with co-resistance patterns and the close human–animal–environment interface, supports further investigation within a One Health framework.

4. Conclusions

Among E. coli strains isolated from domestic pigeons in western Romania, 63.4% (85/134) were assigned an MDR phenotype according to the operational classification applied in the present analysis. XDR and PDR were not reported because the antimicrobial panel did not provide sufficiently complete category coverage for robust classification. Co-occurrence of resistance traits was particularly evident for neomycin, doxycycline, and florfenicol, although the molecular basis of these associations was not investigated.

The results indicate a need for periodic AMR surveillance, prudent antimicrobial use, improved treatment records in pigeon holdings, and molecular characterization of resistance determinants. Because the study was cross-sectional and sampling was not strictly randomized, the findings should be interpreted as evidence from the investigated population rather than as national prevalence estimates.

Integration of veterinary, human, and environmental AMR data within a One Health framework would strengthen surveillance and help assess the potential role of pigeons as reservoirs and disseminators of resistant *E. coli*.

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