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# ANTIMICROBIAL RESISTANCE IN FAECAL *ESCHERICHIA COLI* FROM FARMED OSTRICH (*STRUTHIO CAMELUS*)

## ANTIBIOTICOREZISTENȚA TULPINILOR DE *ESCHERICHIA COLI* IZOLATE DE LA STRUȚI (*STRUTHIO CAMELUS*) DIN FERME

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**ABSTRACT | REZUMAT**

This study aimed to characterize *Escherichia coli* commensal strains isolated from clinically healthy farmed ostriches from Central and North-Western Romania. The samples were analyzed by standard microbiological methods (isolation on selective media and *in vitro* antimicrobial sensitivity testing). Results indicated an asymptomatic carrier state for 97.6% of the ostriches and varying antibiotic resistance levels, depending on the tested antimicrobial, the strain origin (farm), and ostrich age. The highest level of antimicrobial resistance was observed for streptomycin (52.5%), followed by tetracycline (45%), colistin (40 %), doxycycline (37.5%), and ampicillin (32.5%). In total, 17 distinct resistance patterns were recorded, with the multidrug resistance (MDR) feature described for 20% of *E. coli* isolates. These findings underline the human health risks in the case of potentially contaminated ostrich products (carcasses, eggs). Within the One Health integrated approach, further studies are needed to comprehensively evaluate the farmed ostrich's role as a reservoir for multidrug-resistant *E. coli* strains.

**Keywords**: farmed ostriches; *Escherichia coli*; commensal strains; multidrug resistance, *One Health*

Acest studiu și-a propus să caracterizeze tulpinile comensale de *Escherichia coli* izolate de la struți de crescătorie clinic sănătoși din centrul și nord-vestul României. Probele au fost analizate prin metode microbiologice standard (izolarea pe medii selective și testarea sensibilității antimicrobiene). Rezultatele au indicat stare de purtător asimptomatic pentru 97,6% dintre struți și niveluri diferite de rezistență la antibiotice dependent de antibioticul testat, originea tulpinii (ferme) și vârsta struților. Cel mai mare nivel de rezistență antimicrobiană a fost observat față de streptomicină (52,5%), urmată de tetraciclină (45%), colistină (40%), doxiciclină (37,5%) și ampicilină (32,5%). În total, au fost înregistrate 17 modele distincte de rezistență, cu caracteristica de rezistență la mai multe medicamente (MDR) descrisă pentru 20% din izolatele de *E. coli*. Aceste constatări subliniază riscul pentru sănătatea umană în cazul produselor potențial contaminate provenind de la struți (carcase,ouă). În cadrul abordării integrate One Health, sunt necesare studii suplimentare pentru a evalua cuprinzător rolul struțului de crescătorie ca rezervor pentru tulpinile antibioticorezistente de *E. coli*.

**Cuvinte cheie**: struți din ferme; *Escherichia coli*; tulpini comensale; antibioticorezistență, *One Health*

In Romania, ostriches (*Struthio camelus*) are being raised in several small and medium-sized farms for their meat, eggs, leather, and feathers (7). With rearing conditions not yet optimized, pathologies leading to increased mortality in both embryos, chicks, and adults have been constantly reported over the last ten years. In addition, bacterial pathogens were involved, and the use of antimicrobials was performed both as a therapeutic and as a preventive approach. Among the factors associated with ostrich morbidity and mortality, several stressors concerning poor management of the farm, overcrowding, fluctuations in microclimate, nutritional imbalances, and incorrect administration of the feed, as well as organisms such as viruses and bacteria, were suggested (3,29). *Escherichia coli* is listed among the most frequently isolated both as a primary and secondary etiological agent (3,9,22,29).

*Escherichia coli* is well-known for its versatility and complexity, occurring in the intestinal commensal flora of both animals and humans (10, 25). The MDR *E. coli* strain's asymptomatic carriage has been demonstrated in several domestic and wild mammals and birds, underlying their role as potential reservoir species in the epidemiology of associated pathologies (1, 23). Furthermore, given the MDR *E. coli* strains potential transfer and spread to humans, public health risks

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are highlighted (4,6,27). Relatively little information is available on the presence and characteristics of *E. coli* in clinically healthy ostriches (20,25). To the best of our knowledge, this is the first study aimed to evaluate and characterize the antimicrobial resistance of *E. coli* strains isolated from healthy ostriches in Romania.

## MATERIALS AND METHODS

Sample collection: fresh faecal samples were collected from clinically healthy ostriches (*Struthio camelus*) (n=41) originating from three farms in Central and North-Western Romania. In total, 14 male and 27 female ostriches were examined, and their ages ranged between 1 month and 6 years. Prophylactic administration of enrofloxacin and colistin in the drinking water, daily, for a period of 3 weeks was carried out on farm 3 in ostriches younger than 5 months of age.

*Escherichia coli* isolation and identification: preliminary microbiological analyses were made of cloacal swabs and pooled faeces by isolation on selective chromogenic agar (Brilliance™ E. coli/coliform Selective Agar, Oxoid Ltd., Cambridge/UK), after incubation under aerobic conditions at 37°C for 24 hours, and evaluation of biochemical properties using the API20E system (BioMerieux SA, Marcy Etoile, France).

Antibiotic susceptibility testing: the antimicrobial susceptibility of the isolates was assessed *in vitro* using the standard Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (8). The strains were tested towards 9 antimicrobial drugs: ampicillin (AMP, 10 µg) amoxicillin/clavulanic acid (AMC, 20/10 µg), tetracycline (TE, 30 µg), doxycycline (DO, 30 µg), streptomycin (S, 10 µg), gentamycin (CN, 10 µg), colistin (CS, 10 µg), enrofloxacin (ENR, 5 µg), florfenicol (FFC, 30 µg) (susceptibility discs, Oxoid Ltd., Cambridge/UK). Based on the growth inhibition zone diameters (mm), compared to CLSI criteria, the strains were recorded as susceptible (S), intermediate (I), or resistant (R). In the case of colistin, enrofloxacin, and florfenicol (Floron®, KRKA, Novo Mesto, Slovenia), breakpoints previously described were taken into consideration (23). *Escherichia coli* ATCC® 25922 (Oxoid Ltd., Cambridge, UK) was also tested as a quality control organism. The multiple antibiotics resistance (MAR) index of isolates was determined based on the a/b ratio, where 'a' represents the number of antibiotics to which the particular isolate is resistant and 'b' the number of antibiotics to which the isolate is exposed (14,20). A MAR index higher than 0.2 indicated that the strain originated from high-risk sources where antibiotics were often used, while values of less than or equal to 0.2 indicated strains that originated from sources where antibiotics were seldom, if ever, used (19). Statistical analysis was performed using Epi InfoTM7(CDC, Atlanta, GA, USA) software. The chi-square test was applied to compare the antimicrobial resistance rates (%) to antimicrobials. A p-value of ≤ 0.05 was considered to indicate statistically significant differences.

## RESULTS AND DISCUSSIONS

The recovery rate of *E. coli* strains after the microbiological assessment of the faecal samples was 97.6% (40/41). The asymptomatic carrier state was recorded for all, but one ostrich.

**Table 1**

**Zone Diameter Interpretive Standards and antibiotic susceptibility for *E. coli* strains**

| Antimicrobial Agent | Zone Diameter (mm) | | | farm 1 n= 8 strains | | | | | | farm 2 n=7 strains | | | | | | farm 3 n=25 strains | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | I | S | R | | I | | S | | R | | I | | S | | R | | I | | S | |
| | | | | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % |
| **Ampicillin (AMP)** | ≤13 | 14-16 | ≥17 | 0 | 0 | 0 | 0 | 8 | 100 | 3 | 42.9 | 1 | 143 | 3 | 42.9 | 10 | 40 | 2 | 8 | 13 | 52 |
| **Amoxicillin-clavulanic acid (AMC)** | ≤13 | 14-17 | ≥18 | 0 | 0 | 0 | 0 | 8 | 100 | 0 | 0 | 1 | 14.3 | 6 | 85.7 | 3 | 12 | 2 | 8 | 20 | 80 |
| **Tetracycline (TE)** | ≤11 | 12-14 | ≥15 | 3 | 37.5 | 0 | 0 | 5 | 62.5 | 5 | 71.4 | 2 | 28.6 | 0 | 0 | 10 | 40 | 3 | 12 | 12 | 48 |
| **Doxycycline (DO)** | ≤10 | 11-13 | ≥14 | 2 | 25 | 0 | 0 | 6 | 75 | 4 | 57.1 | 3 | 42.9 | 0 | 0 | 9 | 36 | 3 | 12 | 13 | 52 |
| **Streptomycin (S)** | ≤11 | 12-14 | ≥15 | 5 | 62.5 | 0 | 0 | 3 | 37.5 | 5 | 71.4 | 0 | 0 | 2 | 28.6 | 11 | 44 | 8 | 32 | 6 | 24 |
| **Gentamycin (CN)** | ≤12 | 13-14 | ≥15 | 0 | 0 | 0 | 0 | 8 | 100 | 0 | 0 | 0 | 0 | 7 | 100 | 0 | 0 | 0 | 0 | 25 | 100 |
| **Colistin (CT)** | ≤10 | 11-12 | ≥13 | 4 | 50 | 0 | 0 | 4 | 50 | 1 | 14.3 | 2 | 28.6 | 4 | 57.1 | 11 | 44 | 4 | 16 | 10 | 40 |
| **Enrofloxacin (ENR)** | ≤17 | 18-21 | ≥22 | 0 | 0 | 0 | 0 | 8 | 100 | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 | 5 | 20 | 4 | 16 | 16 | 64 |
| **Florfenicol (FFC)** | <20 | - | >22 | 0 | 0 | 0 | 0 | 8 | 100 | 0 | 0 | 0 | 0 | 7 | 100 | 3 | 12 | 2 | 8 | 20 | 80 |

Results of the *in vitro* antimicrobial susceptibility testing of the *E. coli* isolates of ostrich origin are presented in Table 1. Among the total number of 40 *E. coli* isolates, antimicrobial resistance was observed in 72.5% (29/40) of the strains and involved at least one of the nine tested antimicrobials. The antibiotic resistance rates differed, depending on the tested antimicrobial, strain origin (farm), and ostrich age. The highest level of antimicrobial resistance was observed for streptomycin (52.5%, 21/40), followed by tetracycline (45%, 18/40), colistin (40%, 16/40), doxycycline (37.5%, 15/40), and ampicillin (32.5%, 13/40) (Fig. 1). The *E. coli* isolates manifested a reduced resistance towards broad-spectrum antimicrobials, with significantly lower rates (p<0.001) to enrofloxacin 15% (6/40), florfenicol and amoxicillin–clavulanic acid (7.5%, 3/40). A significantly high susceptibility rate was recorded in the case of gentamicin (p < 0.001).

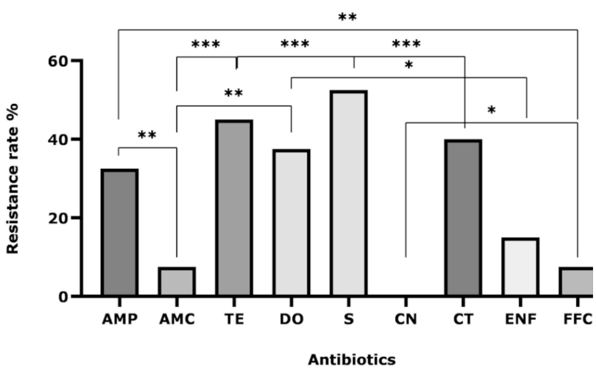


**Fig. 1. Antimicrobial resistance level of *E. coli* isolates**

Statistically significant differences were noticed betwe AMC-TE, AMC–S, AMC-CT, CN-AMP, CN-TE, CN-DO, CN CN-CT, FFC-TE, FFC-S, FFC-CT, ENF-S (***, P<0.001 AMC–AMP, AMC– DO, FFC-AMP, FFC-DO, ENF-TE (** P<0.01), ENF-DO, ENF-CN, ENF-CT (*,P<0.05)

Antimicrobial resistance was at a higher leve strains isolated from ostriches up to 5 months old t had prophylactic antibiotics (enrofloxacin and colistin) administered before this age, than in the 5-6-years-old adults. Multiple Antibiotics Resistance (MAR) index of *E. coli* isolates from ostrich faeces are presented in Table 3. The index ranged from 0.0 to 0.4, 0.0 to 0.6, and 0.0 to 0.9 for farms 1, 2 and 3 isolates, respectively. Overall, 37.5% (15/40) of the bacterial isolates displayed a high MAR index (> 0.2: Table 2).

The resistance profiles of *E. coli* included a maximum number of 8 antimicrobials, with most isolates exhibiting no susceptibility towards 2 and 4 antimicrobials (7 and 5 strains) (Table 3). From the 17 distinct resistance patterns, the most frequently observed was S-CT (17.2%, 5/29 strains), while the rates of antibiotic resistance (6.9%, 2/29 strains) were observed for TE-DO-S, TE-DO-S-CT, AMP-TE-DO-S, and AMP-TE-DO-S-ENF.

**Table 2**

**Multiple Antibiotics Resistance (MAR) Index of *E. coli* isolates**

| | Isolate | AMP | AMC | TE | DO | S | CN | CT | ENF | FFC | MAR INDEX |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Farm 1 25% (2/8) | 5 | S | S | R | R | R | S | R | S | S | 0.4 |
| | 6 | S | S | R | R | R | S | S | S | S | 0.3 |
| Farm 2 57.14% (4/7) | 4 | R | I | R | R | R | S | R | I | S | 0.6 |
| | 5 | R | S | R | R | R | S | I | R | S | 0.6 |
| | 6 | S | S | R | R | R | S | I | S | S | 0.3 |
| | 7 | R | S | R | R | R | S | S | S | S | 0.4 |
| Farm 3 36% (9/25) | 6 | R | R | R | R | R | S | R | R | S | 0.8 |
| | 7 | R | I | R | R | R | S | I | R | I | 0.6 |
| | 8 | R | I | R | R | R | S | I | I | S | 0.4 |
| | 9 | R | S | R | R | R | S | R | I | R | 0.7 |
| | 10 | R | S | R | R | R | S | R | R | R | 0.8 |
| | 11 | R | R | R | R | R | S | R | R | R | 0.9 |
| | 12 | R | R | R | I | I | S | R | R | I | 0.6 |
| | 15 | R | S | R | R | I | S | R | I | S | 0.4 |
| | 21 | S | S | R | R | R | S | R | S | S | 0.4 |
| Total no of strains 37.5% (15/40) | | | | | | | | | | | |

**Table 3**

**Antibiotic resistance patterns of *E. coli* strains**

| No of antimicrobials | Antimicrobial patterns | No of strains | Observation |
|---|---|---|---|
| 1 | AMP | 2 | |
| | S | 2 | |
| | CT | 2 | |
| | TE | 1 | |
| 2 | S-CT | 5 | |
| | S-TE | 1 | |
| | TE-DO | 1 | |
| 3 | TE-DO-S | 2 | |
| 4 | TE-DO-S-CT | 2 | |
| | AMP-TE-DO-CT | 1 | |
| | AMP-TE-DO-S | 2 | |
| 5 | AMP-TE-DO-S-CT | 1 | MDR |
| | AMP-TE-DO-S-ENF | 2 | MDR |
| | AMP-AMC-TE-CT-ENF | 1 | MDR |
| 6 | AMP-TE-DO-S-CT-FFC | 1 | MDR |
| 7 | AMP-AMC-TE-DO-S-CT-ENF | 1 | MDR |
| | AMP-TE-DO-S-CT-ENF-FFC | 1 | MDR |
| 8 | AMP-AMC-TE-DO-S-CT-ENF-FFC | 1 | MDR |
| Total | 17 | 29 | |

The most expanded resistance pattern included 8 antibiotics (AMP-AMC-TE-DO-S-CT-ENF-FFC) and was recorded in an isolate from farm 3.

Based on these results, multidrug resistance (MDR), defined as resistance towards four or more classes of antimicrobial agents (18), was found for 20% (8/40) of *E. coli* isolates (Table 3).

*Escherichia coli* is not only one of the most common bacteria of the intestinal tract in many mammal and bird species (10), but also one of the most widespread pathogens worldwide, causing serious enteric and non-enteric diseases in both animals and humans (4, 6, 11, 14, 22, 27). In terms of the zoonotic potential of *E. coli*, animals and animal-derived products are the main source of human exposure (4,6,11,14), with reports pointing out the emergence of multi-resistant and virulent *E. coli* strains (5, 6, 11, 16, 24).

In ostriches, *E. coli* was described mostly as a pathogen responsible for digestive disorders with severe course and fatal outcome (13, 22), but may also cause conjunctivitis, blepharitis, and respiratory infections (9). Keokilwe *et al.* (2015) reported enteropathogenic *E. coli*, enterotoxigenic *E. coli*, *C. perfringens* Type A and E. *S. Muenchen*, *S. Hayindongo*, and *S. Othmarschen* as the most important bacterial agents inducing enteritis and mortality in ostrich chicks less than 3 months of age (13).

Some information is available on the prevalence, virulence factors, and level of antimicrobial resistance of *E. coli* isolated from ostrich and domestic bird (chicken, turkey, quail) carcasses (5, 11, 20). Ley *et al*. (2001) reported that the rate of isolation from ostrich carcasses and antimicrobial resistance level could be very high: 91% (116/128) with strains resistant to erythromycin (98%), neomycin (66%), netilmicin (34%), oxytetracycline (34%), streptomycin (40%), and trimethoprim (13%). The prevalence rate of *E. coli* in ostrich meat (9.33%) in Iran was lower than in turkey and quail meat (23.52 and 27.77%, respectively (11), but several AMR-encoding genes were detected for ostrich strains (blaSHV (91.66%), tetA (83.33%), aac(3)-IV (83.33%), dfrA1 (41.66%), aadA1 (33.33 %), tetB (33.33%), sul1 (33.33%), qnr (16.66%), CITM (75%), cat1 (16.66%)) (11).

To the best of our knowledge, this is the first study that examined the level and characteristics of antimicrobial resistance in commensal faecal *E. coli* strains isolated from healthy farmed ostriches in Romania.

The *E. coli* carrier state was confirmed for almost all the ostrich faecal samples in this study (97.6%), and this high recovery rate was in agreement with results from 54 captive ostriches in Portugal (5).

A high percentage of *E. coli* commensal faecal strains (72.5%, 29/40) displayed resistance towards the nine tested antimicrobials. As for the antimicrobial resistance characteristics, the differences between resistance rates depended on the tested antimicrobial, the strain's origin (farm), and the ostrich's age. The highest level of antimicrobial resistance was observed against streptomycin (52.5%), while the lowest was against florfenicol and amoxicillin–clavulanic acid (7.5%). Gentamycin was the only antimicrobial effective *in vitro* against all isolates.

The antimicrobial resistance observed in 54 *E. coli* strains isolated from farmed ostriches in Portugal included: ampicillin (nine), tetracycline (seven), streptomycin (three), amoxicillin–clavulanic acid, cefoxitin, and gentamicin (one), with all the strains susceptible towards cefotaxime, ceftazidime, azthreonam, imipenem, nalidixic acid, ciprofloxacin, and trimethoprim/sulfamethoxazole (5).

Faecal *E. coli* isolates from domestic ostriches in Zimbabwe showed reduced susceptibility to ampicillin, chloramphenicol, streptomycin, oxytetracycline, and sulphonamide, with antimicrobial resistance patterns very similar to those found in free-ranging and penned chickens (26).

Tetracycline and streptomycin resistance were described in 126 *E. coli* isolates obtained from cloacal swabs of healthy ostriches in Iran (20). The highest antimicrobial resistance rates were observed for tetracycline (21.4%), followed by cefoxitin (15.9%), streptomycin (11.90%), cefazolin (8.7%), gentamicin (7.9 %), ciprofloxacin and bacitracin (6.34%) (20).

In another study, of 45 commensal strains of *E. coli*, isolated from ostriches in Iran 17.8 % were resistant to oxytetracycline, 15.5% to streptomycin, 8.9% to amoxicillin, and 4.4% to gentamicin (1). Their findings have slightly different values than the ones obtained in our study, in particular for gentamicin, for which we did not find resistant strains.

Enrofloxacin is a second-generation fluoroquinolone with high importance for the treatment of any localized or systemic infection caused by avian pathogenic *Escherichia coli* (12,17). Our study found that 15% of the *E. coli* isolates manifested resistance to this antibiotic, in comparison with higher values (28.9 %) obtained in Iran (1). This aspect is important considering it is becoming increasingly restricted through legislation, as this antimicrobial class is of critical importance for human medicine (30).

The multidrug-resistant *E. coli* strains recovered from the faecal samples in this study emerged most likely as a consequence of the antibiotics use, since several isolates displaying multiple resistance profiles were obtained from samples collected from the farm that uses antimicrobials to prevent bacterial infections in ostrich chicks less than 5 months old. This observation was supported by the multiple antibiotic resistance index values greater than 0.2 calculated for 37.5% (15/40) of the *E. coli* isolates, with both the highest number of strains and values on farms 2 and 3. A MAR index >0.2 suggests that the strains have arisen from sources of high levels of antibiotic selective pressure resulting from non-specific, misuse or abuse of antibiotics (14, 19). Similar results were reported by Amani et al. in a study conducted in Iran in 2020. Their research revealed 20.9% MDR strains from the total number of *E. coli* isolates studied (1).

Not only are the rates of antimicrobial resistance important, but so is the list of antimicrobials towards which the resistance appeared, since these antimicrobial agents are often used in food–producing animals, including poultry, and also for humans (i.e., tetracycline, colistin, doxycycline, and ampicillin).

Antibiotic usage is considered the most important factor promoting the emergence, selection, and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (21, 28). The impact of antimicrobial use is not exclusively restricted to pathogenic bacteria, but also to commensal organisms in humans and animals, and all these bacteria serve as reservoirs of resistance genes that can be transferred to bacteria pathogenic to humans (4). Faecal bacteria can be transferred to humans through the food chain (11, 16, 25).

Antibiotics are commonly used in animals for the treatment and control of bacterial infections to reduce economic losses. For farm animals, especially in intensive breeding systems, mass administration in drinking water or feed is recommended, and in certain situations, metaphylactic use of antimicrobials is accepted (28). These methods, aimed to control and prevent bacterial diseases in animals, increase the antibiotic selection pressure for resistance in bacteria and also the risk to human health from the consumption of animal-derived food products (21, 28).

## CONCLUSIONS

In conclusion, the antimicrobial resistance characterized the commensal faecal *E. coli* strains of ostrich origin, underlining the potential human health risks due to contamination of ostrich products (meat and eggs). To avoid further augmentation of the antimicrobial resistance level, rational use of antimicrobials is mandatory. One Health concept studies the complex ecology of agricultural animals, wildlife, and the environment in zoonotic transmission. Considering the high prevalence of samples in which *E. coli* was detected and the high percentage of antibiotic resistance, the impact can be considered important and part of this concept. Moreover, the feeding behaviour of ostriches, related to faeces, can lead to further contamination of the environment and humans' exposure. For all these reasons, further studies are needed to comprehensively evaluate the farmed ostrich's role as a reservoir for multidrug resistant *E. coli* strains.

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