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EVALUATION OF ANTIMICROBIAL RESISTANCE IN STRAINS OF *E. COLI* ISOLATED FROM BROILER CARCASSES

EVALUAREA REZISTENȚEI ANTIMICROBIENE A TULPINILOR *E. COLI* IZOLATE DIN CARCASE DE BROILER

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ABSTRACT

Chicken products may be a source of infection with ExPEC strains and may present a zoonotic risk through multiple antibiotic resistances. The emergence of multiple antibiotic resistances (resistance to three or more classes of antimicrobial agents) among *E. coli* strains isolated from birds has created major economic and human health problems. A total of 30 strains of *E. coli* have been isolated and identified from fresh chicken carcasses harvested at different time intervals over a period of 12 months from different manufacturers.

Strains identified as *E. coli* were tested on 12 antimicrobial substances and showed multiple resistances. The highest resistance was recorded at erythromycin and doxycycline (96.6%), and the smallest resistance was recorded at gentamicin 10%. Extraintestinal *E. coli* strains with multiple antibiotic resistances are the main cause of infections in humans and birds.

Keywords: *E. coli*, antimicrobial resistance, broiler carcasses

REZUMAT

Produsele din carne de pui pot constitui o sursă de infecție cu tulpini extraintestinale patogene de *E.coli (ExPEC)* și pot prezenta risc zoonotic prin rezistența multiplă la antibiotice. Apariția rezistenței multiple la antibiotice (rezistență la trei sau mai multe clase de agenți antimicrobieni) printre tulpinile de *E. coli* izolate de la păsări, a creat probleme majore economice și pentru sănătatea umană. Au fost izolate și identificate, un număr de 30 de tulpini de *E.coli*, provenite din carcase de pui de carne proaspete, recoltate la intervale diferite de timp, pe o perioada de 12 luni, de la diferiți producători. Tulpinile identificate ca fiind *E. coli* au fost testate la 12 substanțe antimicrobiene și au prezentat rezistență multiplă. Rezistența cea mai mare a fost înregistrată la eritromicină și doxicilină (96,6%), iar rezistența cea mai mică a fost înregistrată la gentamicină 10%. Tulpinile extraintestinale de *E. coli* cu rezistență multiplă la antibiotice, reprezintă cauza principală a infecțiilor la om și păsări.

Cuvinte cheie: *E. coli,* rezistență antimicrobiană, carcase broiler

The number of infections with pathogenic *Escherichia coli* strains (ExPEC) is increasing worldwide and is a continuous concern for human and veterinary medicine. In addition to causing diseases in humans, the extra-intestinal strains of *E. coli* are responsible for significant economic losses in the poultry industry, through mortality and carcass confiscation (3, 12).

Similarities between extra-intestinal human and avian *E. coli* strains, particularly on the basis of virulence genes, suggest that poultry products could serve as a source of infection in humans (women, newborns, the elderly, immunocompromised individuals) to whom it may cause urinary tract infections (UTI), meningitis in newborns, peritonitis and septicemia (12).

E. coli remains the main cause of urinary tract infections and nosocomial and neonatal infections.

Frequently, antibiotics are used in farms to prevent or treat microbial infections produced by ExPEC strains to reduce potential mortality losses (5, 6).

To control infections with resistant ExPEC strains, new treatment strategies are needed.

Recognizing the zoonotic risk represented by *E. coli* strains would greatly contribute to improving food safety and it would have a positive impact on human health.

Frozen or chilled, chicken and turkey meat may be contaminated with *E. coli* pathogenic strains during slaughter and cutting, being an important source of infection for people with virulent and multiple antibiotic resistant strains (2, 7).

MATERIALS AND METHODS

Thirty fresh chicken carcasses at different time intervals over a period of 12 months, from different manufacturers, marketed in hypermarkets and stored under the same conditions (4°C temperature), were studied.

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Determination of the bacterial load of broiler carcasses was performed by harvesting samples on the surface of the carcasses and skin pieces of approximately 10g of the carcass, under sterile conditions. The samples were placed in 0.9% saline solution for a ten-fold dilution of the original.

Isolation was carried out by inoculating one millilitre of the first three decimal dilutions on the Violet Red Bile Glucose Agar VRBG medium. The inoculated plates were incubated at 37°C for 24 hours. After the incubation time, the determination of the bacterial charge was performed by calculating the total number of germs (3, 9, 13).

From each VRBG agar plates, between five and ten colonies were isolated in order to identify them and perform sensitivity tests.

The strains were passed through non-selective media, incubated for 24 hours at 37°C, and were then passed onto TBX (Tryptone Bile Agar) agar and incubated at 44.5°C for 4 hours and Levine agar and incubated for 24 hours at 37°C.

After the isolation of pure bacterial strains, only beta-glucuronidase producing and indole-positive bacteria, identified as *E. coli* strains, were selected. *E. coli* strains have been differentiated from other coliform bacteria by growth and colour reaction on certain types of culture mediums.

Cultures identified as *E. coli* were also transferred to API 20E (Analytical Profile Index) and interpreted according to the methodology (1, 15).

On the TSA (Tryptic Soy Agar) agar with 0.15% bile salts and 0.03% colouring, the fixation of Congo Red was highlighted, which is an epidemiological marker designed to identify pathogenic *E. coli* strains (1, 4).

The antibiotic susceptibility testing of bacterial strains was evaluated through the Kirby-Bauer method (or diffusometric disc) using Müller-Hinton agar medium and microcompressed antibiotics provided by the producing companies (14).

This method is usually used in Romanian laboratories of bacteriology, both in farm and companion animals, to determine the sensitivity of pathogenic bacterial isolates (10).

The antimicrobial substances used were at a standard concentration as provided by the producing companies.

The following antibiotics were used: amoxicillin / clavulanic acid, olistin sulfate, doxycycline, enrofloxacin, erythromycin, florfenicol, gentamicin, lincomycin, neomycin, spectinomycin, tetracycline.

RESULTS AND DISCUSSIONS

In all 30 fresh and chilled carcasses, the bacterial contamination charge was evaluated.

The isolated bacteria have been included in the *Enterobacteriaceae* family and have been identified as *E. coli* and bacteria of different genera which have been identified through biochemical API 20E tests.

On the Levine medium, *E. coli* cultures were dark with metallic luster, or blue on TBX agar.

On the API 20 E system, the affiliation of the isolated *E. coli* strains was confirmed.

The fixation of Congo Red has been present in a number of 29 strains of *E. coli*. These strains have the ability to bind Congo Red, an acid dye, used to differentiate pathogenic strains from commensal strains.

Some researchers have considered the presence of this character associated with other properties of *E. coli* pathogenic strains as an epidemiological marker for identifying APEC strains - pathogenic avian *E. coli* strains (11, 12).

For the sensitivity testing, 30 strains of *E. coli* were isolated and identified. Each bacterial strain was considered susceptible, intermediately susceptible or resistant to a particular antibiotic.

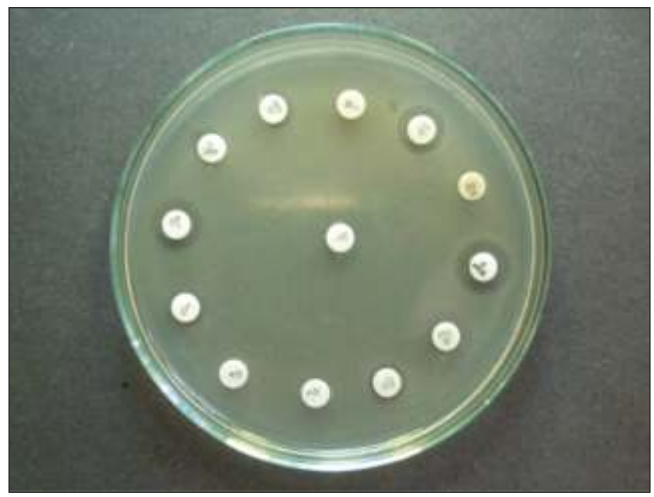

Fig.1. Kirby Bauer *E. coli* disc diffusimetric antibiogram - multiple resistance

To monitor the circulation of *E. coli* strains, it is necessary to monitor the resistance phenomenon of APEC strains, these being considered epidemiological markers.

E. coli strains, not exposed to antibiotic pressure, are susceptible to these substances; instead, *E. coli* strains isolated from birds and mammals, from intensive growth, subjected to antibiotic pressure, exhibit

Table 1

The antimicrobial resistance of 30 *E. coli* isolates

Antibiotics	Sensitive		Intermediate		Resistant	
	No.strains	%	No.strains	%	No.strains	%
Amoxiclav 20/10 µg	0	0	16	53.3	14	46.6
Colistin sulfat10 µg	2	6.6	17	56.6	11	36.6
Doxycyline 30 µg	0	0	1	3.3	29	96.6
Enrofloxacin 5 µg	3	10	7	23.3	20	66.6
Erythromycin15 µg	0	0	1	3.3	29	96.6
Florfenicol 30 µg	18	60	2	6.6	10	33.3
Gentamicin 10 µg	11	36.6	16	53.3	3	10
Lincomycin 10 µg	0	0	6	20	24	80
Neomicyn10 µg	0	0	3	10	27	90
Spectinomycin 10 µg	11	36.6	3	10	16	53.3
Streptomicin 10 µg	0	0	10	33.3	20	66.6
Tetracycline 30 µg	1	3.3	2	6.6	27	90

multiple resistance phenomena as a result of frequent exposure to antibiotics.

The results of the antibiotic resistance of the studied *E. coli* strains are shown in Table 1 and Figures 1 and 2.

The results show that these strains have been shown to be resistant to one or more of the antibiotics used. Analysing these results, it is noted that against 12 antimicrobial substances, the tested strains had a resistance of between 10% and 96.6%.

The highest resistance was recorded for erythromycin and doxycycline (96.6%), followed by resistance to neomycin and tetracycline (90.0%), then resistance to lincomycin (80.0 %), enrofloxacin and streptomycin (66.6%), spectinomycin (53.3%).

Only 46.6% of the tested strains were resistant to amoxicillin / clavulanic acid, compared to 36.6% to colistin sulphate.

In the case of florfenicol, the resistance was 33.3%, and the resistance phenomenon was reported for only 10% of the strains tested against gentamicin.

Our results are similar to those in the literature that mention susceptibility to antimicrobial agents as follows: *E. coli* strains were 100% resistant to quinolones (enrofloxacin and ciprofloxacin); to beta-lactam antibiotics, *E. coli* showed a different resistance, thus reaching 60% for ampicillin, 11.25% for cefazolin and amoxicillin with clavulanic acid, cefotaxime and cefoxitin (8, 9, 11).

The multiple resistances to three or more antimicrobial agents were identified in eight *E. coli* strains, that represent 26.6%, these results being similar to

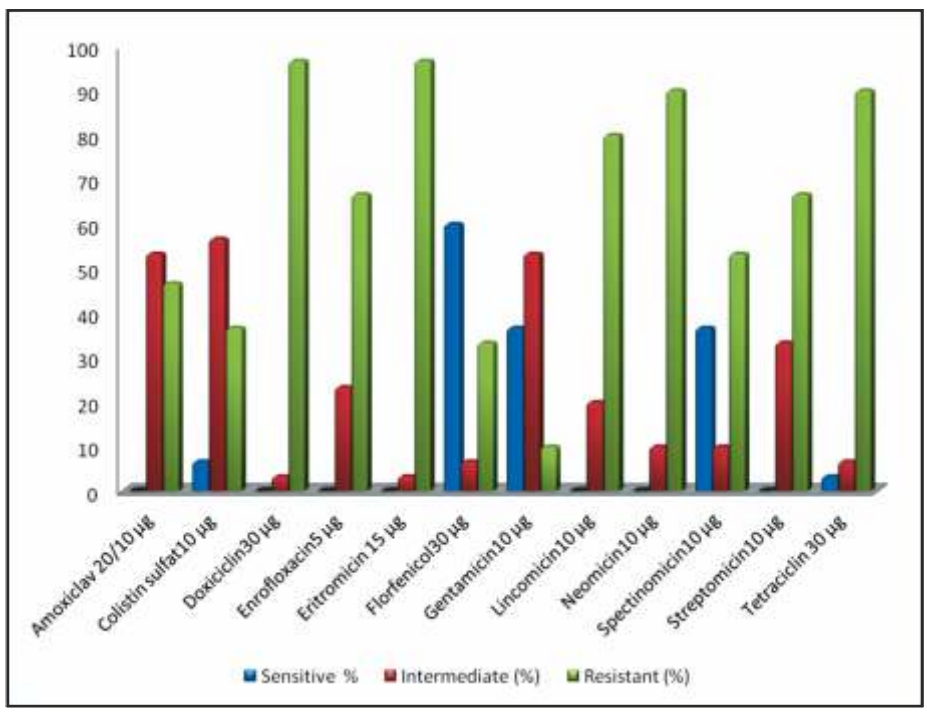

Fig. 2. Resistance to antimicrobial substances of *E. coli* strains

those in the literature (2, 3, 12).

The presence of antibiotic-resistant *E. coli* strains can lead to contamination of poultry products, which could increase the risk of transferring these bacteria or antibiotic resistant genes to humans.

The frequent use of antibiotics in the poultry industry and the increase in poultry meat consumption could have led to the emergence and spread of antibiotic resistance, which is a serious concern for animal and human health.

CONCLUSIONS

A number of 30 strains of *E. coli* were isolated and identified from fresh and chilled carcasses of chicken. The fixation of Congo Red has been present in a number of 29 strains of *E. coli*, this demonstrating pathogenic potential. The *E. coli* strains showed multiple resistances to three or more antimicrobial agents in 26.6% cases. The highest resistance was recorded to erythromycin and doxycycline (96.6%), and the smallest resistance was recorded to gentamicin 10%.

Extra-intestinal *E. coli* strains with the highest resistance and multiple antibiotic resistances are the main cause of infections in humans and birds.

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