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ANTIBIOTIC SENSITIVITY PROFILE OF *E. COLI* ISOLATES FROM POULTRY FLOCKS

PROFILUL SENSIBILITĂȚII LA ANTIBIOTICE A TULPINILOR DE *E. COLI* ÎN EFECTIVE DE PĂSĂRI

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ABSTRACT

Resistance to antibiotics is a problem of public health around the world, consistent with the emergence of many resistant bacteria. Animals are one of the sources for antibiotic resistance bacteria that can be transferred to humans. Aim of the study: Determination of the antimicrobial susceptibility profile of *Escherichia coli* strains from flocks belonging to a unit integrated consumer egg production. Materials and Methods: A number of 27 *E. coli* strains isolated from colibacillosis outbreaks or obtained by routine surveillance were tested for antibiotic susceptibility. Results: The samples were divided into four categories according to their origin: breeding of light breeds, incubation, youth and laying hens. Isolated strains were resistant to spectinomicin, flumequin, erythromycin, trimethoprim, oxytetracycline, ampicillin, amoxicillin, enrofloxacin, doxicillin and sensitive to fosfomycin + tylosin, ceftiofur, lincomycin + spectinomycin, colistin, gentamycin, florfenicol and neomycin. Conclusion: Although antibiotics are used to a lesser extent in chicken flocks for egg consumption compared to poultry meat production, this study found the resistance of *E. coli* strains to different antibiotics and multi-drug resistant strains were identified as well.

Keywords: *Escherichia coli,* colibacillosis, antibiotic susceptibility, antibiotic multi-resistance

REZUMAT

Rezistența față de antibiotice reprezintă o problemă de sănătate publică în întreaga lume, pe măsura apariției în permanență a numeroase bacterii rezistente. Animalele reprezintă una din sursele pentru bacterii antibiorezistente ce pot fi transferate la om. Scopul studiului: Determinarea profilului de sensibilitate antimicrobiană a tulpinilor de *Escherichia coli* din efective de păsări ce aparțin unei unități integrate de producere a ouălor de consum. Materiale și metode: Un număr de 27 tulpini *E. coli* izolate din focare de colibaciloză sau obținute prin supravegherea de rutină au fost testate pentru sensibilitatea la antibiotice. Rezultate: Probele au fost împărțite în patru categorii în funcție de originea lor: reproducție rase ușoare, incubație, tineret și găini ouătoare. Tulpinile izolate au fost rezistente la spectinomicină, flumequin, eritromicină, trimetoprim, oxitetraciclină, ampicilină, amoxicilină, enrofloxacin, doxiciclină și sensibile la fosfomicină + tilozină, ceftiofur, lincomicină + spectinomicina, colistină, gentamicină, florfenicol și neomicină. Din toate tulpinile de *E. coli*, 55,6% au fost rezistente la mai multe antibiotice. Concluzie: Deși antibioticele sunt folosite într-o măsură mai mică în efectivele de găini pentru ouă de consum, comparativ cu cele pentru producția de carne de pasare, în acest studiu s-a constatat rezistența tulpinilor de *E. coli* față de diferite antibiotice și au fost identificate și tulpini multirezistente.

Cuvinte cheie: *Escherichia coli*, colibaciloză, sensibilitate la antibiotice, rezistentă la antibiotice

Escherichia coli is a commensal gram-negative bacillus of the vertebrates' digestive tracts, with several pathogenic strains involved in various intra and extra intestinal infections (2, 8, 20). In poultry intestine, *E. coli* is also considered a commensal bacterium; however avian pathogenic strains, called APEC, are able to produce systemic fatal disease (8, 18). Despite being known for over a century, avian colibacillosis remains one of the major endemic diseases afflicting the poultry industry worldwide (5).

Over the last decades, the number of multi-drug resistant bacteria increased dramatically, and the irresponsible use of antibiotics is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (4, 10, 16, 17, 21).

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World Health and Life Science institutions are concerned about a range of deleterious effects that antimicrobial resistant bacteria may have on human health, like increased duration of illness, treatment failure, and loss of therapeutic options as a consequence of human exposure to resistant bacteria through ingestion of animal derived food products (14).

Most APEC strains do not possess zoonotic potential, but the risk of human infection has been demonstrated for certain strains and their plasmids (7). Avian colibacillosis is caused by different types of *E. coli*. They do not fit in the *E. coli* classification used for mammals: entero-invasive (EIEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), verotoxigenic (VTEC/STEC), enterohaemorrhagic (EHEC), enteroadherent (EAEC), or nephrotoxigenic *E. coli* (NTEC). For this reason, they are referred to as avian pathogenic *E. coli* (APEC). This notwithstanding, no markers have been identified allowing a clear differentiation between APEC and commensal *E. coli*, in spite of extensive research in this area (20).

Avian colibacillosis can evolve as an acute systemic disease with significant economic losses in poultry farms (6), with a complex syndrome characterized by multi-organ lesions, such as: airsacculitis, pericarditis, perihepatitis, peritonitis (6, 18), coligranulomatosis, enteritis, yolk sac infection, omphalitis, swollen head syndrome, cellulitis, salpingitis, respiratory tract infection (8), septicaemia and other extraintestinal diseases (3).

Chicken challenge experiments proofed the pathogenic properties of the virulent APEC strains as the single etiological agent (6), but APEC are usually in the intestinal microflora of healthy birds and the diseases are secondary to viral, environmental, and host predisposing factors (3, 6). In this regard, Kabir et al. (2010) postulated that "APECs are responsible for a considerable number of different clinical illnesses at different ages. The avian colibacillosis was found widely prevalent in all age group of chickens (9.52 to 36.73%) with the highest prevalence rate in adult layer birds (36.73%)" (8). Also, in the pathogenesis and disease syndrome description, Vandekerchove (2004) argues that "Neonatal infection of chicks can occur horizontally, from the environment, or vertically, from the hen. A laying hen suffering from *E. coli* induced oophoritis or salpingitis may infect the internal egg before shell formation. Faecal contamination of the eggshell happens during the passage of the egg through the cloacae or after laying" (20).

The control of APEC infections targets the risk factors (e.g. environmental contamination, microclimate parameters such as humidity and ventilation), antibiotic therapy (still widely used, even if APEC are frequently multi-drug resistant), vaccination with killed or attenuated virulent bacteria (not widely practiced) (3).

The aim of the study was to assess different strains of *E. coli* concerning their antimicrobial resistance in laying hens. The flocks were different by their genetics (layer breeders and layers) and by age (day old chicks, rearing and adult layers/breeders).

MATERIALS AND METHODS

The study was carried during a period of 15 months on poultry flocks in Romania. The flocks from this study belong to an integrated unit and represented four categories: layer breeders, hatchery, layer rearing and layers (commercial adult layers). Layer breeders' flocks were aged 45-48 weeks old during the study. The hatchery comprised day old chicks coming from two groups of layer breeder, observed on this study between 36-46 weeks old (young flocks) and 83-90 weeks old (old flocks). The rearing flocks ranged between 5 and 46 days of age. The layers comprised three farms A, B and C. The first layer farm (A) had white hybrid and it was multi-age, having flocks ranging from 24 weeks to 139 weeks during this study. The second layer farm (B) was also multi-age farm with ages from 58 weeks to 125 weeks but had brown hybrid. The last farm (C) comprised single age brown hybrid followed through the period of 45 weeks to 84 weeks of age.

A number of 27 *E. coli* strains, isolated from, were tested for their antibiotic susceptibility patterns.

The strains have been isolated from tissues / organs, corpses, dead-in-shell embryos, day old culled chicks, dying birds. The samples were individually labelled, packed and transported refrigerated or as such to the laboratory no later than 24 hours after sampling. Isolation and identification of *E. coli* strains was according to published techniques – cultivation on culture media special for *Enterobacteriaceae* (MacConkey agar) and then identified based on biochemical characteristics. In vitro antibiotic activities to different antibiotic molecules of the isolates were determined by the disc diffusion method (Bauer-Kirby), as agreed

Table 1

The dynamic of antibiotic sensitivity in layer breeder flocks

Age (weeks)	SPT	TMP	FLO	CST	CIP	ENR	NOR	DOX	OTC
45	R	R	S		S	S			R
48		S	S	S					
49	R	R	S		S	S	R	R	R

R = resistant; I = intermediate (moderate sensitive); S = sensitive; blanc = no test

by Food and Drugs Administrations and World Health Organization. Interpretation of the results was done as prescribed by the standardized procedures for antibiograms.

The antibiotic discs (OXOID Limited, Basingstoke, Hampshire, England) used in this study and their concentration were: ceftiofur (CEF) 30µg, gentamicin (GEN) 10 µg, neomycin (NEO) 30 µg, ampicillin (AMP) 10 µg, amoxicillin (AMX) 25 µg, erythromycin (ERY) 15 µg, florphenicol (FLO) 30 µg, colistin (CST) 10 µg, flumequin (FLU) 30 µg, enrofloxacin (ENR) 5 µg, doxycycline (DOX) 30 µg, oxytetracycline (OTC) 30 µg, spectinomycin (SPT) 25 µg, trimethoprim (TMP) 5 µg, ciprofloxacin (CIP) 5 µg and norfloxacin (NOR) 10 µg. The fosfomycin+tylosin (FOS+TYL) 250 µg discs were acquired from BEDSON Espana SL, Campanillas (Malaga), Spain and the lincomycin+spectinomycin (LCM +SPT) 109 µg discs were acquired from Laboratoris CONDA, Madrid, Spain.

RESULTS AND DISCUSSIONS

The poultry flocks belong to four breeding categories: layer breeders, hatchery, layer rearing and layers (adult layers). All the farms (including hatchery) and flocks were part of an integrated unit.

I. Layer breeders

The layer breeder flocks consisted in 3 barns (A, B and C) at the same age. Several episodes of clinical colibacillosis were observed during a period of two months (ages 45-49 weeks).

At the age of 45 weeks the first episode of colibacillosis breaks since the moment of transfer to the production farm. Mortality reached 2.4% in barn A and 1.1% in barn C. At laboratory examination *E. coli* was isolated from the bone marrow; the strain was found sensitive to FLO, ENR, CIP and resistant to SPT, NEO, OTC and TMP.

At the age of 48 weeks another episode of colibacillosis was registered. Mortality peaked 2.4% at barn A and 3.0% at barn C. The laboratory isolated *E. coli* from the bone marrow; it was sensitive to FLO, TMP, NOR, CIP and with moderate sensitivity to OTC and DOX.

At 49 weeks age, barns B and C showed colibacillosis with mortality 1.4%, respectively 3%. Laboratory findings revealed *E. coli* isolated from the bone marrow with sensitivity to FLO, ENR, CIP and resistance to TMP, NOR, DOX, OTC and SPT.

Although the three layer breeder barns were located on the same farm and had the same age and origin, different evolution of colibacillosis episodes was found. Barn C was the most affected by colibacillosis outbreaks and, unexpectedly, barn B which was situated between the two other barns experienced only one episode with moderate mortality.

The dynamics of the evolution of antibiotic sensitivity in the layer breeder flocks for *E. coli* strains varied for some antibiotics while for others remained unchanged (Table 1). For streptomycin resistance was noticed, for FLO, CIP and ENR sensitivity was seen. For TMP, NOR, DOX and OTC different results of sensitivity were noticed. It is noteworthy to mention that *E. coli* strains changed their antibiotic phenotype from sensitive to resistant (NOR) or from resistant to sensitive and back again resistant (TMP). In day old chicks (layer breeder) housed on two rearing barns mortality above technology was registered in the first week of life. The laboratory findings revealed *E. coli,* isolated from brain and yolk sac; the strain was sensitive to FLO, ENR, NOR and resistant against DOX, OTC and SPT.

II. Hatchery samples

The investigated samples from hatchery gathered from hatchings during one spring season (3 months) as a routine testing. They included dead in shell and culled hatchlings.

The first hatch came from eggs provided by 36 weeks old layer breeders. The hatch performance was 83.9% compared with the 88.0% expected.

Table 2

The dynamic of antibiotic sensitivity in hatchery samples coming from young flock

Age (weeks)	GEN	NEO	AMP	AMX	ERY	FLO	CST	FLU	ENR	DOX	OTC	FOS+ TYL	LCM+ SPT
36	S	I	R	R	R		S	R	I	S	I		S
41	S	S	R	R	R	I	S	R	R	S	I	S	
43		S	R	R	I		S	R	R	S	S	S	S
46	R	S	R	R	R	S	S	R	R	I	I	S	S

R = resistant; I = intermediate (moderate sensitive); S = sensitive; blanc = no testing

Strains isolated from the yolk sac and identified an *E. coli* strain sensitive to GEN, CST, LCM+SPT, DOX, moderately sensitive to NEO, ENR, OTC and resistant to FLU, AMX, AMP and ERY.

The second hatch comes from laying hens aged 41 weeks. Hatch performance was 81.5% compared with the 88% expected hatch. The laboratory findings revealed the presence of *E. coli* sensitive to FOS+TYL, CEF, GEN, NEO, CST, DOX, moderately sensitive to FLO, OTC and resistant to FLU, ENR, AMX, AMP and ERY.

At the third hatch (43 weeks breeder age) the performance was 83.2% compared with the 88.0% expected.*E. coli* was isolated from the yolk sac; the strain was sensitive to CEF, GEN, NEO, CST, FOS+TYL, LCM +SPT, OTC, DOX, had moderate sensitivity to ERY and was resistant against ENR, AMX, AMP and FLU.

The fourth belongs to the breeders of 46 weeks age. Hatch result was 81.6% compared with the 87.0% expected. *E. coli* was isolated from the yolk sac; it showed sensitivity for CEF, NEO, CST, FOS+ TYL, FLO, LCM+SPT, moderate sensitivity for DOX and OTC and resistance against GEN, FLU, ENR, AMX, AMP and ERY.

The hatching performance of the young breeder flocks were below the expected ones, on average with 5.2% lower than predicted. Among the reasons behind the modest results were hatching eggs production lower by 7.5% on average than foreseen and the multistage type of setters from the hatchery.

The dynamics of the antibiotic sensitivity in samples coming from hatchery registered variation (Table 2). For CST and for the two combinations of antibiotics (FOS+TYL and LCM+SPT) sensitivity was seen whilst for AMP, AMX, FLU resistance was noticed. Only for GEN the sensitivity was lost. For NEO and DOX sensitivity was seen for 75.0% of the *E. coli* strains. For ERY and ENR resistance was noticed for 75.0% of the tested strains.

There were also some hatches from older breeder flocks. From breeders aged 83 weeks the hatch results was 73.4% compared with the 76.0% expected. The *E. coli* was isolated from the yolk sac: it was sensitive to CEF, CST, AMX, it had moderate sensitivity for ENR, LCM+SPT and it had resistance against FLU, DOX, OTC and ERY. Another hatch sampled (breeder's aged 90 weeks) had hatch performance of 62.3% compared with the 70.0% expected. *E. coli* was isolated on laboratory findings and it was sensitive to GEN, CST, LCM+ SPT, it had moderate sensitivity to NEO and it was resistant against ENR, FLU, OTC, DOX, AMX and AMP.

The old breeder flocks were treated separately because of their different location and the data were scarcer than for young breeder flocks. Sensitivity for CEF, GEN, CST, FOS+TYL was observed (Table 3). Resistance against AMP, FLU, DOX and OTC was noticed. For the others antibiotics tested namely AMX, ERY and ENR different result were found.

III. Layer rearing flocks

In the rearing farms for commercial layers samples were taken (dead or culled birds) from flocks with different ages usually when clinical problems emerged.

The samples were gathered during a period of 5 months in a multi-age rearing farm.

Two rearing flocks aged 46 days presented clinica-

Table 3

The dynamic of antibiotic sensitivity in hatchery samples coming from old flocks

Age (weeks)	GEN	NEO	AMP	AMX	ERY	CST	FLU	ENR	DOX	OTC	FOS+ TYL	LCM+ SPT
83				S	R	S	R	I	R	R		I
90	S	I	R	R	I	S	R	R	R	R	S	S

R = resistant; I = intermediate (moderate sensitive); S = sensitive; blanc = no testing

Table 4

The dynamic of antibiotic sensitivity in layer rearing flocks

Rearing flock	Age (days)	GEN	NEO	AMP	AMX	ERY	FLO	CST	ENR	DOX	OTC	FOS+ TYL	LCM+ SPT
#1	46	S	S					S	S			S	S
#2	17	S	S					R		I	R	S	S
#3	5	S	S					S					S
#4	42	S	S	I	I	R	S	S	I	S		S	S
#5	42	S	S	S	S	R	S	S	S	S	R	S	S

R = resistant; I = intermediate (moderate sensitive); S = sensitive; blanc = no testing

lly respiratory signs. On gross pathology, aerosacculitis and catarrhal bronchopneumonia were found. Samples were taken and *E. coli* was isolated from bone marrow and lung. The strain was sensitive to GEN, NEO, CST, LCM+TMP, ENR and FOS+TYL.

One rearing flock aged 17 days had clinical signs of listlessness and loss of appetite. At laboratory examination there were found gross lesions of kidney hypertrophy, urates deposits in ureters and hepatosis. Mortality reached 4.8%. *E. coli* was isolated from the bone marrow and it was sensitive to GEN, NEO, LCM+ SPT, FOS+TYL, moderately sensitive to DOX and resistant against CST and OTC.

Another rearing flock aged 5 days was routinely tested. The *E. coli* was isolated from the yolk sac. The isolate was sensitive to CEF, GEN, NEO, CST, and LCM +SPT. The same flock was sampled at 42 days, facing a clinical episode of coccidiosis with mortality 1.4%. The laboratory findings revealed *E. coli* isolated from the spleen, liver, intestine and bone marrow.

The strain was sensitive to CEF, GEN, NEO, CST, DOX, LCM+SPT, FOS+TYL, FLO, it had moderate sensitivity for ENR, AMX, AMP and it was resistant against ERY.

Another flock having the same age of 42 days and with clinical coccidiosis as well, was sampled and *E. coli* was isolated from bone marrow, heart and caecum. The isolated strain presented sensitivity for GEN, NEO, CST, DOX, LCM+SPT, FOS+TYL, FLO, ENR, AMX, AMP and resistance against ERY and OTC.

The sensitivity or resistance of *E. coli* strains to different antibiotics varied from flocks on the rearing farm (Table 4). Sensitivity for GEN, NEO, FLO and antibiotic combinations (LCM+SPT, FOS+TYL) was noticed. Resistance against ERY and OTC found. For CST sensitivity was observed except for one flock aged 17 days where resistance was seen. For AMP, AMX, ENR and DOX a mix between sensitivity and moderate sensitivity was observed.

IV. Commercial layers

Three farms of commercial layers were studied. The farms had different locations and also layer hybrid (brown, white). Two farms were multi-age, and one farm was single age.

a. The first farm of layers (white hybrid) comprised two groups of age

The old flocks (two barns A and B) had multiple episodes of colibacillosis throughout the production period.

At 88 weeks age a clinical episode of colibacillosis was suspicioned based on the mortality (0.98% barn A and 0.94% barn B) and the necropsy findings. *E. coli* was isolated from bone marrow and it was sensitive to GEN, NEO, LCM+SPT, FOS+TYL, moderately sensitive to ERY, OTC, ENR, FLU and resistant to AMX, AMP and DOX.

At the age of 136 weeks an episode of staphylococcosis was registered that was complicated secondarily by colibacillosis. The *E. coli* isolate was sensitive to GEN, NEO, CST, LCM+SPT, FOS+TYL, FLO, moderately sensitive to DOX, AMX and resistant to OTC, AMP, ERY and FLU. Cumulative mortality for both staphylococcosis and colibacillosis reached 3.6% (barn A) and 2.3% (barn B).

At 139 weeks age the flock housed in barn A experienced another episode of colibacillosis with 1.4% mortality. *E. coli* was isolated from bone marrow. The strain had sensitivity for GEN, NEO, CST, LCM+SPT, moderate sensitivity for ENR, DOX, OTC and resistance against AMX, AMP and ERY.

The *E. coli* strains found on layer farm 1 were sensitive to some antibiotics namely GEN, NEO, FLO, CST and antibiotic combinations (FOS+TYL, LCM+SPT) but resistant against AMP.

For others antibiotics like AMX, ERY, FLU, ENR, DOX and OTC there was an intermix of resistance and moderate sensitivity (Table 5).

Table 5

The dynamic of antibiotic sensitivity in commercial layers (farm 1)

Age (weeks)	GEN	NEO	AMP	AMX	CEF	ERY	FLO	CST	FLU	ENR	DOX	OTC	FOS+ TYL	LCM+ SPT
88	S	S	R	R		I			I	I	R	I	S	S
136	S	S	R	I	S	R	S	S	R		I	R	S	S
139	S	S	R	R		R		S		I	I	I		S

R = resistant; I = intermediate (moderate sensitive); S = sensitive; blanc = no testing

The group of young flocks presented problems since rearing period (aspergillosis, coccidiosis, and colibacillosis). At 24 weeks of age unusually high mortality was reached (1.4% barn C and 3.6% barn D). Necropsy revealed wasting in a context of low body weight and secondary *E. coli* infections. *E. coli* isolated from intestine,ovary and salpinx was sensitive to GEN, CST, NEO, FOS+TYL, LCM+SPT, AMX and AMP.

b. The second layer farm comprised multiage flocks (brown hybrid)

An old barn aged 125 weeks register one colibacillosis episode with mortality 3.0%. *E. coli* was isolated, and it was sensitive to GEN, NEO, CST, LCM+SPT, FOS+TYL.

Other two barns (A and B) in the farm 58 weeks of age faced an episode of colibacillosis with mortality 0.85% and 0.76%.Laboratory findings revealed *E. coli* with sensitivity to NEO, ENR, AMX, AMP, OTC and resistance against ERY and FLU. At the age of 102 weeks the same two barns have developed colibacillosis with mortality 1.3% for each barn. *E. coli* was isolated from bone marrow and heart and it was sensitive to GEN, LCM+SPT, CST, NEO, FOS+TYL, DOX, it was moderately sensitive to AMX, AMP and it was resistant against OTC, ERY, ENR. At the age of 106 weeks colibacillosis relapsed. *E. coli* was found at laboratory investigation; the strain was sensitive to GEN, CST, LCM +SPT, FOS+TYL, moderately sensitive to ENR, OTC, AMP, NEO and resistant against ERY.

On the commercial layer farm 2 which had the same location with layer farm 1 some similarities with farm 1 regarding antibiotic susceptibility of *E. coli* strains can be noticed but also some differences (Table 6).

For GEN, NEO (with one exception), CST and antibiotic combinations (FOS+TYL, LCM+SPT) sensitivity was noticed like in farm 1. For DOX sensitivity was noticed. The evolution of *E. coli* susceptibility during the period of 58-106 weeks altered in the sense that the strains changed their antibiotic phenotype from sensitive to resistant. This is the case for ENR and OTC for which sensitivity was noticed at 58 weeks of age, then resistance was seen at 102 weeks of age and moderate sensitivity was recorded at the sampling from 106 weeks. For AMP and AMX a mix of sensitivity and moderate sensitivity is noticed.

c. The third layer farm comprised single age flocks (brown hybrid)

First isolation of *E. coli* appeared on barns A and B at the age of 45 weeks on routine checking. *E. coli* was isolated from salpinx, liver and bone marrow.

The strain was sensitive to GEN, CST, FOS+TYL, FLO, LCM+SPT, was moderate sensitive to AMX, AMP, NEO and resistant against ENR, FLU, DOX, OTC, ERY.

At the age of 60 weeks an episode of staphylococcosis erupted and then got complicated with colibacillosis. Mortality peaked at 1.65% in barn A and 0.6% in barn B. *E. coli* was isolated from bone marrow and it had sensitivity to FOS+TYL, FLO, LCM+SPT, CST and resistance against ENR, OTC, DOX, and NEO.

Barn C presented an episode of colibacillosis at the age of 76 weeks with mortality 0.6%. The laboratory isolated *E. coli* from bone marrow; the strain was sensitive to LCM+SPT, GEN, CST, FOS+TYL, moderately sensitive to DOX and resistant against OTC, AMP, AMX, ERY, FLU.

Table 6

The dynamic of antibiotic sensitivity in commercial layers (farm 2)

Age (weeks)	GEN	NEO	AMP	AMX	ERY	CST	ENR	DOX	OTC	FOS+ TYL	LCM+ SPT
58		S	S	S	R		S		S		
102	S	S	I	I	R	S	R	S	R	S	S
106	S	I	I		R	S	I	S	I	S	S

R = resistant; I = intermediate (moderate sensitive); S = sensitive; blanc = no testing

Table 7

The dynamic of antibiotic sensitivity in commercial layers (farm 3)

Age (weeks)	GEN	NEO	AMP	AMX	ERY	FLO	CST	FLU	ENR	DOX	OTC	FOS+ TYL	LCM+ SPT
45	S	I	I	I	R	S	S	R	R	R	R	S	S
60		R				S	S		R	R	R	S	S
76	S		R	R	R		S	R		I	R	S	S
84	S	S	R		I				S		R	S	S

R = resistant; I = intermediate (moderate sensitive); S = sensitive; blanc = no testing

At 84 weeks of age routine samples were taken to the laboratory. From barn C, *E. coli* was isolated from joints and bone marrow. The strain presented sensitivity to ENR, GEN, NEO, LCM+SPT, FOS+TYL, moderate sensitivity to ERY and resistance against AMX, AMP, and OTC.

The dynamic of events related to *E. coli* strains isolated in layer farm 3 during a period of 9 months, reveals routine checking at 45 and 84 weeks of age (dead birds from technological mortality), one colibacillosis episode at 76 weeks of age and a combination of staphylococcosis and colibacillosis at 61 weeks of age.

Layer farm 3 was located in a different region than farms 1 and 2. It was a single age layer farm. Sensitivity of *E. coli* strains was noticed for GEN, FLO, CST and antibiotic combinations (LCM+SPT, FOS+TYL). Resistance was noticed for AMP, AMX, ERY, FLU, DOX and OTC (Table 7). For NEO and ENR it was noticed that *E. coli* strains changed their antibiotic phenotype from resistant to sensitive. A very interesting fact to mention was that in general there was more overall resistance to antibiotics compared with farms 1 and 2 despite the fact that farm 3 was a single age farm and isolated from the other farms. Another noticeable matter was that for some antibiotics, i.e. NEO and ENR, a change in antibiotic phenotype of *E. coli* strains was seen from resistant in the beginning to sensitive on the last sampling (age 84 weeks).

In this study a total number of 27 *E. coli* strains were isolated from the farms including hatchery. From the whole number of strains layer breeder counted 4 (14.8%) strains, hatchery 6 (22.2%) strains, rearing farm 5 (18.5%) strains and layer farms 12 (44.4%) strains.

By analysing the dynamic of antibiotic sensitivity in all *E. coli* investigated in this study (Table 8), it can be seen that the highest resistance against antibiotics was seen in hatchery isolates and the ones from farm layer 3 and the highest sensitivity was noticed in the rearing isolates. GEN sensitivity was noticed, except for one isolate in the hatchery which was resistant. NEO was also sensitive, except one isolate from layer farm 3. CST as well was sensitive, except one isolate from the rearing farm. For the combinations of antibiotics (LCM+SPT, FOS+TYL), sensitivity was noticed. FLO, CEF and CIP were tested fewer times for antibiotic susceptibility and sensitivity was seen. Resistance was high for FLU, ERY, OTC, AMP and AMX. SPT was found to have high resistance as well but it was tested only a few times.

One noticeable matter was that for some antibiotics a change in antibiotic phenotype of *E.coli* strains was seen from resistant to sensitive or vice-versa. This observation requires genotypical investigation of the *E. coli* strains in terms of not only the virulence / resistance factors but also for pathotype review.

DISCUSSIONS

Antimicrobial resistance in *Enterobacteriaceae* has increased dramatically over the last decade as a consequence of the extensive usage of antimicrobials in treating both human and animal infections (12). Antibiotic resistance studies have been conducted around the world, in several types and categories of poultry farms. Khodadadi *et al.* (2013) studied the difference in antibiotic resistance in broiler breeder and broiler farms and found that resistance to different antimicrobial agents (FLO, ENR, DOX, TMP) in broiler farms (ranging between 51.4% to 94.3%) was higher than broiler breeder farms (ranging from 23.5% to 58.8%). The study also revealed high sensitivity for both categories of farms for FOS/TYL (9).

Breeder poultry may represent an important reservoir of pathogenic *E. coli* strains and subsequent colibacillosis in newly hatched broiler chicks. A study carried on broiler hatching eggs gathered from different hatcheries in Belgium,the Netherlands and France (11) found that approximately one third of *Entero-*

Table 8

Dynamic of antibiotic sensitivity in poultry flocks from an integrated unit

Category	Age (weeks)	SPT	GEN	NEO	AMP	AMX	CEF	TMP	ERY	FLO	CST	FLU	CIP	ENR	NOR	DOX	OTC	FOS+TYL	LCM+SPT
Layer breeder	45	R						R		S			S	S			R		
	48							S		S	S				S	I	I		
	49	R						R		S			S	S	R	R	R		
	day old	R								S				S	S	R	R		
Hatchery samples	36		S	I	R	R			R		S	R		I		S	I		S
	41		S	S	R	R			R	I	S	R		R		S	I	S	
	43		S	S	R	R			I		S	R		R		S	S	S	S
	46		R	S	R	R			R	S	S	R		R		I	I	S	S
	83					S			R		S	R		I		R	R		I
	90		S	I	R	R			I		S	R		R		R	R	S	S
Layer rearing	46 days		S	S							S			S				S	S
	17 days		S	S							R					I	R	S	S
	5 days		S	S			S				S								S
	42 days		S	S	I	I			R	S	S			I		S		S	S
	42 days		S	S	S	S			R	S	S			S		S	R	S	S
Layers farm 1	88		S	S	R	R			I			I		I		R	I	S	S
	136		S	S	R	I	S		R	S	S	R				I	R	S	S
	139		S	S	R	R			R		S			I		I	I		S
	24		S	S	S	S	S				S							S	S
Layers farm 2	125		S	S			S				S							S	S
	58			S	S	S			R			R		S			S		
	102		S	S	I	I			R		S			R		S	R	S	S
	106		S	I	I				R		S			I		S	I	S	S
Layers farm 3	45		S	I	I	I			R	S	S	R		R		R	R	S	S
	60			R						S	S			R		R	R	S	S
	76		S		R	R	S		R		S	R				I	R	S	SS
	84		S	S	R	R			I					S			R	S	S
Resistant (%)		100	5.0	4.8	58.8	52.9	0	66.7	76.5	0	0	90.9	0	35.0	33.3	35.0	59.1	0	0
Intermediate (%)		0	0	19.0	23.5	23.5	0	0	23.5	9.1	4.8	9.1	0	30.0		30.0	31.8	0	4.8
Sensitive (%)		0	95.0	76.2	17.7	23.6	100	33.3	0	90.9	95.2	0	100	35.0	66.7	35.0	9.1	100	95.2
Strains tested (no.)		3	20	21	17	17	5	3	17	11	21	11	2	20	3	20	22	18	21

bactoriaceae (mostly *E. coli*) isolates showed resistance to AMP, tetracycline, trimethoprim and sulphonamides. On the contrary, most isolates were susceptible to ceftiofur, nitrofurantoin, the aminoglycosides, and florfenicol. For the layer breeder flocks in this study, it was observed that the strains of *E. coli* isolated were sensitive to FLO (100%), CST (100%), CIP (100%), ENR (100%), NOR (66.7%) and resistant against SPT (100%), OTC (75%), DOX (66.7%) and TMP (66.7%). Of all *E. coli* isolates from layer breeder flocks, 75% were multidrug resistant (resistance to ≥ 3 antimicrobials). *E. coli* isolates from day old layer breeder were already resistant against SPT, DOX and OTC, resistance which was noticed also in the existing layer breeder flocks within the same location.

Osman *et al.* (2018) assessed hatcheries in Egypt as reservoirs for antimicrobial resistant pathogenic *E. coli* that have the potential to spread through the food chain and the environment. *E. coli* isolated were examined and resistance was observed for SPT (100%), DOX (100%), OTC (100%) and CIP (30%). Furthermore, Osman *et al.* (2018) say that "the isolates obtained from the hatchlings did not group with the isolates obtained from various sites within the hatchery, indicating hatchlings may become contaminated with *E. coli* primarily through contact with other hatchlings" (15). In the study of Baron et al. (2014), layers were resistant to tetracycline (1).

For the hatchery samples studied here, it was noticed that the strains of *E. coli* isolated were sensitive to antibiotics that do not pass the intestinal barrier, namely CST (100%), GEN (80%), NEO (60%), as well as for antibiotic combinations, i.e. FOS+TYL (100%) and LCM+SPT (80%), and also for DOX (50%), florfenicol (50%). The strains isolated presented resistance against AMP (100%), FLU (100%), AMX (83.3%), ERY (66.7%), ENR (66.7%), as well as for DOX (33.3 %), OTC (33.3%). Multidrug resistance was seen for all *E. coli* isolates from the hatchery.

Obeng *et al.* (2014) revealed that "birds are colonized with resistant bacteria encoding various resistance genes from a very early age". *E. coli* isolates with resistance to AMP and tetracycline were frequently detected in egg layer pullets and free-range meat chickens, and isolates without resistance to NEO, GEN and CIP were detected in intensively raised egg layers (13).

In this study, the rearing farm *Escherichia coli* isolates showed sensitivity for GEN (100%), CST (100%), NEO (100%), LCM+SPT (100%), FOS+TYL (100%), CST (80%), ENR (66,7%), DOX (66,7%), AMP (50%) and resistance against ERY (100%), OTC (100%). Of all *E. coli* isolates from layer breeder flocks 75% were multi-drug resistant (resistance to ≥ 3 antimicrobials). No multidrug resistance was noticed among isolates from rearing. *E. coli* isolates from layer farm 1 were sensitive 100% to aminoglicosides, ceftiofur, florfenicol, CST, FOS+TYL and LCM+SPT. Moderate sensitivity was seen for ENR. Resistance was noticed against AMP (75 %), ERY (66,7%), AMX (50%), FLU (50%), tetracyclines (33,3%). Multidrug resistance was seen in 75 % of the isolates. For the *E. coli* isolates from layer farm 2, sensitivity was noted for GEN (100%), CST (100%), DOX (100%), FOS+TYL (100%), LCM+SPT (100%). Resistance was seen for ERY (100%), ENR (33.3%) and OTC (33.3%). Multidrug resistance was noticed in 25% of *E. coli* isolates. Layer farm 3 showed the highest prevalence of resistant *E. coli* strains from all layer farms although it was a single age farm and located in a different region than farms 1 and 2. Sensitivity of *E. coli* strains reached 100% for GEN, FLO, CST, FOS+TYL and LCM+SPT. Resistance was noted for FLU (100%), OTC (100%), AMP (66.7%), AMX (66.7%), ERY (66.7%), ENR (66.7%) and DOX (66.7%). Multiresistance was seen in all *E. coli* isolates from farm 3.

Overall, the *E. coli* strains isolated in this study were resistant against SPT (100%), FLU (90.9%), ERY (76.5%), TMP (66.7%), OTC (59.1%), AMP (58.8 %), AMX (52.9%),ENR (35%), doxycyxline (35%). Isolates were highly sensitive to FOS+TYL (100%), CEF (100%), LCM+SPT (95.2%), CST (95.2%), GEN (95%), FLO (90.9%), NOR (66.7%) and NEO (76.2 %). Of all *E. coli* isolates in this study, 55.6% were multidrug resistant (resistance to ≥ 3 antimicrobials).

CONCLUSIONS

E. coli strains from the farms in the layer integrated unit were highly sensitive to FOS+TYL, CEF, LCM+SPT, CST, GEN, FLO. The isolates were resistant against SPT, FLU, ERY, TMP, OTC, AMP, AMX, ENR, DOX. Antibiotic susceptibility analysis in hatchery showed high resistance of the *E. coli* isolates which did not corresponded to the low resistance seen in the rearing flocks. There was variation between antibiotic susceptibility among the layer breeder farms although the source of pullets was the same. This variation probably was due to different structure of flocks within each

farm, location, local epidemiological situation and biosecurity. Within the same flocks over a certain period of time some *E.coli* strains changed their anti-biophenotype from sensitivity to resistant or vice-versa.

Further genotypical investigation of the *E. coli* strains is required not only in terms of the virulence /resistance factors but also for pathotype review.

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