

EVALUATION OF THE ANTIMICROBIAN RESISTANCE OF *Escherichia coli* ISOLATED OF HEALTHY HENS (*Gallus gallus*)

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ABSTRACT

Intestinal microbiota of the chickens is composed by many species of bacteria that benefit the host in many ways during its life. When the host is submitted to inadequate treatments with antibiotics, this microbiota suffers a selection trial, becoming resistant to these drugs. It was evaluated the resistance profile of *Escherichia coli* isolated from intestine of healthy posture hens to the antibiotics. From 100 samples analyzed, 83 showed resistance to more than three antibiotics, and two were resistant to all of the tested antibiotics.

Keywords: antibiotics, microbiota, *Escherichia coli*, resistance, hens.

AVALIAÇÃO DA RESISTÊNCIA ANTIMICROBIANA DE *Escherichia coli* ISOLADAS DE GALINHAS (*Gallus gallus*) SAUDÁVEIS

RESUMO

A microbiota intestinal das aves é composta por inúmeras espécies bacterianas que beneficiam o hospedeiro de alguma maneira durante toda sua vida. Quando o hospedeiro é submetido a tratamentos inadequados com antimicrobianos, essa microbiota sofre um processo de seleção, podendo tornar-se resistente a esses medicamentos. Nesse estudo avaliamos o perfil de resistência de amostras de *Escherichia coli* isoladas do intestino de galinhas de postura saudáveis aos antimicrobianos. Das 100 amostras analisadas, 83 apresentaram resistência a mais de três antimicrobianos, sendo que duas amostras foram resistentes a todos os antibióticos.

Palavras-chave: antimicrobianos, microbiota, *Escherichia coli*, resistência, galinhas.

EVALUACIÓN DE LA RESISTENCIA ANTIMICROBIANA DE *Escherichia coli* AISLADA DE GALLINAS (*Gallus de gallus*) SANAS

RESUMEN

La microbiota intestinal de los pollos se compone de muchas especies bacterianas que benefician al huésped de diversas maneras durante toda su vida. Cuando el huésped es sometido a tratamientos antibióticos inadecuados, este microbiota sufre un proceso de selección y puede llegar a crear resistencia a dichos fármacos. En este estudio, evaluamos el perfil de resistencia de *Escherichia coli* aislada del intestino de gallinas de postura sanas. De

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Palabras clave: antibióticos, microbiota, *Escherichia coli*, resistencia, gallinas.

INTRODUCTION

Escherichia coli (*E. coli*) is a Gram negative bacillus, facultative anaerobic, belonging to the family *Enterobacteriaceae*, found colonizing the healthy gut of birds and mammals (1). The pathogenic lineages for birds are named Avian Pathogenic *Escherichia coli* (2), responsible for infections characterized by signs of septicemia, peritonitis, pneumonia, aerossaculitis, pericarditis, onfalitis and salpingitis. The infection caused by *E. coli* represent one of the main problem of the industrial poultry breeding, responsible for economic losses as mortality, decrease of weight and condemnation of carcasses in the slaughterhouse (3). The use of antibiotics in the animal food as "promoters of growth" or in an indiscriminate way under the form of sub or over dosage (3), increases the resistance genes selection pressure to the antibiotics for multiresistant bacterial lineages (4).

Studies have shown the resistance genes transmission between the micro-organisms. This fact has become a concerning a public health problem, as the genes of resistance may be transferred to pathogenic bacteria that can infect the humans (5). Many species of the family *Enterobacteriaceae* present in the gut of healthy birds are frequently exposed to several antibiotics during the life of the animal (6). The present study evaluated the profile of resistance to 12 antibiotics in isolated of *E. coli* obtained from the excrements of adult healthy hens, without intestinal signs.

MATERIAL AND METHODS

One hundred samples of *E. coli* obtained from the healthy posture hens excrements were used. The samples were isolated utilizing swab of cloaca, and cultivated in ágar MacConkey, in conditions of aerobiosis, to 37° C, for 24 hours. The characteristic colonies were submitted to the biochemical analysis. For the achievement of the sensitiveness test to the antibiotics, the colonies identified like *E. coli* were submitted to the diffusion standard test with disks (7), utilizing 12 antibiotics: cefalexin (30µg), gentamicin (10µg), enrofloxacin (5µg), norfloxacin (10µg), doxiciclin (30µg), florfenicol (30µg), cloranfenicol (30µg), neomycin (30µg), amoxicillin (10µg), tetracilin (30µg), ampicillin (10µg) and penicillin (10µg).

The results were submitted to the statistical analysis, using the program SAS (table 1).

RESULTS AND DISCUSSION

Table 1 shows the antibiogram results. From 100 samples of *E. coli*, just two (2%) showed itself sensitive to all the tested antibiotics, 15 presented resistance to one or two antibiotics and the other samples were resistant to three or more antibiotics; two of them were resistant simultaneously to the 12 tested antibiotics. Zao et al (8) described similar results when evaluating 95 isolated samples of *E. coli*, in which 71% were resistant to five or more antibiotics.

Table 1. Sensitivity and resistance by 100 bacterial strains of *Escherichia coli* from the normal intestinal microbiota of hens.

Antimicrobial	% sensibility/ % resistance	Risk of bacterial resistance		
		RAP	CI 95%	Value P
Penicillin	0/100	Reference	Reference	NA
Amoxicillin	17/83	0,049	0,01-0,38	<0,004
Ampicillin	0/100	1,0	0,06-16,21	>0,1
Cefalexin	25/75	0,03	0,004-0,23	<0,001
Cloranfenicol	85/15	0,002	0,001-0,01	<0,0001
Doxiciclin	50/50	0,01	0,001-0,07	<0,0001
Enrofloxacin	75/25	0,003	0,001-0,02	<0,0001
Florfenicol	67/33	0,005	0,001-0,04	<0,0001
Gentamicin	75/25	0,003	0,001-0,02	<0,0001
Neomicin	0/100	1,0	0,06-16,21	>0,1
Norfloxacin	50/50	0,01	0,001-0,07	<0,0001
Tetraciclin	50/50	0,01	0,001-0,07	<0,0001

RAP: Ratio of adjusted proportions;

CI 95%: Confidence interval 95% probability;

NA: Not Applicable.

Neomicin and ampicillin were not effective for the isolated samples. Blanco et al. (4) observed resistance in 46% of the isolated sample for ampicillin and 79% for neomicin when evaluating 310 isolated samples, differing from the results found in this study. The antibiotic which showed the best effectiveness facing the 100 isolated samples was cloranfenicol with 85% of sensitiveness. Zao et al. (8) observed 89% of sensitiveness of *E. coli* to this antibiotic. The prescription of antibiotic for healthy birds, in subtherapeutic concentrations for prolonged time, exposes microbiota to a selective pressure, enabling the acquisition of resistance genes to the antibiotics. The intestinal lumen complex, where a great amount of bacteria interacts in very limited space, represents an ideal niche for studies in vivo of the resistance genes transfer between different species and gender (9).

The occurrence of multiresistant samples of *E. coli* from animal origin is concerning to the world health organization (10). Another prominent point resides in the fact of the majority of the antibiotics available in the market are based on ampicillin or neomicin that, coincidentally are broadly used in the human and veterinary medicine. However, these drugs of the group showed have low or no efficiency against all the samples tested, fact that can be consequence of the inappropriate use of the antibiotics in the poultry breeding.

CONCLUSION

Escherichia coli samples isolated from excrements of healthy birds were resistant to the antibiotics, fact that is a great problem for the poultry breeding and public health with dissemination risk of multiresistant gene to the antimicrobians to other microorganisms including the pathogenic ones.

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