

ANTIMICROBIAL RESISTANCE IN *Escherichia coli* POPULATIONS COLECTED FROM FARM ANIMALS

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ABSTRACT

Antimicrobial resistance is currently one of authorities' major concerns in healthcare, mainly due to the danger that may arise from multiresistant strains in situations of contamination and infection of patients in hospital settings. The origin of this resistance is linked to the dynamics of natural bacteria populations in soil and water, but also to the excessive and inappropriate use of antimicrobials in clinical treatment and as growth promoters in herds. In this study, antimicrobial resistance profiles were analyzed in potentially pathogenic populations of *Escherichia coli* in the gastrointestinal tract of poultry, cattle and sheep. This bacterial specie, although harboring pathogenic pathotypes, is part of the normal microflora of these animals' intestinal tracts. The lowest antimicrobial resistance rates were observed in sheep isolates. Resistance highest rates of were observed among bacterial populations derived from the poultry. In bacterial population from cattle feces, resistance to ampicillin, cephalothin and erythromycin was observed. Resistance to cephalothin was noted to be widespread among analyzed populations. Furthermore, the conscious use of growth promoters, and supported on a proper diagnosis in clinical cases it is essential to inhibit the emergence of multidrug-resistant strains.

Keywords: *Escherichia coli*, antimicrobial resistance, cattle, sheep, broilers.

RESISTÊNCIA A ANTIMICROBIANOS EM POPULAÇÕES DE *Escherichia coli* ISOLADAS DE ANIMAIS DE PRODUÇÃO

RESUMO

A resistência antimicrobiana é atualmente uma das principais preocupações das autoridades em saúde, principalmente devido ao perigo que pode surgir de cepas multirresistentes em situações de contaminação e infecção de pacientes em ambientes hospitalares. A origem desta resistência está ligada à dinâmica das populações de bactérias naturais no solo e na água, mas também ao uso excessivo e inadequado de antimicrobianos em tratamento clínico e como promotores de crescimento nos rebanhos. Neste estudo, os perfis de resistência antimicrobiana foram analisados em populações potencialmente patogênicas de *Escherichia coli* no trato gastrointestinal de aves, bovinos e ovinos. Esta espécie bacteriana, embora abrigando patotipos patogênicos, faz parte da microbiota normal do trato intestinal destes animais. As menores frequências de resistência antimicrobiana foram observadas em isolados de ovinos. Foram observadas as maiores frequências de resistência entre as populações bacterianas derivadas dos frangos de corte. Na população bacteriana oriunda das fezes dos bovinos, observou-se

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resistência à ampicilina, cefalotina e eritromicina. A resistência à cefalotina foi generalizada entre as populações analisadas. Além disso, o uso consciente dos promotores de crescimento, e o uso apoiado em um diagnóstico adequado em casos clínicos, é essencial para inibir o surgimento de cepas multirresistentes.

Palavras-chave: *Escherichia coli*, resistência antimicrobiana, bovinos, ovinos, frangos de corte.

RESISTENCIA A LOS ANTIMICROBIANOS EN POBLACIONES DE *Escherichia coli* AISLADOS DE ANIMALES DE PRODUCCIÓN

RESUMEN

La resistencia antimicrobiana es actualmente una de las principales preocupaciones de las autoridades sanitarias, sobretodo a causa del peligro que puede surgir a partir de cepas resistentes a múltiples fármacos en situaciones de contaminación e infección de los pacientes en el ámbito hospitalario. El origen de esta resistencia está vinculada a la dinámica de las bacterias naturales en el suelo y el agua, y también por el uso excesivo e inapropiado de antibióticos en el tratamiento médico y como promotores del crecimiento en el ganado. En este estudio, los perfiles de resistencia antimicrobiana fueron analizados en cepas potencialmente patógenas de *Escherichia coli* en el tracto gastrointestinal de los pollos, ganado y ovejas. Esta especie bacteriana, apesar de albergar patógenos, es parte de la microflora normal en el tracto intestinal de los animales. La frecuencia más baja de la resistencia antimicrobiana se observaron en las ovejas aisladas. Se observaron las mayores frecuencias de resistencia entre las poblaciones bacterianas derivadas de los pollos. En la población bacteriana procedente de las muestras de heces de ganado, se observó resistencia a la ampicilina, cefalotina y eritromicina. La resistencia a cefalotina estaba extendida entre las poblaciones estudiadas. Por otra parte, el uso consciente de los factores de crecimiento y el uso apoyado en un diagnóstico adecuado de los casos clínicos es esencial para inhibir la aparición de cepas resistentes a múltiples fármacos.

Palabras clave: *Escherichia coli*, resistencia a los antimicrobianos, ganado, ovejas, pollos.

INTRODUCTION

Farm animals are usually raised in large groups and frequently housed in high density, many are fattened up for quick slaughter, which often occurs before physical maturity. Animals' youth, raising in large-scale groups, and the movement and mixing of animal groups ends up facilitating the entry and spread of disease in lots (1). To prevent the damage caused by diseases, antibiotics are used therapeutically, prophylactic or as growth promoters. Currently, a major debate involves the relationship of the subtherapeutic use (growth promoters) of antimicrobials in animals used for food and the problem of antimicrobial resistance in bacterial populations found in humans. Drug resistance presents a global growing threat to public health, which involves all major microbial pathogens (2).

Resistant bacteria, active antimicrobial or its metabolites are spread throughout the farms' environment, by animal feces (manure) that are produced there. However, the direct transmission of resistant bacteria among animals and workers of farms has been observed (3). As in hospital settings, the agricultural use of antibiotics selects the resistance, certainly a more disseminated form due to the use in water and animal food (4). And antibiotics, either from urban or rural source, may persist in soil and aquatic environments, producing a selective pressure

that may affect the treatment of human disease, for example by transferring resistance genes for human pathogens (5). Indeed, the use of antibiotics can extend beyond their point of use, for example, antibiotics used in animals may, through their droppings, contaminate the soil and groundwater (4). Schwarz et al. (6) report that dynamics of antibiotic resistance population depend on the drugs used, but is also influenced by other factors, such as the availability of pre-existing resistance genes, the exchange of resistance genes and their functional activity in different bacterial hosts and selective pressure.

Escherichia coli (*E. coli*) is a bacteria that is part of the intestinal tract commensal microbiota of animals, but some strains can exhibit pathogenicity as *E. coli* Shiga-like toxin (STEC) and pathogenic avian *E. coli* (APEC). The spreading resistance profiles among commensal and pathogenic bacteria present in the intestinal tract can occur, where commensal bacteria constitute a reservoir of resistance genes to potential pathogenic bacteria, additionally, resistant bacteria commensal from animal production, such as bacteria zoonotic, may contaminate meat products and so reach humans' intestinal tract (7).

Using antibiotics as growth promoters in animals creates a larger population of resistant microorganisms that can be transmitted to humans, either by ingestion of food or produced by direct contact with the animal. Furthermore, the transmission of resistant bacteria originated from animals to humans results in more people carrying resistant bacteria among populations (1). Barros et al. (8) emphasize that monitoring resistance to antibiotics in isolated animal bacteria becomes a determining factor for election and successful treatment, as well as the possibility to eliminate those that have plasmids to avoid transfer related to pathogenicity genes. Allen et al. (4) report that there is evidence that the antibiotic resistance genes are common in natural environments, even in plasmids, before the use of antibiotics. Therefore, this study aimed to evaluate the antimicrobial resistance profile in potentially pathogenic populations of *E. coli* in different animal species.

MATERIAL AND METHODS

For six months in the southwestern region of Goiás State, stool samples were first collected by rectal swabs from healthy cattle (45), sheep (23) and cloacal swabs from poultry (80), the animals were not on antimicrobial therapy at the time of sample collection. Soon after the samples (swabs) were plated on MacConkey agar and incubated for 24 hours at 37°C, from each plated sample on MacConkey agar, three typical colonies were picked for biochemical identification based on lactose fermentation testing, indole production, red reactions methyl and Voges-Proskauer, citrate utilization, urease production and gas production hydrogen sulphide (H₂S), after incubation for 24 and 72 hours at 37 °C (9). The microbial DNA template was obtained according to the thermal lysis technique (10). Isolates from poultry identified as *E. coli* were subjected to PCR in order to detect the presence of *fiutA*, *hlyF*, *ompT* and *cvaC* (11). Additionally, *iss* and *iroN* were detected using primers and conditions previously described (12). Only those strains that have at least one of six genes related to virulence factors investigated were selected for the study, which, in this case, were 91 strains. Isolates from cattle and sheep identified as *E. coli* were subjected to PCR in order to detect the presence of *stx1* and *stx2* (13), in this case 88 STEC from cattle and 99 STEC from sheep were isolated.

All bacterial strains, 88 from cattle, 91 from poultry and 99 from sheep were subjected to sensitivity antimicrobial tests (14). Aliquots of the cultures were inoculated aseptically in tubes containing 4ml BHI broth and incubated "overnight" at 37°C until turbidity reached 0.5 on the MacFarland scale. Next, the cultures were plated with the help of sterile swab on Mueller-Hinton agar and after about 3 minutes, time required for drying the surface of the medium discs that were placed containing antimicrobials.

The reading was performed after 24 hours of incubation at 37°C by measuring inhibition halos, using a millimeter ruler. The obtained diameter in millimeters was compared with the table provided by the manufacturer of the used disks. The tested antimicrobial agents were ampicillin, cephalothin, enrofloxacin, streptomycin, erythromycin, gentamicin, neomycin, tetracycline and trimethoprim + sulfa.

RESULTS

A wide antimicrobial resistance was observed in *E. coli* population of in poultry feces (Table 1), being that seven from nine analyzed antimicrobials have efficacy lower than 50% in the studied population, this large resistance was observed with ampicillin (84,6%), cephalothin (78%), streptomycin (79,1%), erythromycin (96,7%), neomycin (51,6%), sulfa + trimethoprim (68,1%) and tetracycline (67%). Among the cattle, over 50% of the bacterial population was resistant to the antibiotics, ampicillin (73.9%), cephalothin (95.4%) and erythromycin (97.7%). Considerable resistance in this population was also found to antibiotics neomycin (31.8%) and tetracycline (38.6%). Interestingly, we observe the lowest resistance index among the bacterial population derived from feces in sheep (Figure 1). Among the antimicrobials tested, only one showed a high resistance rate, the cephalothin (61,6%).

Table 1. Patterns of antimicrobial resistance and host in *Escherichia coli*.

Antibiotics	Animal host and resistance profile (%)			Overall resistance profile (%)
	Cattle	Sheep	Poultry	
AMP ^a	73,9 (65/88)	15,1 (15/99)	84,6 (77/91)	56,5 (157/278)
CEF ^b	95,4(84/88)	61,6 (61/99)	78 (71/91)	77,7 (216/278)
ENR ^c	11,3 (10/88)	8,0 (8/99)	22 (20/91)	13,6 (38/278)
EST ^d	15,9 (14/88)	22,2 (22/99)	79,1 (72/91)	38,8 (108/278)
ERI ^e	97,7 (86/88)	2 (2/99)	96,7 (88/91)	63,3 (176/278)
GEN ^f	7,9 (9/88)	6,1 (6/99)	9,9 (9/91)	8,6 (24/278)
NEO ^g	31,8 (28/88)	5 (5/99)	51,6 (47/91)	28,7 (80/278)
SXT ^h	11,4 (10/88)	1 (1/99)	68,1 (62/91)	26,2 (73/278)
TET ⁱ	38,6 (34/88)	9,1 (9/99)	67 (61/91)	37,4 (104/278)

^aAmpicilin, ^bCephalothin, ^cEnrofloxacin, ^dStreptomycin, ^eErythromycin, ^fGentamicin, ^gNeomicin, ^hSulfamethoxazole+trimethoprim, ⁱTetracycline

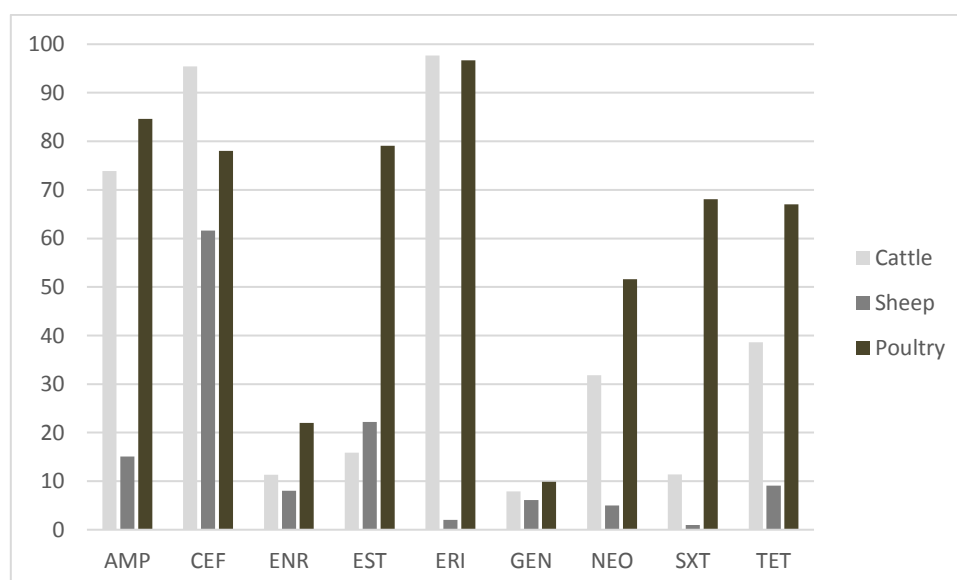


Figure 1. Frequency of antimicrobial resistance to specific antibiotics in isolates from cattle, sheep and poultry. ^{AMP}Ampicilin, ^{CEF}Cephalothin, ^{ENR}Enrofloxacin, ^{EST}Streptomycin, ^{ERI}Erythromycin, ^{GEN}Gentamicin, ^{NEO}Neomicin, ^{SXT}Sulfamethoxazole+trimethoprim, ^{TET}Tetracycline.

DISCUSSION

Resistant bacterial strains are a major concern in global animal production, their presence in animals and in the environment is by the great use of antimicrobials during periods of intensive raising of the animals. These antimicrobials eventually select resistant strains, which, through plasmids, can quickly disseminate this profile. In Netherlands, analyzing *E. coli* populations in the feces of broiler Van den Bogaard et al. (15) related that a high prevalence of resistance was also found such as neomycin (72%), oxytetracycline (78%) and trimethoprim (80%). In this same study, high prevalence of resistance in *E. coli* populations derived from feces of workers of broiler farms was observed, where the rates were 20% to neomycin, 61% to oxytetracycline and 47% for trimethoprim. Van den Bogaard et al. (15), then, strongly suggests that there is a spread of *E. coli* resistant to antibiotics from animals to people, not only among farm workers, but also among poultry meat consumers.

Pessanha and Gontijo Filho (16), in their study, demonstrated that broiler chickens may act as resistance gene reservoirs to important antibiotics in veterinary and human medicine, reporting lower resistance to our work, except for gentamicin (26%); other resistance percentages reported in their work were 48% to cephalothin, 45% to tetracycline, 42% to ampicillin and 28% to trimethoprim + sulfa. Selective pressure from the use of antimicrobials plays a key role in the emergence of resistant bacteria. Where mixed bacterial population is exposed to an antimicrobial agent, there will probably be resistant bacteria for the applied concentration. The number of these bacteria will increase and some of these will pass their resistance genes to other members of this population (6).

In Britain, Enne et al. (17), analyzing *E. coli* from bovine feces, found lower resistance than in this work, ampicillin (1.3%), gentamycin (0.1%), neomycin (0.6 %) and tetracycline (4.1%). Mora et al. (18), analyzing non O157 STEC strains isolated from cattle in Spain, also report lower frequency resistance, ampicillin (6%), cephalothin (1%), gentamycin (0.2%), neomycin (3%) and tetracycline (29%), however, for streptomycin resistance, 24% is reported, which is higher when compared to 15.9% reported in our work. Interestingly, Wegener (1) mentions that the practice of administering antibiotics in feeding large batches of animals has the obvious disadvantage that the sick or weak animal will consume a smaller amount of antibiotic, allowing those who need (patients) it to consume subtherapeutic amounts. This same author points out that animals and humans are superposed resistance reservoirs, and consequently the use of antimicrobials in animals can have an impact on public health. For example, the occurrence of vancomycin resistant enterococci in farm animals, is linked to the use of avoparcin, an antibiotic used as a food additive to enhance the weight gain in animals.

Schwarz and Noble (19) reported that the main factors to be considered in the rapid spread of antibiotic resistance are: the location of the resistance genes on mobile genetic elements and the close contact between the bacteria in polymicrobial environment (intestinal tract). In addition, Roberts (20) points out that the use of a single antibiotic can apply not only resistance to this drug in particular, but can also result in resistance to other antibiotics, especially when structurally related. However, even antibiotics of different classes, when sharing the same site of action, cross resistance, can develop (19). An antibiotic used exclusively in veterinary medicine may impact the level of resistance to bacteria in relation to important antibiotics used in human medicine. So we have a disturbing cycle: development of resistant bacteria in animals, spread of resistant bacteria from animals to humans and clinical disease in humans with subsequent failure in treatment with antibiotics.

In bacterial population derived from feces in sheep, we observed the lowest resistance index (Table 1). Among tested antimicrobials, only one showed a high resistance rate, the cephalothin (61.6%), though the resistance levels are high, compared to Enne et al. (17), reporting a lower frequency than our data resistance, ampicillin (1%), gentamycin (0%),

neomycin (0,6%) and tetracycline (2,5%). In Argentina, Panzzotti et al. (21) also reported low levels of resistance in sheep, as 10,5% to ampicillin, 5,3% to streptomycin, 5,3% to cephalothin and 21% to tetracycline. Antimicrobials that used a high resistance to cephalothin stands out because of their wide distribution in the three bacterial populations analyzed (78% in poultry, 95,4% in cattle and 61,6% in sheep). This probably indicates a great distribution of bacteria that is resistant to cephalothin in the animal production environment. Schwarz et al. (6) reported that the transfer of resistance genes of commensal bacteria to pathogenic bacteria is highly probable to occur and should be considered. Considering tetracycline, poultry formed the group that had the highest resistance rate (67%). Wegener (1) reports that is difficult to determine the impact of using tetracycline as a growth promoter in animals, because this antimicrobial agent is also used in prophylaxis and therapy. Van den Bogaard and Stobberingh (7) punctuate that the emergence and spread of resistant bacteria and genes is inevitable due to use of antibiotics in animals and humans as prophylaxis or prevention of disease. These authors also cite that the introduction of antibiotics raises not only the level of resistance of pathogenic bacteria, but also the commensals, and the level of resistance in these bacteria groups is a good indicator of the selection pressure exerted by the use of antibiotics and future problems related to resistance to pathogenic bacteria.

Levy (22) reiterates that, although antimicrobial resistance is not a new event, the number of resistant microorganisms, the affected geographic locations and extent of resistance in individual organisms are serious and unprecedented. However, Allen et al. (4) argues that some organisms and environments harbor resistance genes regardless of the use of antibiotics. Wegener (1) points out that the selection of resistant bacteria in farm animals, by the growth promoters and the consequent spreading among the animals in the farm environment, are important factors in the spreading of these microorganisms between animal reservoirs. The same author mentions that the spreading rate of resistant bacteria from animals to the environment and producing the food chain are decisive factors to the spreading in human population.

CONCLUSIONS

Among *E. coli* potentially pathogenic populations, the smallest antimicrobial resistance rates were observed in isolated bacteria from sheep. The highest resistance rates were observed among populations from poultry feces. In bacterial population derived from cattle feces, resistance to ampicillin, cephalothin and erythromycin stands out. Resistance to cephalothin showed to be widespread among studied populations. In view of the results, and due to few data related to the presence of resistant bacterial isolates in animal production in our country, it is essential to establish future monitoring program for one responsible and prudent use of antibiotics in animals.

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