

**INVESTIGATION OF SENSITIVITY AND RESISTANCE TO ANTIBIOTICS AND  
CHEMOTHERAPEUTICS IN *E. COLI* STRAINS ISOLATED FROM ANIMALS BRED IN  
INTENSIVE FARMING CONDITIONS**

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*Being an important pathogenic and indicator microorganism, *E. coli* has been included in all the resistance follow up programs worldwide. We carried out sampling on 42 cattle, pig and poultry farms. After sampling the material was delivered to the laboratories, where it was exposed to standard treatment and streaking by standard methods of microbiological diagnostics with the aim to isolate and identify *E. coli* strains. On the selected isolated *E. coli* strains an investigation of its sensitivity to a certain number of antimicrobial agents (ampicillin, tetracycline, chloramphenicol, streptomycin, neomycin and sulphametoxazol) was carried out. After obtaining the results from each farm for the three different animals categories (young healthy, young unhealthy, adult), 60 *E. coli* strains for each category were isolated to be examined for sensitivity to 17 antibiotics and chemotherapeutics. The investigation included a total of 180 *E. coli* strains originating from cattle, pigs and poultry. For the investigation of isolated *E. coli* strains Mueller Hinton broth and agar, antibiogram tablets (Torlak, Belgrade) as well as discs by Becton Dickinson, BBL (USA) were used. In isolated *E. coli* strains resistance to investigated antimicrobial agents was significantly different depending on the species age and health status of the animals.*

*The majority of the isolated *E. coli* strains examined for 17 antibiotics and chemotherapeutics showed a resistance to 15, and sensitivity only to cephalosporines of the III generation and colistin.*

*Key words: *E. coli*, calves, pigs, poultry, antimicrobial agents, sensitivity, resistance.*

INTRODUCTION

Siegel and collaborators reported in Montana in 1974. that *E. coli* strains isolated from calves showed sensitivity to tetracycline and only 1% of them was resistant to ampicillin. Ten years later, Harnett and Gyles while investigating the resistance of *E. coli* strains to chloramphenicol and canamycin in regard to

tetracycline, streptomycin, ampicillin, neomycin and sulphonamides, found out a smaller percentage of resistance to the mentioned antibiotics and chemotherapeutics and concluded that it was the consequence of their less frequent application in domestic animals. However, Helmuth and Protz reported in 1997 that due to masive application of antibiotics and chemotherapeutics farms, along with hospitals, take a very high position as reservoirs of resistance genes in the global ecosystem. Usage of antibiotics and chemotherapeutics depends on the method of animal breeding and significantly differs from country to country. According to the Committee on Human Health, 1989, in conditions of intensive animal breeding much larger quantities of antibiotics and chemotherapeutics are used. Consequently the resistance of *E. coli* strains originating from animals, ranges from 49 % to 96 %, that is, from 13 % to 77 % on a large number of farms in the USA. Gellin *et al.* (1989), on the basis of investigations of sensitivity of *E. coli* strains isolated from pigs, found out in a significantly higher percentage of resistant and multiresistant bacteria at farms where antibiotics and chemotherapeutics have been constantly used in subtherapeutic and therapeutic doses.

The very problem of bacterial resistance, attracted the attention of numerous investigators (Coates and Hoopes, 1980, Das, 1984, Gellin *et al.*, 1989). In Serbia, as well as worldwide, a number of different antibiotics and chemotherapeutics in farm animals, both in prevention and therapy are used. A selective effect of certain preparations on pathogenic bacteria, and the appearance of resistance not only in pathogenic but also in indicator bacteria, lead to great health problems, as well as to economic losses (Krnjaic, 2000). In their research, Krnjaic and Asanin, (2001), reported on the importance of monitoring bacterial resistance, as well as on the need for responsible application of antibiotics and chemotherapeutics in domestic animals. Data on the resistance to chloramphenicol of beta-chemolytic strains of *E. coli* originating from pigs suffering from diarrhea, Bischoff *et al.* reported in 2002. Investigating *E. coli* strain 0157 originating from calves, pigs, people and feed, in the period from 1985 to 2000, Schroeder *et al.* found out the resistance to ampicillin in 21% of *E. coli* isolates originating from people, in 12% to sulphamethosazol and tetracycline and in 15% to cephalothyn. Similar results were also obtained in strains isolated from calves: 3% to ampicillin, 14% to sulphamethosazol and 20% to tetracycline, while the percentage of resistance to chloramphenicol, cephalothyn and nalidixic acid varied from 2,3% to, and for amoxycylin with clavulon acid from 1% to. Bywater, (2004) investigated the application of antibiotics and chemotherapeutics, as well as the resistance of indicator and pathogenic bacteria strains originating from animals in EU countries. According to the results concluded that the percentage of resistance in the examined bacteria varied from country to country, thus represents a warning to all veterinarians for a necessity of controlled usage of antimicrobial agents. His data refer to the lack of bacterial resistance to fluorquinolone and cephalosporines of the III generation. The presence of resistance and multiple resistance of *E. coli* strains isolated from calves and pigs at farms in Serbia and Montenegro was determined by Ruzica Asanin *et al.* (2004).

## MATERIAL AND METHODS

### *Investigation material*

This investigation included 42 farms (cattle, pigs and poultry) of different categories from which samples were taken. The most frequent material for laboratory investigations were rectal swabs and feces of healthy and unhealthy individuals of different categories (young healthy, young unhealthy and adult), and in the case of dead animals ligated intestines and parenchymatous organs, cloacal swabs and eggs.

### *Bacteria isolation procedure*

For the isolation of *E. coli* strains a large number of nutrient culture medium were used: nutrient agar, nutrient broth, tryptose agar with 6% of sheep blood and MacConkey agar (Torlak). Identification of bacteria was carried out by a large number of biochemical reactions and appropriate mediums and reagents. Beside conventional tests, determination of the isolated strains was carried out by the commercial system BBL Crystal Enteric/nonfermenter kit from Becton Dickinson and API 20 E from BioMerieux. For investigations of the sensitivity of the isolated *E. coli* strains, as well as of the referent *E. coli* ATCC 25922 strain to antimicrobial agents a disc diffuse method according to Kirby Bauer was used along with the application of antibiogram tablets (Torlak), antibiogram discs (Becton Dickinson), Mueller-Hinton agar (Torlak, Beograd) and Mueller Hinton broth (Becton Dickinson).

Interpretation and estimation of the sensitivity results was carried out by measuring the diameter of the zone without bacterial growth i.e. the zone of inhibition around the tablets and discs. Determination of the therapeutic categories S, R, and I was carried out by comparing the inhibition zones with the referent values given by the producer.

## RESULTS

The results of the investigation of sensitivity and resistance of *E. coli* strains isolated from domestic animals to antibiotics and chemotherapeutics by applying a disc diffuse method are presented in Table 1 and Figure 1.

Prevalence of resistance of the isolated *E. coli* strains to certain antimicrobial agents was determined disregarding animal species, age and health status. A high percentage of resistance from 85% to 100% was found in the strains originating from unhealthy calves, pigs and poultry. Especially the strains isolated from unhealthy pigs and healthy chicken showed a 100% resistance to tetracycline. The lowest percentage of resistance to tetracycline was determined in cows and healthy calves, (30% and 40% respectively). The investigated resistance of *E. coli* strains to chloramphenicol, which is no longer in use, ranged from 5% to 30% of all the animals, and 55% strains from unhealthy calves and pigs. Such a finding is the result of cross reaction combined of chloramphenicol and tetracycline. A high resistance to aminoglycoside antibiotics was found out in

Table 1. Resistance and multiple resistance to antibiotics and chemotherapeutics of *E. coli* strains isolated from animals

Kind and category of animals	Amp	Akk	Cl	Cll	Clll	Tet	Chlor	Col	Str	Neo	Gen	Spec	NK	Cipr/Enr	Sul	Tmp	Stm	% Multires. Strains
Calves	50	15	25	0	0	40	5	0	55	40	0	10	15	0	25	5	5	30
Sick calves	85	80	45	25	0	85	45	0	70	60	40	25	30	20	70	65	65	80
Cows	40	10	5	0	0	30	10	0	35	25	0	5	0	0	5	5	5	15
Pigs	60	35	25	0	0	70	25	0	90	80	30	60	35	0	85	50	50	90
Sick pigs	60	45	30	0	0	100	55	0	90	85	20	65	35	10	90	35	35	95
Swine	40	0	10	0	0	60	5	0	70	35	5	25	5	0	45	0	0	40
Chicken	95	45	20	0	0	100	0	0	95	60	5	15	50	15	70	60	60	85
Dead chicken	90	90	25	10	0	85	30	0	95	40	10	35	45	35	95	85	85	95
Hens	40	10	5	0	0	75	5	0	75	50	0	15	30	0	30	0	0	45

Legend: Amp – ampicillin; AKK- Amoxicillin with clavulone acid; Cl- cephalaxin; Cll- cephalaxin; Clll- cephalaxin; Cll- cephalaxin; Clll- cephalaxin; Tet- tetracycline; Hlor- chloramphenicol; Kol- colistin; Str- streptomycin; Neo- neomycin; Gen- gentamicin; Gen- gentamicin; Spek- spectinomycin; NK- nalidixic acid; Cipr/Enr- cyprofoxacin/enrofloxacin; Sul- sulphamethoxazol; Tmp- trimethoprim; Stm- sulphamethoxazol + trimethoprim

90% of isolates originating from pigs and poultry only to streptomycin or to streptomycin and neomycin. In *E. coli* strains originating from pigs, disregarding their health status, a high percentage of resistance only to neomycin or only to gentamycin was found, while considering the related aminocyclotole antibiotic – spectinomycin resistance was determined in all the strains. Although fluoroquinolones are widely used in treating domestic animals, resistance was present only in strains originating from young unhealthy animals (15% to 50%).

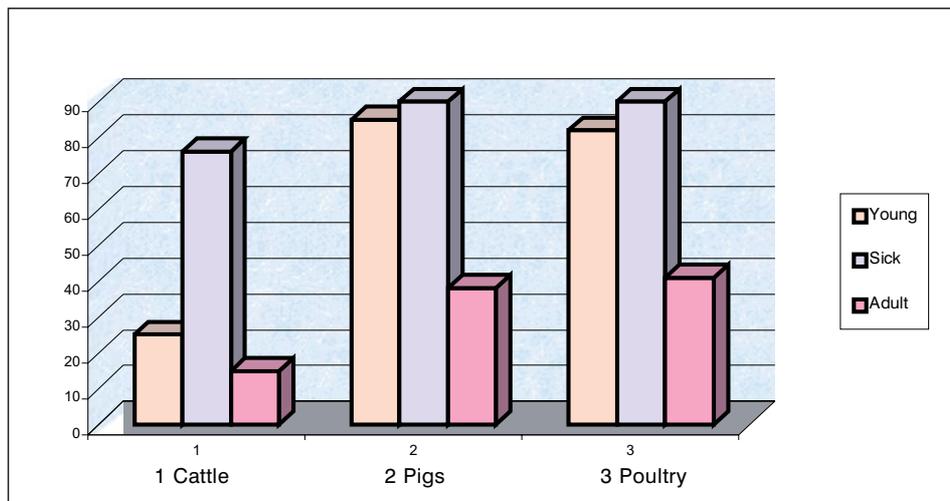


Figure 1. Prevalence of multiresistant *E. coli* strains isolated from domestic animals

*E. coli* resistance to fluoroquinolones is a consequence of mutation, where the first mutation is shown by a resistance to nalidixic acid, while after a greater number of mutations the resistance to enrofloxacin and norfloxacin appears. The resistance in the isolated strains of *E. coli* to nalidixic acid in all the examined animal groups ranged from 5% to 50% in cows, which is probably a consequence of their frequent application in our country. The resistance to enrofloxacin was found out in *E. coli* strains isolated from unhealthy domestic animals, i.e. 10% in pigs, 20% in unhealthy calves and 35% in dead chicken. However, it can not be neglected that even in 15% of healthy chicken, there was found a *E. coli* resistance to fluoroquinolones.

It is interesting that *E. coli* strains isolated from all animal groups showed a resistance to sulphonamides, while the lowest percentage of resistant strains was in cows 5%, the highest in pigs (85% - 90%), and chicken (70% - 95%). The resistance to trimetoprim, with the exception of only one strain, was shown in *E. coli* strains isolated exclusively from young animal (pigs and chicken, 35% to 85%), disregarding animal health status. In contrast to pigs and chicken, in *E. coli* strains originating from calves, a high percentage (65%) of resistance to

sulphamethosazol and trimetoprim 65% was found out, while in healthy cows that percentage was only 5%. The determined resistance of *E. coli* strains to sulphonamide and trimetoprim together, was in a high correlation with the resistance to trimetoprim. The multiple resistance to four and more antibiotics and chemotherapeutics was found out in *E. coli* strains originating from all the examined categories of animals. It is important to emphasize that the percentage of multiresistant *E. coli* strains isolated from cattle is lower in regard to the strains originating from pigs and poultry. Depending on species, age and health status of animals, multiple resistance was present in a different percentage in isolated strains of *E. coli*, as shown in (Figure 1). In the isolated *E. coli* strains, multiresistance differed in dependance to animal species and category. A considerably lower percentage of multiresistant strains was found out in cattle in regard to pigs and poultry (5% to 65% calves and cows, 85% to 90% pigs and poultry), respectively. Extremely high percentage of resistance was found out in *E. coli* strains isolated from calves suffering from diarrhoea, pigs and poultry. Multiple resistance of *E. coli* strains isolated from cows was found out only in 15% strains, from sows in 40% and from laying hens in 45% strains. This points out to a greater use of certain antimicrobial agents in different animal species – tetracycline and sulphonamides in pigs and poultry, spectinomycines in swine, and neomycine and fluoroquinolones in poultry. The finding of 90% multiresistant strains of *E. coli* in healthy pigs, 85% in healthy chicken and only 30% in healthy calves confirms the fact that there is a mutual dependance of resistance and quantity of the applied antibiotics and chemotherapeutics in animals. In our investigation of the sensitivity of *E. coli* strains isolated from animals in intensive livestock production, we found out a resistance to all antimicrobial agents, except to cephalosporines of the III generation and colistin.

#### DISCUSSION

The appearance and spread of *E. coli* resistance was in a high correlation with the quantity of used antibiotics. For this reason the prevalence resistance of *E. coli* strains, disregarding their pathogenic characteristics, is the indicator of antimicrobial agents usage in breeding domestic animals. Gellin *et al.* (1989), followed up the sensitivity of enterobacter spp. isolated from pigs and found out a considerably higher percentage of resistance and multiresistance in *E. coli* strains isolated from animals on farms where antibiotics are constantly used in subtherapeutic or therapeutic doses.

The appearance and spread of resistance of bacteria isolated from farm animals is considerably influenced also by technological conditions of production.

Between young and adult healthy animals there is a great difference in resistance prevalence of isolated *E. coli* strains to the majority of antimicrobial agents. The percentage of multiresistant strains is considerably higher in sows and laying hens in regard to cows. Such data are the result of application of antibiotics, not only in therapy but also in prophylactic treatments of infectious diseases in young animals. This is confirmed by a prevalence of multiresistant *E.*

*coli* strains in healthy pigs 90%, healthy chicken 80%, and only 30% in healthy calves. A high resistance prevalence in pigs was discovered by Das in 1984, after investigating 138 strains of *E. coli*, as well as by Coates and Hoopes (1980) by investigating 98 strains isolated from calves. Wray *et al.* (1993) found out multiple resistance in most of *E. coli* strains isolated from domestic animals, especially in calves suffering from colibacillosis. According to investigations of Langlois and Dawson (1999), a high resistance prevalence of *E. coli* strains originating from pigs is the result of antibiotics and chemotherapeutics application in prevention and therapy, but also a consequence of stress caused by transport and lower atmospheric temperature (Moro *et al.* 1998).

According to our investigations, the obtained results are completely in accordance with the results of a great number of authors (Murray *et al.* 1986, Wray *et al.*, 1993, Nair *et al.*, 1995; Baggesen and Aarestrup, 1998; O'Connor *et al.*, 2002; Schroeder *et al.* 2002).

Intensive livestock production and application of antimicrobial agents in prophylaxis and therapy considerably influence a higher prevalence of resistant strains isolated in poultry and pigs than of the ones isolated in cattle. Massive application of fluoroquinolone agents in poultry husbandry both in healthy and unhealthy individuals contributed to a high resistance of *E. coli* strains to nalidixic acid and enrofloxacin. The above mentioned points out to the fact that a usage of antibiotics and chemotherapeutics in prophylaxis and therapy in certain categories and species of animals in intensive livestock production leads to selective pressure and elimination of sensitive bacterial strains, and consequently to a higher prevalence of resistant and multiresistant *E. coli* strains in domestic animals.

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**ISPITIVANJE OSETLJIVOSTI I REZISTENCIJE NA ANTIBIOTIKE I  
HEMIOTERAPEUTIKE SOJEVA *E. COLI* IZOLOVANIH OD ŽIVOTINJA GAJENIH U  
USLOVIMA INTENZIVNE PROIZVODNJE**

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SADRŽAJ

U svetu je *E.coli* kao važan patogeni i indikatorski mikroorganizam uključena u sve programe praćenja rezistencije i upravo iz tih razloga je i u našim uslovima sprovedeno uzorkovanje materijala na 42 farme goveda, svinja i živine. Materijal je po uzorkovanju dopreman do laboratorije nakon čega je vršena obrada i zasejavanje primenom uobičajenih metoda mikrobiološke dijagnostike u cilju izolacije i identifikacije sojeva *E.coli*. Na odabranim izolovanim sojevima *E. coli* izvršeno je ispitivanje osetljivosti - rezistencije na sledeća antimikrobna sredstva: ampicilin, tetraciklin, hloramfenikol, streptomycin, neomicin i sulfametoksazol. Nakon dobijanja rezultata sa svake farme iz tri različite kategorije životinja (mlade zdrave, mlade bolesne, odrasle) izdvojeno je po 60 sojeva *E.coli* za ispitivanje osetljivosti – rezistencije na 17 antibiotika i hemioterapeutika. Ispitivanjem je obuhvaćeno ukupno 180 sojeva *E. coli* poreklom od goveda, svinja i živine. U ispitivanju su korišćeni Mueller Hinton agar, antibiogram tablete i diskovi proizvođača Torlak i Becton Dickinson, BBL (USA). Kod izolovanih sojeva *E.coli* prevalencija rezistencije na ispitivana antimikrobna sredstva značajno se razlikovala u zavisnosti od vrste, starosti i zdravstvenog stanja životinja.

Kod većeg broja izolovanih sojeva *E.coli* ispitanih na 17 antibiotika i hemioterapeutika ustanovljena je rezistencija na 15 a osetljivost samo na cefalosporine III generacije i kolistin.