Journal of Animal and Veterinary Advances 11 (24): 4571-4577, 2012
ISSN: 1680-5593

Antibiotic Resistance of *E. coli* and *Salmonella* sp. Strains and Their Tendency According to Isolate Characteristics

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Abstract: The study has been undertaken in the frame of an initiative of WHO to see the activity and efficacy of the antibiotics that are in use and currently available on human medicine service and veterinary medicine service. The methodology of the study has to do with evaluation of resistance against antibiotics of Enterobacteriaceae family circulating in Albania in order to clinically control colibacillosis and salmonellosis antigenic characterization of *Salmonella* sp. and *Escherichia coli* isolated from birds clinically affected by colibacillosis and salmonellosis through serotypization process for epidemiological purposes. The study attracts the attention of the producers of antibiotics to produce antibiotics that have greater aggressiveness against microbes. Resistance of 284 isolates of *E. coli* and *Salmonella* sp. against 14 tested antibiotics. The highest levels of resistance for *Salmonella* sp. was seen for Tetracycline (100%) and Furazolidon (100%). For *E. coli* isolates the highest level of resistance were noticed against Oxytetracicline (98.1%), Tetracicline (97.2%) and Doxyciclin (90.2%). Presence of antibiotic resistance strains of *E. coli* and *Salmonella* sp. in poultry breeding industry in Albania is very high. This fact is a major concern for veterinary public health.

Key words: Microbe, antibiotic resistance, isolation serotype, active principle, antibiograms test

INTRODUCTION

The routine diagnose followed by isolation of *E. coli* and *Salmonella* sp. in laboratories cannot lead in classification or full recognition of circulating strains in a particular territory. Moreover, it cannot be concluded whether these strains behave as primary pathogens or are part of secondary infections which in fact are quite common in intensive poultry breeding. Now a days, breeding birds in Albania has a very heterogeneous character. The development of poultry industry and breeding of many avian species is mainly based in the existence of modern intensive farms with large capacity that often are mixed in a road capillary with another widespread form throughout Albania such as rural breeding, extensive or family breeding complexes. Therefore, further researches aim deepening of knowledge on epidemiology and pathogenesis of colibacillosis and salmonellosis in poultry in intensive and rural breeding in Albania. For this reason, this study will complement the pharmacokinetic and antigenic profile of members of Enterobacteriace family isolated from affected poultry by colibacillosis and salmonellosis but are clinically healthy by taking into consideration:

- Evaluation of resistance against antibiotics of Enterobacteriaceae family circulating in Albania in order to clinically control colibacillosis and salmonellosis
- Antigenic characterization of *Salmonella* sp. and *Escherichia coli* isolated from birds clinically affected by colibacillosis and salmonellosis through serotypization process for epidemiological purposes

This study aims to detail the serotype affiliation, the profile of antibiotic resistance of strains group that belong to Enterobacteriaceae family isolated during 2006 to 2010 period from affected poultry by colibacillosis and salmonellosis, in order to create an epidemiologic dataset on the phenotypic profile of *Salmonella* sp. and *Escherichia coli* that are prevalent in Albanian territory.

MATERIALS AND METHODS

About 284 strains of *E. coli* and *Salmonella* sp. passed the antibiogram test in order to identify their sensitivity or resistance towards selected antibiotics. Selected antibiotics for this test, their amount and concentration of principe actives are presented as follow:

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- 35 μg Amoksicilina (AMX)
- 50 μg Enrofloksacina (ENR)
- 10 μg Gentamicina (GN 10)
- 50 μg Colistin Sulfat (Cl50)
- 30 μg Neomicina (Ne30)
- 30 μg Tetraciklina (TE 30)
- 25 μg Trimetoprim Sulphamethoxasole (STX)
- 10 μg Linkospektia (L)
- 30 μg Flumequina (F)
- 10 μg Norfloxacina (NOR)
- 30 μg Furazolidon (FL)
- 25 μg Chloramphenicol
- 30 μg Oxytetracyclina
- 25 μg Doxyciclina

Majority of antibiotic discs were made possible by OXOID (Unipath Ltd. Basingstoke, UK) while only discs of Enrofloksacine (ENR) were provided by Bayer. According to Bauer and Kirby, the sensitivity and resistance of *E. coli* and *Salmonella* sp. isolates obtained from poultries towards 14 different antibiotics were defined by measuring the inhibition wave of their grow in Mueller-Hinton agar (OXOID), (Bauer *et al*., 1966).

In this study, the diffusion method in agar permits categorization of bacterial isolates in resistant and sensible. The diffusion procedure for testing bacters with rapid growth is standardized (Bauer *et al*., 1966; NCCLS, 2002). The inhibition diameter zone depends on the diffusion rhythm of antimicrobic agent.

Antibiogram test: This test was conducted as follow: Terrain Mueller-Hinton agar was autoclaved and then distributed in Petri's plates with 150 mm diameter. In each Petri's plate was dropped 60-70 mL median in order to form a uniform layer by 4 mm. The 4 mm thickness is important, as a greater thickness may produce false resistant results while thickness <4 mm may influence the growing inhibition size zone (falls-sensible) (NCCLS, 2002). The media pH has to be within the limits of 7.2-7.4 because every value outside these borders may influence in giving false results. The antibiogram test was performed based on routine protocol of NCCLS.

Inoculation microbic burden: The NCCLS standard requires an inoculation by 0, 5 McFarland (Bauer *et al*., 1966; NCCLS, 2002; D'Amato and Hochstein, 1982). There exist two methods to prepare the inoculation. In this study was used direct inoculation of colonies in plates with rigid media (SS agar). Four to five colonies by SS agar dish were planted directed in broth or physiologic solution, in certain amount in order to create a suspension with 0, 5 McF. Plates with media Mueller-Hinton agar are inoculated within 15 min from preparation of suspension. A sterile swab is immersed in bacterial suspension, turning several times and outstanding suspension is removed by easily pressing the tube surfaces. The swab rubbed across the media surface for 3 times by turning the Petri Plate at angles of 60 degrees. Plate left for 3-5 min in order to absurd the excess liquids but not >15 min.

Antimicrobic discs: The amount of antimicrobic agent in the disc, respects the standards of NCCLS (2002). According to this standard, disc diameter is 6 mm and the amount of microbes agent is calculated in a way that inhibition zone for resistant strains to be no <10 mm and for sensitive strains to be not >40 mm. Discs are preserved in a fridge at 2-8°C and placed in the environment temperature one hour before use. Within 15 min from plate inoculation are placed discs with antibiotics. Discs were placed in equidistant from each other with a sterile forceps. In the NCCLS standard disks should be placed at a distance not <24 mm and as a rule they should not be placed >12 discs in Petri Plate with a diameter of 150 mm.

Inoculation: In this study, inoculated plates along with used discs with antibiotics were placed in thermostat at 37°C, per 24 h. Normally, the inhibition zones appear in oval shape. The diameter of inhibition zone was measured with a ruler including the diameter of antibiotic disc and results were expressed in millimeters. Interpretation of results such as resistance, sensible or intermediate are done in accordance with inhibition diameter zone published by manufacturing company of each antibiotic discs. Discs with antibiotics were placed in inoculated plates. After 24 h incubation at 37°C the growing incubation zones were measured and interpreted in full accordance with criteria's established by manufacturing Mueller-Hinton (OXOID) media and Bauer and Kirby techniques. In this study in accordance with reference methodic, all strains with intermediate resistance towards antibiotics panel were considered as resistant strains of *E. coli* dhe *Salmonella* sp.

RESULTS AND DISCUSSION

In this study, as it is shown in the Table 1 and the resistance of 284 isolates of *E. coli* and *Salmonella* sp. towards 14 tested antibiotics, resulted in values that varied from 36.4% (Lyncospectina per *E. coli*) until 100% (Tetracyclina and Furazolidoni per *Salmonella* sp.). The high level of resistance for *Salmonella* sp. strains during the study period (2006 to 2010) was seen mostly towards Tetracycline (100%) and Furazolidon (100%). While for isolates of *E. coli* the high values of resistance

Table 1: Resistance of *E. coli* and *Salmonella* sp. isolates in intensive poultry breeding complexes

	Genres (No. of strains tested)				
	E. coli (183)		Salmonella (51)		
	Resistant		Resistant		Enterobacteri-aceae (284)
Antibiotics	Strain	%	Strain	%	Total (Strain)
Chloramphenicol	162	74.6	31	46.2	284
Norfloxacin	90	41.4	42	62.7	284
Enrofloxacin	142	65.4	42	62.7	284
Doxycycline	166	76.4	54	80.5	284
Colistin	92	42.4	42	62.6	284
Neomycin	124	57.1	41	61.1	284
Flumequin	137	63.1	37	55.2	284
Linco-Spectin	72	33.1	30	44.8	284
Tetracycline	179	82.5	51	76.1	284
Oxytetracycline	180	83.0	49	73.1	284
Trimethosulpha	130	59.9	43	64.2	284
Amoxycillin	154	71.0	48	71.6	284
Furazolidon	103	47.4	51	76.1	284
Gentamycina	136	62.6	53	79.1	284

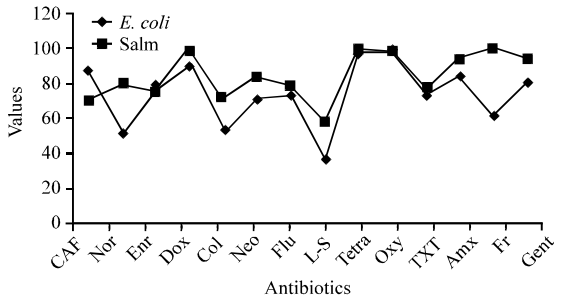

Fig. 1: Distribution of antibiotic resistance in 284 strains (217 isolates of *E. coli* and 67 strains of *Salmonella* sp.) towards the panel of 14 tested antibiotics

were seen for Oxytetraciclina (98.1%), Tetraciclina (97.2%) and Doxyciclin (90.2%). Regarding Amoxicylina, these values were 84.3 and 94%, respectively in isolates of *E. coli* and *Salmonella* sp. Also, high percentage values were seen towards the effectiveness of Gentamicina (80.1% for isolates of *E. coli* and 94% for *Salmonella* sp. strains) and Enrofloxacina (76.9% for isolates of *E. coli* and 74.6% *Salmonella* sp. strains) (Fig. 1).

In this study, the strains of *E. coli* and *Salmonella* sp. resulted amoxicillino-resistant, respectively 83.4% dhe 94%. Amoxiciline is amino-peniciline, part of Maktam Antibiotic class (Neu, 1992; Palmer *et al*., 1976). As result of wide spectrum of its antimicrobic action, good absorbation in organism, tissue penetration and fast bactericid action (Bada-Alambedji *et al*., 2006) this antibiotic finds wide use in veterinary medicine. Antibiotic resistance towards sulphonamides such as trimethoprim-sulfamethoxazole (73.7% per *E. coli* and 77.6% per *Salmonella* sp.) in strains of Enterobacteriaceae family has been reported in many countries all over the world (Bada-Alambedji *et al*., 2006; Zaidi *et al*., 2006; Zhao *et al*., 2007).

Among 14 antibiotics used in this study, Colistin Sulphat showed level of resistence of 52.9 and 72.6% for isolates of *E. coli* dhe *Salmonella* sp. The problem of this antimicrobic substance stays at the fact that many studies bring a low sistemic activity for this antibiotic which in fact is quite contrary with the ekstraintestinal nature of colibacillosis infection in poultry, classifying this medical substance as not selected one for colibacillosis therapy. Regarding the 72.6% of isolates of *Salmonella* sp. researchers can say that Colistine Sulphat is not one the elite antibiotics for salmonella treatment, even though these infections are localized in gastro-intestinal tract of poultry. On the other hand, problematic remains the levels of antibiotic resistance showed against Gentamicine (80.1% for isolates of *E. coli* and 94% for strains of *Salmonella* sp.) which in fact for the reason of non frequent administration in poultry, as results of lack of this product as additive nutrition in polver manner (Shtylla *et al*., 2009) was assumed to had a sensitive reaction on isolates of *E. coli* and *Salmonella* sp.

The almost absolute resistance towards Tetracikline (92.7% for isolates of *E. coli* and 100% for strains of *Salmonella* sp.) is in the same line with previous studies regarding these class of antibiotics in other parts of the world which in fact report the same level of resistance for >90% of strains of *E. coli* isolated from poultries against tetracycline groups (Geornaras *et al*., 2001).

The antibiotic resistance against Tetracyclina is often influenced by continue use and for a long time of this therapeutic substance in poultry breeding industry. Tetracycline is a substance with natural ingredients which is used in animals and poultry for therapeutic and profilactic purposes. Often, Tetracyclina is administered in an empiric way without carrying out the antibiogram test. While in respect of isolates of *E. coli* in poultry data obtained from this study presents similar reports regarding resistance towards Tetracycline groups, sulphamides and aminoglycosides but in high levels, compare with those reported by earlier studies, (Bazile-Pham-Khac *et al*., 1996; Blanco *et al*., 1997; Cormican *et al*., 2001; Saenz *et al*., 2003; Yang *et al*., 2004; Vandemaele *et al*., 2002).

Displayed high resistance showed against Neomycina (70.5%) for isolates of *E. coli* and 83.5% strains of *Salmonella* sp. results four times >20% level reported by Geornaharas.

Flumequine is a synthetic composition of nalidixic and oxolinic acid with a pretty good action against gram negative pathogen microogranisms. For more than three decades this substance is used often for treating infections of urinary tract in humans caused by *Escherichia coli*. In this study, resistance against Flumequine was 74.1% for isolates of *E. coli* and 77.6% for strains of *Salmonella* sp. results that raise concern regarding non effectiviness of this quinolone not only in treating infections in poultry breeding but also for the veterinary public health because this antibiotic is being used in human medicine. Many studies have shown that misuse of antimicrobic substances leads in growing and distribution of antibiotic resistance strains (Levy *et al*., 1976; Van de Bogaard and Stobberingh, 2000) that can be transmitted to humans via food with animal origin or through contacts with animals or poultries that are carriers of these microorganisms. For this problem, number of reports is increasing, detailing circulation and amplification of antibiotic resistance genes including monitoring of antibiotic residues in poultry environment (Sayah *et al*., 2005) which facilitate the spread of antibiotic resistance in microorganisms. Resistance to antimicrobial substances in enteric bacterial pathogens originating from animal food is due to the inevitable use of antibiotics in animal husbandry and poultry industry, especially in developing countries where such use is often applied inappropriately (Threfall *et al*., 2000).

Antibiotic resistance in non typhoid serotypes of Salmonella genres is a worldwide concern. Identification of antibiotic resistance Salmonella strains in foods with animal origin, in many studies is considered to be with frequent use of Fluoroquinolone groups for treating salmonella in poultry. Globalization of food business has facilitated the rapid spread of strains of Salmonella genres which impacts the health of human consumers in different geographic locations (Butaye *et al*., 2006).

Although, antibiotics such as chloramphenicol and trimethoprim-sulfamethoxazoli have not been used since, mid 80s for treating salmonellosis in poultry, many recent studies in different countries classify these antibiotics as substances that encounter resistance at levels over 50%. While antibiotic resistance to Fluoroquinolons has been growing up after 1991 (Chiu *et al*., 2004).

The emergent spread of antibiotic resistance of Salmonella strains is quite disturbing because has implications on hardness and failure of controlling for these invasive infections in poultry flocks. In developing countries, as Albania is classified, it is widespread poultry breeding in the family wards which in fact indicates for higher chance of human contact with birds and their sub products which in fact can lead transmission of antibiotic resistance strains from birds to humans and vice versa. Controlling creation and spread of antibiotic resistant of *Salmonella* strains can be reached only by reducing administration of antimicrobic substances in poultry. Preventive and controlling measures for bacterial infections in poultry should be taken in combination with typhoid and adequate disinfections, rigid application of best practices, hygiene in slaughterhouses and in the processing industry of products with animal origin. Similar results for antibiotic resistance of *Salmonella* sp. isolates against neomycin and tetracycline for prophylactic and auksinic purposes come from Spain as well. In developing countries, an increase in antibiotic resistance for strains of *Salmonella enterica* in poultry has stressed the importance of overuse of different antibiotics such as: chloramphenicol, amoxycylina, trimethoprim-sulfamethoxazole and fluoroquinolone for controlling of Salmonellas in poultry where only 16-40% of isolates were sensible against treatment.

Antibiotic resistance of *E. coli* isolates seen according to breeding type, showed similar results with earlier studies (Guerra *et al*., 2003; Saenz *et al*., 2003) where the level of antibiotic resistance as it is shown in this study as well was several times higher in breeding poultry industry. The high level of resistance in breeding poultry industry come as result of frequent use of antibiotics for controllin colibacillosis and salmonellosis in poultry. Isolates of *E. coli* obtained from intensive poultry breeding expressed high levels of resistance mainly towards Oxytetracicline (98.3%), Tetracycline (77.8%), Chloranphenicol (88.5%), Amoxycicline (84.1%), Enrofloxacine (77.5%) and 78.4% for Flumequine (Table 1). Strains of *Salmonella* sp. obtained by this type of breeding, expressed high value regarding the resistance mainly towards Lyncospektine (100%), Gentamycine (100%), Amoxycicline (100%) and 93.7% for Trimethoprim-sulphametadasole.

Antibiotic resistance levels of *E. coli* varies by characteristics of isolate, length of administration and development of multiresistence against other antibiotics which are all related with the presence of plasmid/factor R. Also, transimission of antibiotic resistance plasmids from poultries to humans has been mentioned by different researchers (Mansouri and Shareifi, 2002).

The obtained results testify for a moderate resistance against antibiotics in rural breeding complexes this is due to the fact that birds in rural complexes are breeded in flocks with low density and the financial cost of antimicrobic drugs is unaffordable for owners of such bussineses. In the meantime, as it is shown in Fig. 2 were identified high levels of antibiotic resistance of *Salmonella* sp. strains, in comparison with isolates of *E. coli*. Resistance of *Salmonella* sp. strains agains Quinolone groups (Enrofloxacine and Norfloksacine) were expressed in almost similar levels either in intensive breeding type as well as in the rural one. This results may

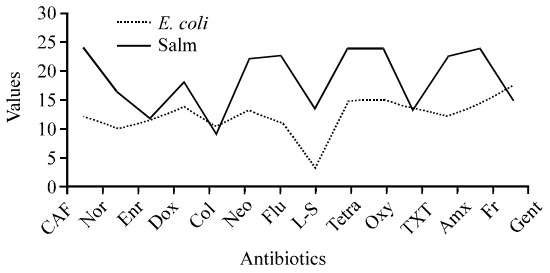

Fig. 2: Resistance of *E. coli* isolates and strains of *Salmonella* sp. in rural poultry breeding

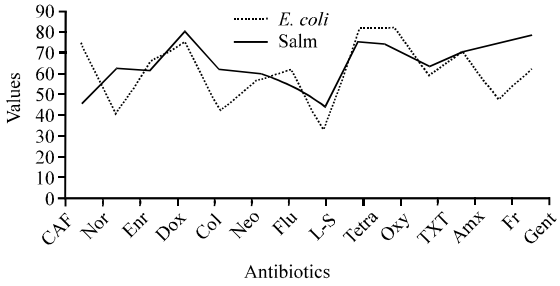

Fig. 3: Trend of antibiotic resistance level during study period (2006 to 2010)

be indicated by direct reports of multiresistance towards an antibiotic class, despite type of poultry breeding.

In this study, the phenomenon of displaying antibiotic resistance simultaneously for Enrofloksacine and Norfloksacine in average values and distribution by type of intensive and rural breeding resulted to be similar. These values, correspond with other studies in this field on the resistance of Enterobacteriaceae family strains toward Quinolone group as in poultry and in humans as well (Alimehr *et al*., 1999) however, it should be noted that this study reveals higher level of resistance compare with those reported by other developing countries. Also, these results are becoming more empirical because in therapeutic treatment of poultry, enrofloksacine is one of the most florinated quinolone with greater use for fighting many infections caused by gram negative microorganisms, linked as well with its bacterial ability and wide antimicrobic specter.

Pharmacokinetics, efficacy and metabolism of quinolone varies for different mammals and poultry species (Anadon *et al*., 1996; Ovando *et al*., 1999). High levels of antibiotic resistance were noticed not only in isolates of *Salmonella* sp. prelevated by intensive breeding poultry but also among those prelevated by rural breeding poultry.

Towards Lyncospectine, Trimetoprim-sulphatmetaxosol and Gentamicine, strains of *Salmonella* sp. prelevated by intensive breeding poultry resulted several fold resistant (100, 93, 7 and 100%), in comparison with strains of *Salmonella* sp. which were sampled by rural breeding poultry (68.7, 56.2 and 62.5%). High rates of antibiotic resistance in *Salmonella* sp. strains, prelevated by intensive breeding poultry compared with those from rural breeding poultry brings to attention the important role of intestinal microbiota some of which are members of Enterobacteriacae family as reservoir of codic plasmids of responsible genes for antibiotic resistance. Furthermore, theser results indicate the difficulty to eradicate the antibiotic resistance in poultry flocks whose organisms are constantly in conctact with antibiotic preparations.

High levels of antibiotic resistance of strains of Enterobacteriaceae family, in both intensive and rural poultry breeding is assumed to come as result of easy access of antimicrobic substances which on the other hand have low price and therefore lead in their widely use in poultry breeding industry.

As matter of fact, the above results are very similar with other studies conducted in many developing countries, results that raise the problematic of inappropriate and misuse of antibiotics in poultry in both breeding complexes. The Fig. 3 presents the progress of antibiotic resistance for 284 isolates of *E. coli* and *Salmonella* sp. during the study period.

As it can be noticed in the above figure distribution level of antibiotic resistance of *E. coli* dhe *Salmonella* sp. strains are caotic which in fact raised doubts about the effectiveness of medicamentation control in cases of colibacillosis outbreak. This significant increase of antibiotic resistance level for *E. coli* and *Salmonella* sp. strains is due to uncontrolled administration and empiric treatment scheme for controlling infections, auxinic purposes and without conducting antibiogram test as well as transferance of resistance among migroorganisms of intestinal microflora. A careful interpretation of risk factors related with appearance of colibacillosis caused by *E. coli* and *Salmonella* sp. antibiotic resistance will serve as a rational tool for empirical therapy, therefore by limiting the evolution of this phenomenon and transferance of responsible plasmids for species of Enterobacteriaceae resistance by animals or foods with animal origin. For this reason, antibiotic selection should be done by using antibiogram test as well following strictly the guidelines on dose and length of administration of medicamentos preparations, in controlling colibacillosis and salmonellosis outbreaks in poultry. The presence of antibiotic resistance of *E. coli* dhe *Salmonella* sp. strains in poultry breeding industry in Albania is very high, widespread in all study areas and in breeding types as well. This fact, makes this phenomenon a major concern for veterinary public health.

CONCLUSION

Resistance of 284 isolates of *E. coli* and *Salmonella* sp. against 14 tested antibiotics, resulted in values from 36. 4% (Lyncospectine per *E. coli*) until 100% (Tetracycline and Furazolidon per *Salmonella* sp.). While sustainability against other 10 tested antibiotics resulted to be at the same leveles. The highest levels of resistance for *Salmonella* sp. during study period (2006 to 2010) was seen for Tetracycline (100%) and Furazolidon (100 %). For *E. coli* isolates the highest level of resistance were noticed against Oxytetracicline (98.1%), Tetracicline (97.2%) and Doxyciclin (90.2%). Towards Amoxicylines these values were 84.3% (*E. coli*) and 94% (*Salmonella* sp.). Also, high levels of antimicrobic resistance were seen for efficacy of Gentamicine (80.1% for isolates of *E. coli* and 94% for isolates of *Salmonella* sp.) and Enrofloxacine (76.9% for isolates of *E. coli* and 74.6% for isolates of *Salmonella* sp.).

High degress of antibiotic resistance in intensive poultr breeding industry is due to inappropriate use of medicamentos preparates for colibacillosis control. Isolates of *E. coli* obtained by intensive poultry breeding displayed high level of resistance mainly towards Oxytetracicline (98.3%), Tetracycline (77.8%), Chloranphenicol (88.5%), Amoxycicline (84.1%), Enrofloxacine (77.5%) and Flumequine (74.8%). *Salmonella* sp. strains obtained from intensive poultry breeding, presented higher level of resistance agains Lyncospektine (100%), Gentamycine (100%), Amoxycicline (100%) and Trimethoprim-sulphametaxosol (93.7%). High level of antibiotic resistance were also observed in isolates of *Salmonella* sp. prelevated by both intensive poutry breeding and rural ones. Towards Lyncospectine, Trimetoprim-sulphatmetaxosol and Gentamicine, strains of *Salmonella* sp. prelevated by intensive poultry breeding were several folds more resistant (100, 93 and 7% dhe 100%), in comparison with those from rural poultry breeding (68.7 and 56.2 dhe 62.5%).

High rates of antibiotic resistance in strains of *Salmonella* sp. prelevated by poultries that were intensively breeded in comparison with their rural counterparts, bring to attention the importance role of intestinal microbiota, part of which are members of Enterobacteriacae family, as reservoir of codic plasmids of genes that are responsible for antibiotic resistance. Results of this study, express the difficulty of eradicating the antibiotic resistance in poultry flocks, organisms of which are continusly in contact with antimicrobic preparates. Obtained results, express a moderate resistance towards antibiotics in rural breeding farms, this due to fact that in rural farms poultries are breeded in low density flocks and the financial cost of antibiotics is unaffordable for owners of such bussineses.

Resistance towards Quinolone group (Enrofloxacine and Norfloksacine) was present in quite similar levels in both breedings (intensive and rural). This result may be indicated by fair reports of multiresistance against one class of antibiotics from majority of *Salmonella* sp. strains despite their origin. Presence of antibiotic resistance strains of *E. coli* and *Salmonella* sp. in poultry breeding industry in Albania is very high, widespread in all geografic study area for both intensive and rural poultry breeding. This fact is a major concern for veterinary public health.

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