ANTIMICROBIAL MULTIPLE RESISTANCE OF AVIAN ESCHERICHIA COLI IN ALBANIA

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Abstract
In this study 101 E. coli from broilers, laying hens, and turkeys death for colibacillosis coming from intensive and rural farms of Albania were tested for antimicrobial susceptibility toward 12 different molecules. An higher level of resistance has been observed for E (100 %) AMX (99, 1 %), TE 30 (96, 07 %), STR (93, 07 %) and N30 (85,15 %). A relevant resistance was detected for fluoroquinolones. Moreover the 73,33 % of *E. coli* resistant to at least one fluoroquinolone were also resistant to the 2 other checked fluoroquinolones. No evident differences were found between the *E. coli* from intensive and rural farms. Multiple antibiotic resistance was expressed by all the E. coli tested. The 23,63 % and 17,39% of *E. coli* from respectively intensive and rural farms were resistant towards all the tested drugs. These data lead to retain possible an incorrect use of antibiotics in poultry farms of Albania.

Key words: E. coli, poultry, Albania, antimicrobial resistance

Riassunto
In questa ricerca 101 stipiti *E. coli* isolati da broilers, galline ovaiole e tacchini morti per colibacillosi e provenienti da allevamenti e rurali dell'Albania sono stati testati per valutare la sensibilità nei confronti di 12 differenti antibiotici.
Un livello di resistenza elevato è stato riscontrato in particolare nei confronti di E (100 %) AMX (99, 1 %), TE 30 (96,07 %), STR (93,07 %) and N30 (85,15 %). Anche i fluorochinoloni (ENR, CIP5, MAR), si sono rivelati spesso inefficaci in vitro. Inoltre, il 73,33% dei ceppi resistenti ad almeno un chinolone manifestava resistenza anche nei confronti degli altri due testati.
Non sono state riscontrate sostanziali differenze tra i ceppi provenienti dagli allevamenti intensivi e quelli rurali. Resistenze multiple sono state osservate in tutti gli *E. coli* testati. Rispettivamente, il 23,63 % ed il 17,39% dei ceppi provenienti dagli allevamenti intensivi e rurali sono risultati resistenti a tutte le molecole testate. Questi dati fanno ritenere che in Albania sussista un uso poco accorto degli antibiotici negli allevamenti di pollame che riduce l'efficacia delle terapie nei confronti di E. coli ed amplifica rischio di immettere sul mercato prodotti con residui di farmaci..

Parole chiave: E. coli, pollame, Albania, antibioticoresistenza

Introduction

Albania is among the third world countries that during the past 15 years has developed a lot in animal production industries. At the present, the poultry production is ensured by six intensive eggs and broilers brands. Moreover, is very common in Albania the poultry rural and free range breeding, often to private consumption.

Colibacillosis is one of more diffused diseases both in intensive and rural Albanian poultry farms. The disease is economically relevant for poultry producers because it causes high mortality, increased condemnations and poor eggs quality respectively in broilers and laying hens flocks. Especially in the rural farms *Escherichia (E.) coli* infections influence seriously the production and the surviving of the birds because the biosecurity and the hygiene are frequently unheeded. The control of the disease is obtained using antimicrobials for therapy and prophylaxis.

Actually the administration of antimicrobials is very common in Albania and is frequently abused and applied without a real veterinary control, both in intensive and in rural farms.

This makes unlike a real reduction of the disease or his effective eradication.

This incorrect use of antibiotics may cause high level of resistance both in the pathogenic microrganisms (Amara et al. 1995) but also in the normal poultry microflora (Allan et al. 1993). These bacteria may also act as a possible source for the transfer of antimicrobial resistance to human pathogens (Bebora et al. 1994).

Currently no data are available on the resistance of *(E.) coli* isolated from poultry in Albania and consequently on the efficacy of the therapies applied for the treatment of the colibacillosis.

In this paper, for the first time, E. coli isolates from intensive and rural poultry farms were checked for antimicrobial resistance toward the antibiotics more frequently used in the therapy of the colibacillosis.

Materials and Methods

E. coli collection. The researches were performed on a total of 101 *E. coli* isolated from broilers, laying hens, and turkeys death for colibacillosis in 36 different intensive (n.7 from laying hens farms, n.5 from broilers farms) and rural poultry farms (n.12 from laying hens and n.13 from broilers/turkeys farms). In particular, n.55 and n.46 *E. coli* come from intensive and rural farms respectively. Birds were sampled from sept 2007 to dec 2008. All the suspected strain were isolated from liver or spleen. Each sample, was plated on Agar Mac Conkey (Oxoid) at 37°C for 24h. All the suspect bacterial colonies were isolated on nutrient agar and confirmed as *E. coli* by the API 20E Test (bioMerieux). Each *E. coli* strain was cultivated on Brucella Broth added with 20% glycerol and kept in criovials at –80°C until the execution of the following tests.

Antibiotic resistance evaluation. Laboratory tests were performed in accordance with the principles described in the standard method of the National Committee for Clinical Laboratory Standards (NCCLS). Drug resistance was tested by checking the *E. coli* isolates with the following range of antimicrobials with the Kirby-Bauer disk diffusion method (Bauer et al. 1966). The following panel of antimicrobial agents was used: 35µg Amoxicillin (AMX), 500µg Ciprofloxacin (CIP5), 50 µg Enrofloxacin (ENR 5), 10µg Gentamicin (GN 10), 50µg Colistin Sulphat (CS), 30 µg Tetracycline (TE 30), 25µg Trimetroprim sulphamethoxasole (STX), 10 µg Lynco-

spectin, 25µg Erythromycin (E), 10 µg Marbofloxacin (MAR), 10 µg Streptomycin (STR), Neomycin (N30, 30 µg).

Results and Discussion

As expected the highest rate of resistance has been against E (100 %). High levels of resistance have been observed for AMX (99,1 %), TE 30 (96,07 %), STR (93,07 %) and N30 (85,15 %). These results are alarming in particular for tetracycline and amoxicillin; these compounds, heavily used in the poultry industry in Albania, are currently of little efficacy in the treatment of E. coli infections in intensive and rural farms.

Gentamicin seems one of the more efficacious molecules, especially in the rural farms (table 1). A medium activity was expressed even from Colistin Sulphate (52,48 %) in comparison with the other very high resistance rates. As known this drug plays its activity in the intestinal tract meanwhile E. coli causes mainly an extraintestinal infection in poultry (Barnes et al. 2003).

The results coming from fluoroquinolones test are very interesting; the avian *E.coli* isolates showed a resistance rate of 74,26 %, 69,31 %, 63,37 % respectively for CIP5, ENR and MAR. Moreover, the 73,33 % *E. coli* resistant to at least one fluoroquinolone were also resistant to the 2 other checked fluoroquinolones.

These resistance levels have been similar in broiler and layer intensively reared, and also in layers of rural farms (table 2). This result may be due to an uncontrolled drug availability even in rural owners.

There is a strong evidence that populations of *E. coli* Ciprofloxacin-resistant were selected in the poultry farms of Albania. This antibiotic is frequently used for the therapy of bacterial infections in humans.

Multiple antibiotic resistance was expressed by all the *E. coli* tested. In the intensive poultry farms the 23,63 % *E. coli* were resistant towards all the tested drugs.

The same multiple resistance *AMX,CIP5,CL50,ENR5,GN10,NE30,STX,TE30,L,E ,MAR,STR* was also detected in 8 *E. coli* strains (17,39 %) from the rural farms. Moreover 11 strains (23,91%) showed were susceptible only to gentamycin.

The high prevalence of multiple resistance may be emphasized by the use of antimicrobials for preventive purpose. This practice is common in Albania and applied sometime to cover falls in the farms management.

Conclusions

The results of this study provide evidence for significant antimicrobial resistance of *E. coli* isolates from poultry and suggest a possible incorrect use of antibiotics both in intensive and rural farms in Albania.

The high resistance rate observed lead to suppose their inefficacy during the outbreaks of the disease.

The inefficacy of fluoroquinolones observed in this study is of relevance; in fact some of these molecules are used for therapy in human.

A more accurate management of the poultry farms is essential to avoid an excessive use of antibiotics which may lead to multiple resistance development especially in birds with a longer production cycle. A strict surveillance of the use of drugs during the production cycles in the poultry farms is fundamental to reduce the risk of residues in eggs and meat.

References
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Table 1. Antimicrobial resistance of avian *E.coli* from intensive and rural farms

Antimicrobial agent (µg)	Intensive farms (n= 55)		Rural farms (n=46)	
	S	R	S	R
Amoxicillin (35)	0,00	100,00	2,17	97,82
Ciprofloxacin (50)	24,45	74,55	26,09	73,01
Colistin Sulphat (50)	54,54	45,46	50,00	50,00
Enrofloxacin (50)	20,10	70,90	32,61	67,39
Gentamicin (10)	46,46	54,54	71,74	28,26
Neomicin (30)	20,00	80,00	8,70	91,30
Trimetroprim S (25)	21,82	78,18	23,91	76,09
Tetracycline (30)	1,82	98,18	6,52	93,48
Lyncospectin(50)	23,64	76,36	21,74	78,24
Erithromycin(25)	0,00	100,00	0,00	100,00
Streptomycin(10)	5,45	94,56	8,70	91,30
Marbofloxacin(10)	34,55	65,45	39,13	60,87

Key words: S = sensitivity; R = resistance

Table 2: Antimicrobial resistance of *E.coli* from broilers/turkeys and laying hens reared in intensive and rural farms

	Intensive farms (n= 55)		Rural farms (n=46)	
Antimicrobial agent (µg)	Broilers/Turkeys *E. coli* (n=25)	Laying hens *E. coli* (n= 30)	Broilers/Turkeys *E. coli* (n=20)	Laying hens *E. coli* (n= 26)
Amoxicillin (35)	100,00	100,00	95,00	100,00
Ciprofloxacin (50)	76,00	73,33	50,00	92,31
Colistin Sulphat (50)	56,00	36,67	70,00	34,62
Enrofloxacin (50)	68,00	73,33	45,00	84,62
Gentamicin (10)	72,00	30,00	10,00	42,31
Neomicin (30)	88,00	73,33	90,00	92,31
Trimetroprim S (25)	88,00	70,00	40,00	88,46
Tetracycline (30)	100,00	96,67	95,00	92,31
Lyncospectin(50)	88,00	66,67	55,00	96,15
Erithromycin(25)	100,00	100,00	100,00	100,00
Streptomycin(10)	96,00	93,33	80,00	100,00
Marbofloxacin(10)	60,00	70,00	35,00	80,77

Key words: S = sensitivity; R = resistance