Multiple antimicrobial resistance among Avian Escherichia coli strains in Albania

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ABSTRACT

In this study, 101 Escherichia (E.) coli isolates from broilers, laying hens and turkeys which had died from colibacillosis, collected from 37 intensive and rural farms in Albania, were tested for antimicrobial susceptibility toward 12 different molecules. The highest levels of resistance were observed for Erythromycin (E) (100%) Amoxicillin (AMX) (99.1%), Tetracycline (TE 30) (96.07%), Streptomycin (STR) (93.07%) and Neomycin (N30) (85.15%). Considerable resistance was also detected for fluoroquinolones. Moreover, 73.33% of E. coli resistant to at least one fluoroquinolone were also resistant to the two other fluoroquinolones checked. No evident differences were found between the E. coli from intensive and from rural farms. Multiple antibiotic resistance was expressed by all the E. coli tested. 23.63% and 17.39% of E. coli isolated from intensive and rural farms, respectively, were resistant towards all the drugs tested. These data would seem to indicate incorrect use of antibiotics on poultry farms in Albania.

Key words: E. coli, Poultry, Albania, Antimicrobial resistance.

RIASSUNTO

ANTIBIOTICORESISTENZA IN CEPPI DI AVIAN ESCHERICHIA COLI ISOLATI IN ALBANIA

In questa ricerca 101 stipiti E. coli isolati da broilers, galline ovaiole e tacchini morti per colibacillosi e provenienti da 37 allevamenti intensivi 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 Eritromicina (ER) (100%) Amoxicillina (AMX) (99,1%), Tetraciclina (TE) 30 (96,07%), Streptomicina (STR) (93,07%) e Neomicina (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 il rischio di immettere sul mercato prodotti con residui di farmaci.

Parole chiave: E. coli, Pollame, Albania, Antibiotico resistenza.
Introduction

Albania is among the developing countries that during the past 15 years have grown hugely in terms of animal production industries. At present, poultry production is ensured by six intensive egg and broiler brands. Moreover, rural and free range poultry breeding are very common in Albania, with dozens of birds being housed not only for private consumption but also for commercial purposes.

Colibacillosis is the most frequently reported disease in the poultry industry (Barines et al., 2003). The disease is economically relevant for poultry producers because it causes high mortality, increased condemnations and poor egg quality, respectively in broilers and laying hen flocks. Especially on rural farms, *E. coli* infections seriously affect production and bird survival since biosecurity and hygiene are frequently unheeded. The disease can be controlled using antimicrobials for therapy and prophylaxis.

Currently, the administration of antimicrobials is very common in Albania. They are used for therapy or prophylaxis, frequently without any real veterinary control, both on intensive and on rural farms.

This may make it unlikely that any real reduction in the disease or its effective eradication will occur.

Such incorrect uses of antibiotics may cause high levels of resistance both in the pathogenic microorganisms (Amara et al., 1995; Blanco et al., 1997) and in 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 colibacillosis.

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

Material and methods

*E. coli* isolates

The research was performed on a total of 101 *E. coli* isolated from poultry (broilers, laying hens, and turkeys) which had died from colibacillosis and collected from a total of 37 intensive (n=12 farms) and rural farms (n=25 farms).

Birds were sampled from September 2007 to December 2008.

Each sample was plated on MacConkey Agar (Oxoid) at 37°C for 24h. Suspected colonies were isolated on nutrient agar and confirmed as *E. coli* by the API 20E Test (BioMerieux). Each *E. coli* strain was inoculated into Brucella Broth added with 20% glycerol and kept in cryovials at -80°C until the execution of the antimicrobial test. All the *E. coli* tested were recovered from the liver or the spleen of the birds examined post mortem.

Antibiotic resistance evaluation

Laboratory tests were performed using the disk diffusion method (Bauer et al., 1966) in accordance with the principles described in the standard method of the National Committee for Clinical Laboratory Standards (NCCLS,1999). The following panel of antimicrobial agents was used: 35 μg Amoxicillin (AMX), 500 μg Ciprofloxacin (CIP5), 50 μg Énrofloxacín (ENR 5), 10 μg Gentamycin (GN 10), 50 μg Colistin Sulphate (CS), 30 μg Tetracycline (TE 30), 25 μg Trimethoprim sulphamethoxazole (STX), 10 μg Lincospectin, 25 μg Erythromycin (E), 10 μg Marbofloxacín (MAR), 10 μg Streptomycin (STR), 30 μg Neomycín (N30).
Results and discussion

As expected, the highest rate of resistance was against E (100%). High levels of resistance were also observed for AMX (99.1%), TE30 (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 on intensive and rural farms.

Gentamycin seems to be one of the more efficacious molecules, especially on rural farms (Table 1). A medium activity was expressed even by Colistin Sulphate (52.48%) in comparison with the other very high resistance rates. This drug is known to be active in the intestinal tract, whereas E. coli mainly causes extraintestinal infection in poultry (Barnes et al., 2003).

The results of fluoroquinolone tests 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, 73.33% of the E. coli resistant to at least one fluoroquinolone were also resistant to the other two fluoroquinolones surveyed.

These resistance levels were similar in broilers and intensively reared layers, as well as in layers from rural farms (Table 1). These results may be due to an uncontrolled drug availability even for rural owners.

There is strong evidence that Ciprofloxacin-resistant populations of E. coli were selected in the poultry farms of Albania. This antimicrobial is frequently used to treat bacterial infections in humans.

Multiple antimicrobial resistance was expressed by all the E. coli tested. On the intensive poultry farms, 23.63% of E. coli were

| Antimicrobial agent (μg) | Intensive farms (n=55) | Rural farms (n=46) |
|-------------------------|------------------------|--------------------|
|                         | 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 Sulphate (50)  | 56.00                  | 36.67              | 70.00                   | 34.62                   |
| Enrofloxacin (50)       | 68.00                  | 73.33              | 45.00                   | 84.62                   |
| Gentamycin (10)         | 72.00                  | 30.00              | 10.00                   | 42.31                   |
| Neomycin (30)           | 88.00                  | 73.33              | 90.00                   | 92.31                   |
| Trimethoprim S (25)     | 88.00                  | 70.00              | 40.00                   | 88.46                   |
| Tetracycline (30)       | 100.00                 | 96.67              | 95.00                   | 92.31                   |
| Lincospectin (50)       | 88.00                  | 66.67              | 55.00                   | 96.15                   |
| Erythromycin (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                   |
resistant towards all the drugs tested.

The same multiple resistance AMX, CIP, 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%) were susceptible only to gentamycin.

The high prevalence of multiple resistance may be emphasized by the use of antimicrobials for preventive purposes. This practice is common in Albania and is sometimes applied to cover up failures in farm management.

Conclusions

The results of this study provide evidence for significant antimicrobial resistance by E. coli isolates taken from poultry in Albania. These are similar to the findings of previous studies carried out in developing countries (Amara et al., 1995; Yang et al., 2004) and suggest incorrect use of antibiotics both on intensive and rural farms.

The high resistance rate observed raises doubts as to their efficacy during outbreaks of disease.

The inefficacy of the fluoroquinolones observed in this study is of relevance; indeed, some of these molecules are also used to treat humans.

More careful management of poultry farms is essential to avoid excessive use of antibiotics, which may lead to multiple resistance, especially in birds with a longer production cycle. Strict surveillance of drug use during production cycles on poultry farms is essential to reduce the risk of residues in eggs and meat.

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