MONITORING THE PRESENCE OF ESCHERICHIA COLI AND SALMONELLA SPP. IN INDUSTRIAL GROWING POULTRY IN ALBANIA

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Abstract

During 2006 – 2010, out of many poultry farms located in different geographic areas within the country (Fier, Kavaje, Durres, Elbasan, Shkoder, Korce, Lezhe and Lushnje) were championed/selected visceral organs and intestinal materials. In this study, were isolated a total of 1.496 strains of *E. coli and Salmonella spp.* The findings obtained from this study, provide a clear picture on the presence, distribution and the behavioral of poultry pathogenosity of *E.coli and Salmonella spp.*, based on the usage of innovative diagnostic methods. Even though, attenuated and live vaccines are continuously produced for immunization of poultry against *enterobacterias*, *salmonellosis* and *colibacillosis*, these diseases remain among the most encountered bacterial infections in poultry industry. Nowadays, poultry breeding in Albania has a very heterogeneous characteristic. The development of poultry industry and breeding of many avian species is mainly based on the existence of intensive modern farms with huge capacities, which often are mixed in another form- widely distributed in country, such as rural breeding, extensive and family ones. Many *in vivo* and *in vitro* studies have not yet clarified the mechanisms with which pathogen enterobacters in poultry are able to cause the infection. The routine diagnose in the field, followed by isolation of *E. coli* and species of Salmonella genres in reference laboratories cannot lead in classification or full recognition of circulative strains in a territory, if it is not performed a differentiation among the present microorganisms in intensive farms and those in rural areas. Foremost, it cannot be concluded the fact whether these strains are acting as prime pathogens or are part of secondary infections, which occur very often in intensive poultry breeding industry.

Key words: poultry, E.coli, Salmonella spp

1. Introduction

Colibacillosis and Salmonellosis are acute and chronic diseases of poultry, with clinical outbreaks in chickens and turkeys. In the poultry industry, colibacillosis are displayed regardless of the type of breeding (rural or intensive), delivering high morbidity and mortality in flocks, and therefore significant economic loss [1,8,11,13,14].

Bacteria of E. coli and Salmonella spp. normally colonize the digestive tract of mammals and poultry [11]. In most mammals colibacillosis is one of the main enteric diseases, while in poultry it is mainly an extraintestinal and systemic disease, with the outbreaks after breaking the host defensive barriers from other primary diseases or as a result of the presence of virulent strains of E. coli residing in the micro intestinal flora of macroorganism [7,8,9,10,16].

Worldwide, for the control and prevention of many bacterial diseases in poultry industry, prevention and treatment doses of antibiotics are commonly used, along with administration of their food ration and / or drinking water. It was noted that this practice has a positive effect on growth of the additional weight and food conversion [18]. Empirical antibiotic management agents in poultry, has exerted a selective pressure, which in itself explains the phenomenon of antibiotic resistance, encountered in a large number of resident bacteria in the organism of birds [2,5,6,12,15].

The antibiotic resistance comes as a result of complex and multifactorial process, which relies on the involvement of cellular genetic elements, which are carriers of the resistance transfer factors. Acquisition of R plasmid codifying genes is due to the exchange of genetic material from one bacterium to another. Some R plasmid may also carry other virulence factors as well, such as bacteriocins, siderofors, citotoxins and adherence factors [3,5,15].

The inappropriate use of Fluoroquinolone in poultry breeding industry promotes the appearance of a cross-resistance to the drug used in the treatment of enteric infections in humans [1,12]. Also, many studies take into consideration the numerous cases of the cross-antibiotic resistance towards the Tetracycline group (Chlortetracyclina, Oxytetracyclina and Tetracycline) in animals and poultry breeding to produce products with animal and human origin [18].

Bibliographic sources present an obvious increase in the occurrence of poultry antibiotic resistance, as a result of uncontrolled use of antimicrobial agents both during drug treatment of many bacterial infections, as well as their use as additives in food rations [15,18].

Moreover, this microbial resistance is similar to E. coli isolated from people who have direct contact with these birds. Such strains are seen to be similar in the possession and expression of virulence factors in humans, as well as in birds. These data provide evidence for possible transmission of resistant microorganisms or plasmids, from poultry to people [5,20].

2. Material and Methods

In the time frame of 2006 - 2010, by poultry farms located in different geographical areas within the territory of Albania (Fier, Kavaje, Durres, Elbasan, Shkoder, Korce, Lezhe dhe Lushnje), were championed/selected visceral organs and intestinal materials.

The materials were randomly selected. It was based on the clinical outbreak cases of colibacilliosis and salmonellas and sporadic reports of infections screening by Bacterologic Laboratory, Food Safety and Veterinary Institute, I.S.U.V.

The pathological material taken from (dead birds) chicken carcasses was used for isolation of E. coli and Salmonella spp. In the beginning, the material was taken by burning the organ's surface to prevent them from mixing with banal flora, and then planted was carried out in the culture plates and differentiation terrains, such as: broth, Endo and McConkey. The planted terrains were placed to be incubated in thermostat with temperature 37 ° C for a period of 24 to 48 hours. Then, the planted cultures were controlled/checked out after a 24 hours of the incubation period.

For E. coli, the differentiating Endo medium will grow the average colony of red shiny metal (the terrain acidification, lactose positive); while in McConkey will grow pink colonies, colonies that are of type S. In microscopic layouts with Gram method will see average gram negative rods which are uniform in size. In order to separate bacterium E. coli from broth cultures, it was transferred in Gasnar and XLD selective terrains, and placed for cultivation in thermostat at 37 degrees C for 24 hours. A typical coliform colony based on morphological characteristics (lactose - positive) through a sterile needle is transferred in a sterile test tube, containing 10 ml broth and placed for incubation at 37 ° C for 24 hours. After incubation, the indol test is carried out by dropping one (1) drop of Erlih solution in the test tube (epruvet parets) filled with broth culture (24 hrs). In positive cases, a red ring creation will be created, on the broth culture surfaces. By selective DC terrain through a sterile needle a colony for the each culture of E. coli is taken and transferred to 10 ml broth Brilliant Green Bile 2% (OXOID), where a Durham bell was previously reversed. The test is considered positive because after 24 hours incubation, was noticed the presence of gas inside the Durham bell. An Enterotube or API 20E system is used for the characterization of E. coli [4, 11, 13].

For Salmonella spp., initially proceeded with the burning of surface organs taken from carcasses with a spatula and then their surface cutting is carried out with scissors in the cube form. The cutting pieces are inoculated in growth and differentiating endo, blood agar and broth terrains and then placed to be incubated in a thermostat at 37 ° C for 18 - 24 hours. After incubation in broth, a diffuse increase will be

seen: in blood agar will be seen small grey shiny colonies, which are of type S; while in differentiating Endo terrain, salmonella colonies are small, smooth and with color of the respective terrain. Agar Mc.Conkey is inhibitory terrain for non enteric microorganisms. Their cultivation in this terrain make possible the differentiation of microorganisms that ferment the lactoze by microorganisms that do not ferment the lactoze. The II-nd phase has to do with transferring of an amount culture taken from a 24 hrs broth culture in selective terrains. SS terrain is inhibitory for non enteric microorganisms. In this terrain will grow only salmonella colonies, which are small, colorless, smooth and with black centers respectively. To identify the casual the API 20 system is used, where the reading of biochemical reactions that occurs in API 20 system is made through respective coding manuals that follow the kits [11,13,18].

3. Results and Discussion

For the purpose of this study, a total of 1.496 E. coli and 378 Salmonella spp., strains were isolated during the period of 2006 to 2010.

The all 1.496 E. coli and 378 Salmonella spp. obtained strains were diferentiated according to years and the presence of E. coli, Salmonella spp. in the isolates which were divided acording to grop's age.

The figure 1 presents the obtained strains divided by years of study period:

The isolates of E. coli (1.496) and those from Salmonella spp. (378) were grouped according to the age group and their obtained source. Therefore, the figure 2 shows the number of isolated strains in chicken eggs, broilers, turkeys and ostrichs. It is important to note that the number of obtained isolates is higher in matured poultry, emphasizing the fact that the poultry lifespan is related with the presence of many infection sources.

As it can be seen from the figures 2 and 3 E. coli and Salmonella spp. isolates are grouped according to their production sort: chicken for eggs or broilers. The aim of this differentiation is to help us for other study objectives, especially those related with antibiotic resistance.

As it can be noticed from the figure 2, during the 2006 – 2010 periods, a total of 1.496 E. coli and 378 Salmonella spp. strains were isolated. The all E. coli strains were isolated from chicken carcasses by colibacilliosis or with similar clinical signs of this infection, as well as from other poultries carrion from other viral infections. While 378 Salmonella spp., strains were also isolated from poultries with clinical signs of salmonella infection or from poultries carrion from other infections.

The obtained E. coli and Salmonella strains were differentiated according to their group-age and production type, which are summarized as follow:

- 705 (47%) isolates of E. coli strains from the chicken eggs belong to the group- age: chicken/birds and mature;
- 675 (43.9%) isolates of E. coli strains from broilers belong to the group- age: chicken/birds and mature birds;
- 208 (84.9%) isolates of Salmonella spp. from chicken eggs belong to the group- age: chicken/birds and mature birds;
- 15 (3.96%) isolates of Salmonella spp. from broiler belong to the group- age: chicken/birds and mature birds;
- 130 (8.68%) strains of E. coli and 42 (11.2%) strains of Salmonella spp. were obtained from turkey;
- Only a small number of E. coli strains (0.26) were isolated from ostriches.

Clear identification and differentiation associated with the presence of commensally E. coli in the digestive tract of poultry is still a problem for the science of diagnostic laboratory of salmonellosis and colibacillosis. This was the motive for undertaking this study by collecting data on the epidemiological situation in the poultry industry according to infections caused by APEC and Salmonella spp. [19, 20].

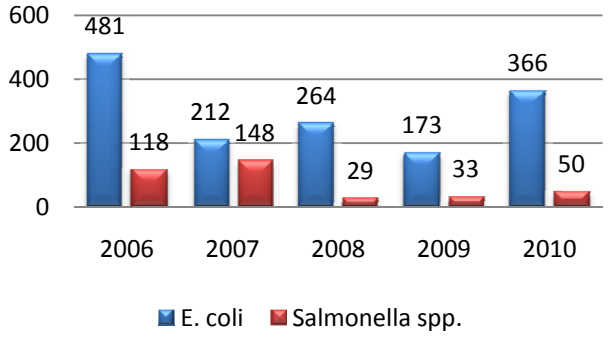

Figure 1: The distribution of E. coli and Salmonella spp. isolates divided according the period of studying.

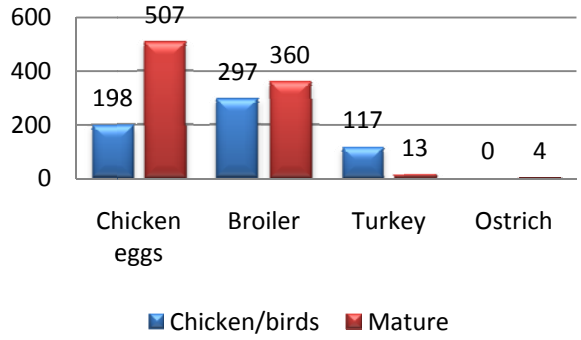

Figure 2: The presence of *E. coli* according to group-age.

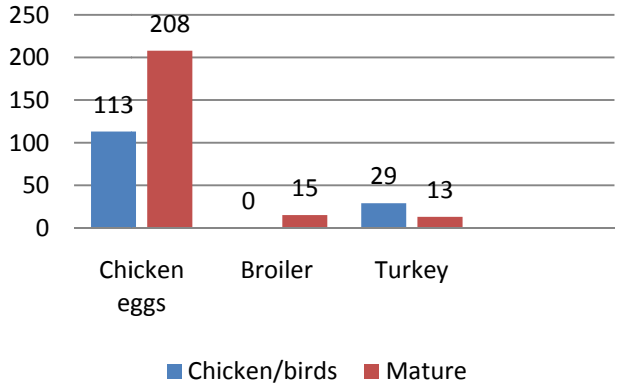

Figure 3: The presence of *Salmonella spp.* according to group-age.

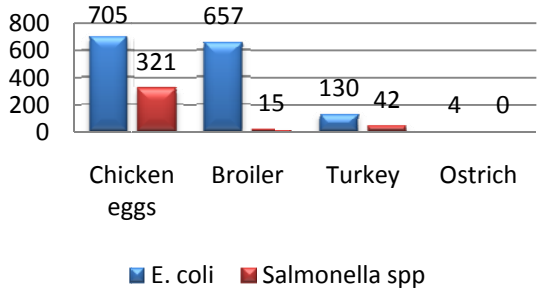

Figure 4: The presence of E. coli and Salmonella spp. isolates according to their production type.

The results of this study provide knowledge regarding the presence, distribution and behavior of E. coli and Salmonella spp., which are pathogenic for poultry, based on the use of innovative diagnostic methods.

Although attenuated and live vaccines are continuously produced for immunization of poultry against salmonellosis and colibacillosis, these diseases remain among the most encountered bacterial infections in poultry industry [17].

4. Conclusions

- The E. coli and Salmonella spp. strains, isolated from analyzed poultry in this study, presented morphological and characteristics typical for Escherichia and Salmonella genus.
- E. coli and 378 Salmonella spp. isolates were obtained from poultries carrioned from colibacilliosis, salmonellosis or other infections with similar clinical signs of these infections.
- 1.496 E. coli and 378 Salmonella spp. strains, served as database for further analyzing, regarding with their serotypisation and antibiotic resistance.

5. References

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