Monitoring of the epidemiological situation of avian salmonellosis in poultry marketing units

Olga JUNCU, Nicolae STARCIUC, Natalia OSADCI, Tatiana ANTOHII
State Agrarian University of Moldova
MD- 2049, Republic of Moldova, Chișinau, str. Mircești 44, n.starciuc@uasm.md

Abstract
The aim of the proposed investigation was to establish the bacterial microflora present on poultry carcasses and eggs sold in the food network of the central agricultural market from Chisinau; especially, bacteria of the genus Salmonella spp. During the investigation was studied the type and number of bacterial colonies and was serotyped the bacteria of the genus Salmonella spp. At the same time was performed the sensitivity of the isolated microflora to some more frequently antibiotics used in poultry farms. The research samples were taken from refrigerated carcasses of broilers chikens and eggs from poultry farms from the republic. The investigation's result confirmed the presence of an associated microflora with Srteptoccocus, Staphylococcus, E. Coli and bacteria of the genus Salmonella spp. in poultry carcasses and eggs of current consumption. From the total number of samples taken, in 12% of them was detected the bacteria of the genus Salmonella spp. The serotyping confirmed the presence of the following Salmonella spp. serotypes: S. Infantis, S. Enteritidis and S. Typhimurium. The antibiotic resistance tests confirmed a low sensitivity of the isolated microflora to some of the most common antibiotics used in birds' raising. The area of inhibition ranged from 8mm to 0 mm in most tested antibiotics; the most sensitive antibiotic proved to be florfinicol with a maximum inhibition area of 18 mm. The obtained results demonstrate the presence of some pathogenic serotypes of Salmonella spp. which can have a major risk for public health.
Key words: *chickens, carcasses, antibiotic resistance, contamination, samples, serotyping.*

Introduction

Chicken meat is one of the highest consumed meat across the globe. Global Livestock Counts report that there are almost 19 billion chickens in the world, making it the most common species of birds. Nowadays, worldwide, poultry meat occupies an important place in human nutrition due to its benefits in terms of biological components. Compared to meat produced by other domestic animals, poultry meat has varios advantageous aspects which will be further discussed. Chickens have a low body weight which allows to obtin fresh meat production in a short timeframe. Poultry meat and organs represent a rich source of mineral salts and vitamins. From biochemical aspect, this type of meat contains all the essential amino acids necessary for the human nutrition [7,10].

However, there are also potential risks associated with the consumption of poultry meat because it can be contaminated with various conditionally pathogenic or pathogenic micro-organisms. These micro-organisms may be present in the finished products, meat or eggs, following the contamination during the growing chain or slaughter, storage and marketing as well as during preparation. Compliance with all veterinary health rules, technological maintenance and feeding indices are the main criteria in maintaining the health of poultry flocks as well as reducing the risk of transmitting communicable diseases to humans, either through direct contact with poultry flocks or through contaminated poultry products (meat and eggs). Currently, more than 200 diseases are included in the category of zoonoses of which over 60% are diseases with infectious etiology [2,6].

Protecting poultry from contamination with unwanted microorganisms is an essential component of the poultry industry. The application of daily biosecurity procedures represent the best management practices on poultry farms as they contribute significantly to reduce the possibility of contacting zoonotic microbiological infections such as *Salmonella* and

C*ampylobacter*, as well as other infectious diseases such as avian influenza, newcastle disease, Gumboro diseaseand et all [5,9].

Taking a retrospective of bacterial diseases, in the group with a major risk to the poultry health, but especially to human health, bacteria of the genus *Salmonella spp*. represents up to 25% of foodborne infections causes in humans due to poultry contaminated with this genus of bacteria. Although it is known that there are currently about 2500 different *Salmonella* serotypes, only about 200 serotypes are associated with foodborne infections in humans. Vertical transmission of Salmonella serotypes from breeding flocks to commercial poultry flocks was analyzed for two of the most important serotypes, *Salmonella Enteritidis* and *Salmonella Typhimurium*. However, recently, disimination of these two serotypes have been reduced in many countries due to the introduction of strict biosecurity measures, effective surveillance, but also because vaccinations has been introduced; which, usually causes some difficulties in serologic monitoring system of salmonellosis. General veterinary sanitary measures are a good start, but they may not be enough to completely eliminate the infection in most situations. Still, a major importance of avian salmonellosis is to prevent the contamination of poultry products with this type of bacteria through the prism of systematic bacteriological investigations on poultry meat and the exclusion of its penetration into the public alimentation [1,8].

Objectives

In this context, our scientific research activities have focused on monitoring the epidemiological situation of avian salmonellosis and contamination of poultry products with bacteria of the genus *Salmonella spp*. and establishing the presence and diversity of pathogenic serotypes of the genus *Salmonella spp* [3,4].

Materials and methods

Samples were taken from the carcasses of birds sold in the Central Agricultural Market of mun. Chisinau and from eggs for current consumption. In total, were collected 80 samples from the poultry carcasses and 40 from eggs of current consumption. The samples from the poultry carcasses were delivered from the following poultry units: “Valcovschii Iurii”, Ialoveni district, v. Dmbreni; GŢ “Marandici Serghei”, Teleneşti district, v. Mîndreşti; SRL “Dobrocolischii”, mun. Chisinau, v. Singera; GŢ “Goroşenco Angela”, Anenii Noi district, v. Chetrosu; SRL "Viamar", Teleneşti district, v. Mîndreşti; II ”Gachiuţa Elena, Ialoveni district, v. Ulmu; SRL ”Lutam Com”, SRL “Primantol Grup”, SRL “Genevis Grup”, SA “Floreni”, SRL “Margaritar Impex”, ÎI Poperecnaea Elena ”, SRL“ Procolnis ”. The examinations were performed in the laboratory of microbiology of the faculty of Veterinary Medicine, SAUM and in the laboratory of microbiology of the Animal Health and Welfare Department of the Republican Center for Veterinary Diagnosis. In laboratory conditions the inoculations were performed on culture media as: Nutrient agar, Endo Agar, Salmonella Shigella Agar (SSA), Sabouraud Dextrose Agar, Bismuth sulfite agar (BSA). The presence and morphological structure of bacterial colonies grown on culture mediaserved as monitoring indicators. Subsequently from the bacterial colonies were prepared smears that were stained using the Gram method and examined under a biological microscope, 10x100 objective. Serotyping of bacteria of the genus *Salmonella spp*. was performed in the Republican Center for Veterinary Diagnosis.

The washes from the consumer eggs were taken from the units specialized in the eggs production and sold in the Central Agricultural Market of mun. Chisinau, delivered from poultry companies as: SRL “Intervetcom”, Cimişliea district, SRL Redi Agro, Donduşeni district,

v.Tîrnova, SRL Dant Agro, Ungheni district, v. Pîrliţa, SRL "Solar Nord", Edineţ diastrict, v.Gordineşti, SRL Avicola Rişcani, v. Corlăteni, SRL Pasărea Silver ", mun. Chisina, v. Ciorescu.

Results

The laboratory investigations were focused on the microbiological investigations done in order to determine the level of contamination of bird carcasses and eggs with the bacterial flora. These investigations were afterwards combined with the microscopic investigations to establish the identity and association of bacterial forms isolated from examinated samples, and performe antibiogamas of the isolated microflora to more common antibiotics used in birds grows. Some of the of laboratory research results are presented in figures 1-9. Figures 1-4 show some of the colonies of microorganisms that have predominantly grown on culture media.

Fig. 1 Associated colonies of *Strepto* and *Staphylococcus* (the peptone agar medium)

Fig. 2 *E. Coli* colonies on the Endo medium

As a result of bacteriological investigations was established that from all samples taken from poultry carcasses and the current consumption eggshells the isolated microflora was associated with predominance of bacterial forms as *E. coli, Salmonella spp., Streptococcus* and *Staphylococcus.* Figure 1 shows the colonies of *Streptococcus* and *Staphylococcus* that have a round or oval shape, white-gray color, placed separately or in piles, being spread over the entire surface of the Petri dish, with predominantly central localisation. Their number ranged from 105 to 215 colonies. An intensive growth of *E. coli* colonies was present simultaneously in all samples examined (Fig. 2), the color of the colonies varying from red with intensity up to burgundy, with metallic luster, placed on the entire surface of the Petri dish, numerically they constituted from 78 to 155 colonies.

Fig. 3 Colonies of *Salmonella spp*. on the SSA medium (pure culture)

Fig. 4 Colonies of *Salmonella spp*. on the BSA medium

In the examined samples *Salmonella spp*. colonies increased from moderate to intensive growth. Some of the results of Salmonella spp. colonies are shown in Figures 3 and 4. On the Salmonella Sigiella Agar medium, *Salmonella spp*. colonies have a dark brown color, with a more intense center and a lighter periphery, placed evenly on the surface of the Petri dishes, with a numerical variation from 168 to 374 colonies.

At the same time, were prepared the smears from the colonies of microorganisms and examined under a biological microscope. The results of this study are shown on figures 5-8. Figure 5 and 6 show bacterial forms as *Streptococcus* and *Staphylococcus* isolated from the eggshells and from chicken carcasses. They were dispersed almost uniformly throughout the microscope field, placed in a chain, separated one by one, in groups of two or in piles in different shapes, having a spherical shape and blue color.

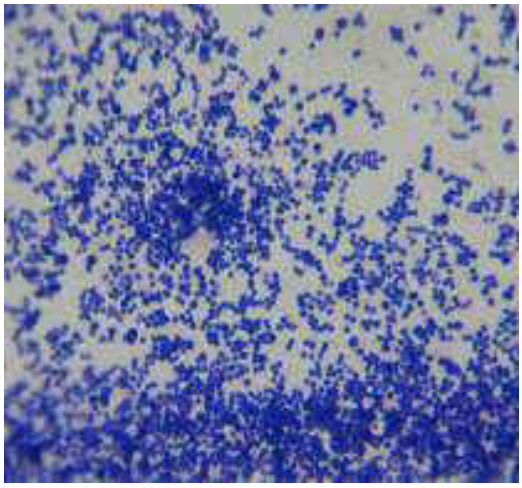


Fig.5 *Streptococcus* and *Staphylococcus* (colonies on nutrient agar, ob. 10x80)

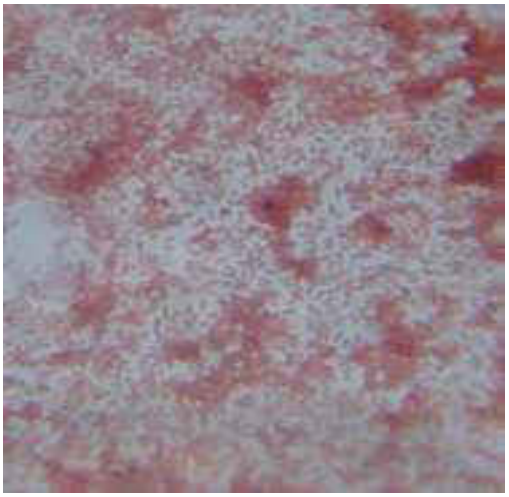


Fig. 6 *Salmonella spp* (colonies on the medium SSA, ob. 10x80)

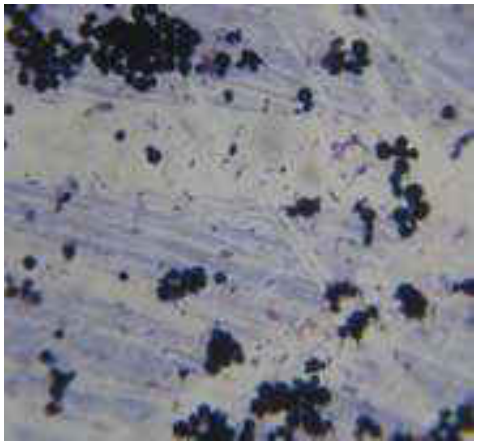


Fig.7 Forms of *Streptococcus*, *Salmonella spp*., (colonies on SSA medium, ob. 10x80)

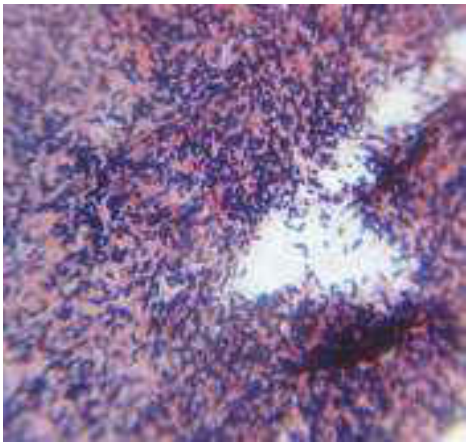
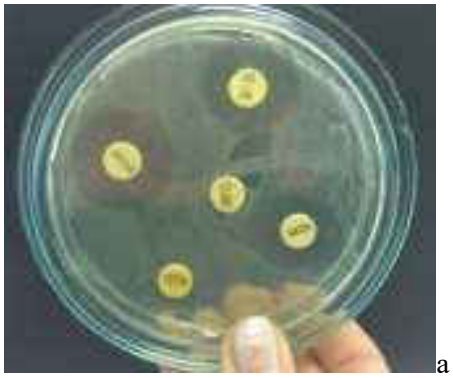


Fig. 8 Fungal and yeasts (colonies on *E.coli*, Sabouraud Dextrose Agar, ob. 10x80)

When the smears were prepared from microbial colonies wich have grown on the Salmonella Shigella agar environment (Fig. 6), the microorganism colonies were associated with bacteria of the genus *Salmonella spp*., *E coli*, which was shaped like sticks, with pink color and oval form. Figure 7 and 8 show some forms of fungi and yeasts in association with *Streptococcus* bacteria. In this case, the smears were prepared from the colonies that have grown on the SSA and Sabouraud Dextroze Agar.

Microbiological investigations performed on poultry carcasses and eggs sold in the Central Agricultural Market of mun. Chisinau show that from the total number of samples taken, 12% of the samples demonstarte the presence of colonies of *Salmonella spp*. Serotyping of *Salmonella spp*. cultures confirmed the predominant presence of serotypes *S. Infantis, S. Enteritidis, S. Typhimurium*.

Subsequently, the isolated bacterial flora was tested for sensitivity to some antibiotics that are more commonly used in poultry farming.



a

b

Fig. 9 a) and b) Antibiogram (area of inhibition of microorganism colony growth)

As a result of this study has been established a moderate sensitivity of the isolated microflora to some antibiotics that have the following parameters: Eurosol 5mg - 4 mm, Florfenicol

30mg - 18 mm, Oxytetracycline 30mg - 8mm, Genta plus 10mg - 3mm, Tilmivap 15mg - 0 mm. The results of this study is represented in figures 9 a) and b. The most sensitive antibiotic against the isolated microflora proved to be Florfinicol, where the inhibition zone of bacterial colonies was 18 mm, and the lowest action was obtained using Tilmivap having an index of 0 mm.

Conclusions

1. The results of the research demonstrate the presence of pathogenic serotypes of *Salmonella spp*. in poultry products; therefore, confirming the existence of risks of contamination of poultry products at some stages of production, processing or marketing, which thus favors the occurrence of toxin infections in humans.
2. The bacteriological investigations of poultry carcasses and eggs of current consumption indicate the presence of an associated microflora which was represented by bacterial forms as: *Srteptococcus, Stafilococcus, E. Coli* and bacteria from genus *Salmonella spp*.
3. From the total number of examined samples, in 12% of them was isolated the bacteria of genus *Salmonella spp.* and the serotiping procedure confirmed the presence of serotipes as: *S. Infantis, S. Enteritidis and S. Typhimurium.*
4. The bacterial forms isolated from the poultry carcasses as well as the common consumption eggs have shown a reduced sensitivity to some antibiotics commonly used in poultry grows, however a higher sensitivity was determined using Florfinicol which demonstrated a maximum inhibition area of 18 mm.

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