RISK ASSESSMENT OF SALMONELLA CONTAMINATION OF POULTRY CARCASSES DURING TRADING PERIOD

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Abstract
The goal of the proposed research was to determine the load and diversity of bacterial flora of poultry carcasses delivered to the Central Agricultural Market of Chisinau from different poultry companies inside the country and analyze the risks of contamination of carcasses with pathogenic microorganisms. Samples were taken from refrigerated and frozen poultry carcasses (chicken, broiler, duck) which were delivered for sale from private and individual poultry farms from different districts of the country. Samples were performed on nutrient media like: peptone agar, nutrient medium Endo, Levin, Salmonella Shigela Agar, Saburo agar. The culture medias were inseminated from the surface as well as the inside of the carcasses samples. As a result, the bacteriological investigations demonstrated the presence of various combinations of conditional pathogenic micro-organisms in all samples collected from the surface of poultry carcasses and 35% from samples collected from the inside of the carcasses. The associations found where: Streptococcus, Staphylococcus, E. coli, Salmonella and microscopic fungal. The result of the antibiotic resistance test confirmed the presence of antibiotic-resistant forms of bacteria; thereby, the highest sensitivity of the isolated microbial flora from poultry carcasses was established for florfinecol (27mm) and tilmycosiny (19mm) and the lowest sensitivity was established for neomycin and tetracycline (0-2mm).

Key words: *antibiotic resistance, microbial colonies, culture media, samples.*

Introduction

The consumption of poultry meat is very important for the human body as it provides a moderate energy content, has highly digestible proteins of good nutritional quality, unsaturated lipids, B-group vitamins and minerals which makes it a valuable food. Therefore, it is very important for it to be derived from slaughtered poultry species, which have not undergone any treatment except treatment of cooling - refrigeration or freezing. The meat quality in terms of hygiene is an essential factor which can be affected by contamination with pathogenic microorganisms or chemical pollution. The freshness and the hygienic condition of the meat are the first features that induce its consumption. Sanitation of meat is an essential requirement before it can be prepared and consumed; however, current technologies for slaughter do not provide products free of pathogens (3,6). Theoretically, a healthy and rested bird must not contain in the muscles and internal organs bacterial flora. In practice, this condition cannot be achieved because the contamination sources of poultry meat are multiple and difficult to remove completely; therefore, to obtain a sterile meat production is not possible (1,7,10). The most common sources of microbial contamination of chicken could be water, air, bird feathers, equipment and tools, insufficiently cleaned and disinfected vehicles.

Despite the "healthy" image that consumers get, often, poultry is contaminated with pathogens like Campylobacter spp. and Salmonella spp. The pathogens found in poultry meat in order of frequency are: Salmonella enteritidis, Campylobacter jejuni, Yersinia enterocolitica, Clostridium perfringens, Staphylococcus aureus, Listeria monocytogenes, and some species of Bacillus (2,6,9). Although Salmonella is recognized as the most important pathogen associated with poultry meat, it is estimated that Salmonella illness occurring because of poultry consumption is approx. 20-25% of all cases of salmonellosis (2,4,5,8). The evolution of microorganisms that

contaminate meat is dependent on many factors; thus, reducing the level of contamination with pathogens can be achieved only by respecting measures focused on adopting good working practice codes, having standard operating procedures for sanitation and the preventing dangers associated with critical points of contamination (3,11). Taking into consideration the above, the aim of our scientific investigation was to establish some of the microbial indices of the poultry carcasses sold in the Chisinau Central Agricultural Market and to perform a risk analysis of possible contamination of carcasses with pathogenic microorganisms.

Material and methods

As a research material served meat samples taken from carcasses of poultry (chickens, broiler, duck) which were delivered for sale to the Chisinau Central Agricultural Market from various poultry farms as well as from private and individual breeders from different districts of the country (IE "Valcovschii Yuri " Ialoveni district, v. Dmbreni; GT" Sergei Marandici " Telenesti district, v. Mindresti, LLC" Dobrocolischii " mun. Chişinau, v. Sîngera; GT" Angela Goroşenco " Anenii Noi district, v. Chetrosu, LLC "Viamar", Telenesti district, v. Mindresti, IE "Gachiuţa Elena, Ialoveni district, v. Ulmu, LLC" Lutam Com ", LLC " Primantol Grup ", LLC " Genevis group ", AC" Floreni " LLC "Margaritar Impex, IE "Poperecnaea Elena ", LLC " Procolnis "). There were used random methods to take samples from various parties of refrigerate and frozen carcasses which afterwards were put in sterile polyester bags and shipped to the laboratory for examination.

In addition. samples were taken also from tables, tools and scales. In total, during the period from May 1^{-st} 2018 to August 1^{-st}, 2018 were collected 72 samples. The samples was examined at the Republican Veterinary Diagnostic Center, mun. Chisinau and in the laboratory of Microbiology, department Clinics II, of Veterinary Medicine Faculty of SAUM. The samples were performed on nutrient media like: peptone agar, nutrient medium Endo, Levin, Salmonella Shigela Agar, Saburo agar. After 48 hours of incubation in a thermostat at temperature of + 370C, were analyzed the type and morphology of microbial colonies of the samples, were prepared the smears from colonies stained by Gram method and examined on biological microscope (10x80) and was studied the sensitivity of isolated microorganism towards some commonly used antibiotics in poultry industry.

Results and discussion

Culture media were inseminated with the samples taken from the surface as well as the inside of the carcasses. After 48 hours of incubation was examined the morphological characteristics of the microorganism's colonies. Some results of the bacteriological investigations are shown on the figures from 1 to 10, which were randomly selected from the total number of microbial cultures on Petri plates.

On the fig.1 and 2 are presented Streptococcus colonies which have spherical or oval forms, with white or gray color and glossy surface, placed on Petri plates separated or fused. There can be observed an increased number of colonies of Streptococcus on both parts of the Petri plates when inseminations were carried out from the surface and from the inside of the carcasses samples. In the case where inseminations were carried out from the surface of the samples, the number of colonies of Streptococcus varied within the limits from 67 to 178, and in the cases from the inside of the samples the colonies number varied from 11 to 126.

Figure 1 Colonies of *Streptococcus* (insemination from the surface and inside of the carcasses, on peptone agar).

Figure 2 Colonies de *Streptococcus* on peptone agar (multiple colonies from the surface of carcasses and unique - from inside)

In cases when the inseminations were performed on the bismuth sulfite agar (fig. 3 and 4) from all samples was established an increased number of colonies of Salmonella, with spherical and oval forms placed in form of chains or separate, whose number ranged from 66 to 168 colonies in samples taken from the surface of carcasses and from 12 to 57 colonies in the those from inside of carcasses.

Figure 3 Colonies of *Salmonella* (medium bismuth sulfite agar), insemination from the surface and inside of the carcasses.

Figure 4 Colonies de *Salmonella* (medium bismuth sulfite agar), analogical intensity of colonies' growth from the surface and inside of the carcasses.

On Salmonella Sighella Agar (fig. 5 and 6), the number of Salmonella colonies varied within 89 to166 colonies from samples taken of the surface of carcasses and from 0 to 42 colonies on those taken from the inside of the carcasses.

Figure 5 Colonies of *Salmonella* (medium SSA), insemination from the surface of the carcasses.

Figure 6 Colonies of *Salmonella* (medium SSA), insemination from the surface and from the inside of the carcasses.

On the medium Saburo (fig. 7 and 8), the microscopic fungal colonies increased particularly in samples taken from the surface of the carcasses, they have white or gray color and oval or round forms, fluffy and overgrown on the surface of the culture medium. The number of the fungal colonies on the surface taken samples varied within 80 to194 colonies and 0-21 colonies on the inside taken samples.

Figure 7 Fungal colonies (medium Saburo), insemination from the surface of the carcasses.

Figure 8 Fungal colonies (medium Saburo), insemination from the surface and inside of the carcasses.

An intensive growth of E. coli colonies was established on the nutrient medium Endo (fig.9 and 10). Morphologically the colonies were dark-red color with metallic glossy aspect. The number of colonies was significant in both situations, when the inseminations were performed from the surface as well as the inside samples, their number ranged from 45 to 188 colonies on surface samples from carcasses and from 17 to 76 colonies on the inside of carcasses samples.

Figure 9 Colonies of E. coli (medium Endo), insemination from the surface and inside of the carcasses.

Figure 10 Colonies of E. coli (medium Endo), analogical intensity growth of the colonies from the surface and inside of the carcasses.

From microbial colonies were prepared smears which were stained by Gram method. On fig.11 is shown the combination of microbial flora consisting of Streptococcus, Staphylococcus, which are gram-positive and placed separate, in chain or in piles. There are also some fungal filaments and hyphae. The associated micro flora consisting of E. coli, Salmonella (pink color) and Streptococcus (blue color) is shown in fig. 12.

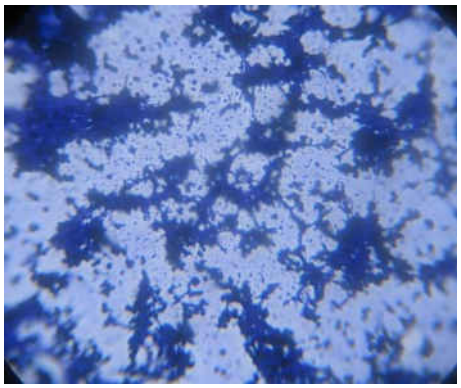

Figure 11 Associate microbial flora (*Streptococcus, Staphylococcus,* and fungal), ob. 10x80.

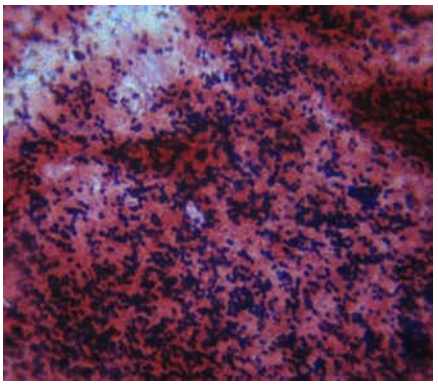

Figure 12 Associate microbial flora (*Streptococci, Stafilococi E. coli, Salmonella),* ob. 10x80.

The antibiotic resistance test of isolated microbial flora (fig. 13) demonstrated that the highest sensitivity of the isolated microbial flora from poultry carcasses was established for florfinecol (27mm) and tilmycosiny (19mm). The lowest sensitivity was established for neomycin and tetracycline (0-2mm).

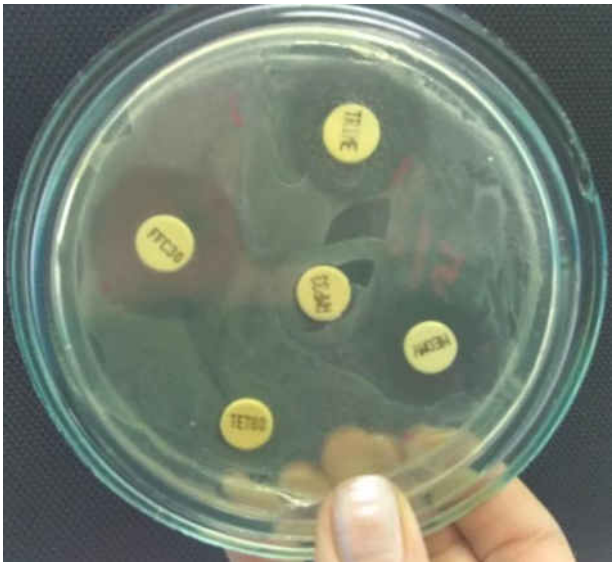

Figure 13 Antibiotic resistance test.

Conclusion

1. The bacteriological investigations demonstrated the presence of various combinations of conditional pathogenic micro-organisms as association of Streptococcus, Staphylococcus, E. coli, Salmonella and microscopic fungal in all samples collected from the surface of poultry carcasses and 35% from samples collected from the inside of the carcasses.
2. Isolation of Salmonella and E. coli colonies present in the inside of meat samples indicate that the is a high level of risk that food intended for consumption can be contaminated with pathogens. Consequently, there should be a more careful monitorization of the poultry growth process, slaughter, and transportation and marketing practices of poultry carcasses that would help to establish the critical points leading to the contamination of carcasses with pathogens.
3. The result of the antibiotic resistance test confirmed the presence of the antibiotic-resistant forms of bacteria showing that the highest sensitivity of the isolated microbial flora from poultry carcasses was established for florfinecol (27mm) and tilmycosiny (19mm) and the lowest sensitivity was established for neomycin and tetracycline (0-2mm).

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