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St.Aureus And Listeria Spp. In Chicken Meat In Slaughterhouses And At Retail Shops In Tirana

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***Abstract*—Chicken is a nutritious, healthy food, which is low in fat and cholesterol compared to other meats but an excellent source of protein. Meat must be of a high microbiological quality in order to ensure that the consumer receives a product that is not spoilt, or does not carry food-borne disease. Food borne diseases associated with the consumption of poultry meat and its processed products are of public health significance worldwide. This paper investigated the incidence of poultry meat contamination with Listeria spp and St. aureus in the slaughterhouses and retail shops in Tirana during different seasons.**

Keywords—poultry carcass; microbes; chicken meat; microbial contamination

I. INTRODUCTION

Pathogens in Poultry Meat

Poultry and poultry meat are often found contaminated with potentially pathogenic microorganisms such as Salmonella, Campylobacter, S.aureus, E.coli and Listeria. In some occasions also Yersinia enterocolitica, Aeromonas and Cl. Perfringens have the potential to be important pathogens in poultry products. However, Salmonella, Campylobacter and to a lesser extent Listeria, are considered to be the major food-borne pathogens in the poultry industry. The meat surface do not normally, inherently contain pathogenic organisms but can acquire the organisms from fecal matter or from cross contamination during slaughter.

The organisms tend to remain on the surface or just under it. Meat is an ideal medium for bacterial growth, because of high moisture content, richness in nitrogenous compounds (essential amino acids, proteins), good source of minerals, vitamins and other growth factors. Furthermore, its pH is favorable for the growth of microorganisms. The water activity (aw) of poultry meat is about 0.98 to 0.99 depending on, if and how long the meat has been stored in dry air. The pH of chicken breast muscle is 5.7 to 5.9, while that of leg muscle is 6.4 to 6.7. Both poultry muscle and skin are excellent substrates for supporting the growth of a wide variety of microorganisms (ICMSF, 2005).

Staphylococcus aureus is one of the most common agents in bacterial food poisoning outbreaks and symptoms of staphylococcal food intoxication generally occurs within one to six hours after the ingestion of food and the common symptoms are nausea, vomiting, abdominal cramps, and diarrhea (Adwan et al., 2005). Contamination with *S. aureus* is important in the evaluation of safety and hygienic quality of chicken meat, but also in the aetiology of food poisoning (JABLONSKI and BOHACH, 1997). Epidemiological reports all over the world incriminate poultry meat as a source of outbreaks of human food-borne disease. Since poultry meat is not usually consumed raw, these outbreaks are caused by undercooking or cross-contamination of ready-to-eat products with microbial contaminants from the raw poultry or others, introduced during preparation of the food.

Ubiquity of bacteria of the genus *Listeria* is an important factor influencing the possibility of poultry meat contamination. Presence of *L. monocytogenes* in fresh broiler meat varies from 0% to 64% (LONCAREVIC et al., 1994). ŽIVKOVIĆ et al. (1997b) have isolated *Listeria* spp. in 27.8% of fresh chicken samples. To the authors knowledge there is no information on the incidence of Listeria spp contamination in poultry meat in Tirana.

The aim of the poultry industry is to find ways to avoid contamination of live poultry and poultry products with potential pathogens. Furthermore they should be able to deliver live poultry free of pathogens to the processing plant.

Description of the organism

S. aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the Staphylococcus genus. S. aureus produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (Montville and Matthews 2008; FDA 2012 The growth and survival of S. aureus is dependent on a number of environmental factors such as Temperature °C (bacterial growth) Optimum, 37 Range4-48. Enterotoxin Production Optimum 40–45,Range 10-48°C. water activity (aw) Bacterial Growth optimum 0.9 range 0.83–099.Enterotoxin Productio optimim 0.98 range 0.87–, pH Bacterial Growth optimum 6-

7range 4-10, Enterotoxin Productio optimim 7-8,range 4.5-9.6, the presence of oxygen and composition of the food (refer to Table 1). These physical growth parameters vary for different S. aureus strains (Stewart 2003). S. aureus is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart 2003)

L.monocytogenes is present in soil, water, vegetables, and intestinal contents of a variety of birds, fish, insects and other animals. Human listeriosis is a sporadic disease, which is associated with consumption of contaminated milk, soft cheese, under-cooked meat, unwashed raw vegetables and cabbage (Schuchat et al 1992).

II. Materials and Methods

A. *FOOD-SYSTEM*

Food system is a 24-well system containing desiccated biochemical substrates and culture media for detection and presumptive identification of microorganisms from meat, milk and cheese, fish and other food products.The system provides detection and presumptive identification of:
Salmonella spp., Proteus spp. / Providencia spp., Pseudomonas spp., Staphylococcus aureus, E. coli, Bacilluscereus, Listeria spp.,yeasts and moulds, and particularly it is validated to ISO 16140 standard for the detection of Salmonella spp. and Listeria spp.
The system is inoculated with a suspension of food sample and incubated at 36 ± 1 °C for 18-24 hours.
The tests for detection and presumptive identification of the microorganisms present in the sample are interpreted by assessing the colour change in the various wells. This system is used for the investigation of incidence of poultry meat contamination with Listeria spp and compared with the traditional method.

B.Sampling

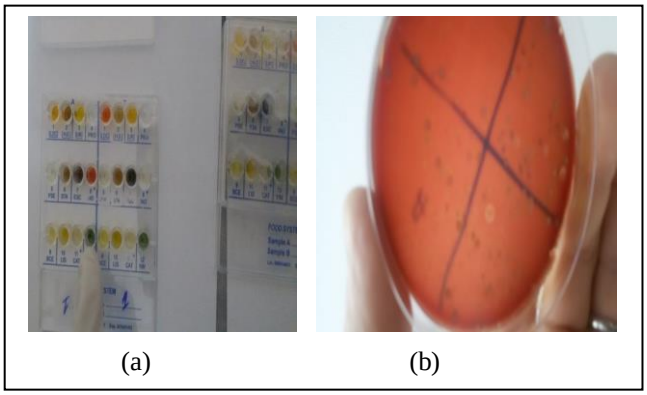
Samples were collected during March 2015-February 2016. A total of 96 broiler carcasses, n= 96 were randomly collected in Tirana. From those, 48 samples were collected from different shops (market and supermarkets where the slaughterhouses distribute in Tirana), the other 48 samples were collected from industrialized slaughterhouses at 2 week intervals. All samples were sent to the laboratory in sterile bags at 4°C within 2 hours. A portion 25g of each sample were homogenized with 225 ml of buffered peptone water. The final dilution of the sample was 1:10. 10 ml of the homogenized sample were transferred into a suitable tube and incubated (dil. 1:10) at 36±1°C for 4-6 hours. 0.5 ml was transferred into a vial of physiological solution provided with the kit. Into each well of the system was distributed 0.2 ml and incubated at 36±1°C for 18-24 hours. In order two identify Listeria spp, two drops of reagent H2O2 is added in the well 11 to catalase test ad we look for the formation of bubbles.

The test was considered positive for Listeria presence if there was a change to black in the 10 LIS well and also a positive catalase test in well number 11-CAT. For the st aureus by food system in well 6 was a change to
For the St aureus loads it was used the traditional technique as follows. The samples were diluted at dilution rate 1:10 and 0.1 ml from the diluted sample was inoculated into CTNA (coagulase thermo nuclease agar) and incubated for 48 hours in 37°C. The colonies of St aureus were identified and counted.

Fig.1 *Food system test*

C. Results and Discussions

There were 96 samples collected in slaughterhouses and at the retail shops in Tirana and analyzed with the traditional technique, in order to assess the St aureus loads and also with the Food system in order to asses if the positivity rates between these two different methods are the same.



(a) (b)

Fig.2 (a) food system during the test, (b)The procces during the interpretation of the test

From the samples 22.91% (11/48) collected from the slaughterhouses were positive for St aureus. From the samples collected from the retail shops 41.6% (20/48) resulted also positive for St aureus. Bacterial loads for St. aureus resulted from 8 cfu / g to 9x10²cfu / g, a level within the limits of Regulation 2073/2005 of the European Committee. There was an increase of positivity in the samples collected from the retail shops, in comparison to the samples collected from the slaughterhouses for both operators. Samples collected from the Operator A resulted positive for St auresus in 25% (6/24) of the samples collected from

the slaughterhouse and 41.6% (10/24) of the samples collected from the retail shops.

Samples collected from operator B resulted positive for St aureus in 20.83% (5/24) of the samples collected from the slaughterhouse and 41.6% (10/24) of the samples collected from the retail shops.

It is interesting to note that there are different contamination patterns between the two slaughterhouses but the contamination patterns are similar for the samples collected from the retail shops (Tab1). This differences between the slaughterhouses may depend from the different hygienic processing conditions, but the number of the samples collected is not sufficient to be statistically significant in order to asses the hygienic processing conditions of the operator's involved. It has to be noted that the higher contamination in the retail shops is a consequence of the longer storage, manipulation and the lack of individual sealed packaging of the products. According to the EU regulation 2073/2005 on St aureus loads in the poultry meat, all the positive samples tested are within the regulation limits. However the high rate of positivity in the slaughterhouse and the increment at the retail shops should be a warn for the producers, sellers and Public Health authorities in order to improve the packaging and the storage conditions of the poultry meat before it is sold to the consumers. The samples were collected in different seasons in order to analyze the impact of the season on the contamination patterns, but there were no differences in the contamination patterns noted.

	Positive sample/ Analysed sample				
	Slaughterhouses		Market		Total
	B	A	B	A	
No samples	24	24	24	24	96
No.positiv sample	5	6	10	10	31
Range of staph. aureus	16-9x10^2	10-2x10^2	10-9x10^2	8-900	
Expressed in%	20.83%	25%	41%	41%	32.29%
Norm cfu/g	5x10^2-5x10^3	5x10^2-5x10^3	5x10^2-5x10^3	5x10^2-5x10^3	

Table I. *Staph. aureus* in %

All the samples were also tested with the food system for the presence of Listeria spp, but none of the samples resulted positive for this specific pathogen. This is in contrast with different authors who found positivity for Listeria monocytogenes from 27.8% to 64% (Loncarevic et al., 1994, Živkovic et al. 1997b).

Table II. The monthly and seasonal distribution of *Staph. Aureus*

St. Aureus						
	Positive sample/ Analysed sample					
	Slaughterhouses		Market		Total	
	OBU					
	B	A	B	A	B	A
03/2015	0/2	0/2	1/2	0/2	1/4	0/4
04/2015	0/2	0/2	2/2	2/2	2/4	2/4
05/2015	0/2	0/2	1/2	0/2	1/4	0/4
–Spring	0/6	0/6	4/6	2/6	4/12	2/12
06/2015	0/2	1/2	2/2	2/2	2/4	3/4
07/2015	2/2	2/2	0/2	1/2	2/4	3/4
08/2015	0/2	1/2	0/2	0/2	0/4	1/2
–Summer	2/6	4/6	2/6	3/6	4/12	7/12
09/2015	0/2	0/2	0/2	0/2	0/4	0/2
10/2015	0/2	0/2	1/2	2/2	1/4	2/2
11/ 2015	2/2	1/2	0/2	0/2	2/4	1/2
–Autumn	2/6	1/6	1/6	2/6	3/12	3/12
12/2015	0/2	1/2	2/2	1/2	2/4	2/4
01/2016	1/2	0/2	1/2	2/2	2/4	2/4
02/2016	0/2	0/2	0/2	0/2	0/4	0/4
–Winter	1/6	1/6	3/6	3/6	4/12	4/12
Total	5/24	6/24	10/24	10/24	15/48	16/48

There was no difference in the positivity rates for Staph. aureus between the traditional method and food system, pointing out the advantage of the Food system as a rapid technique to asses the presence or the absence of different bacterial pathogens in different foodstuff.

CI. Conclusion

According to the literature and to the results of bacteriological analyzes there is constant evidence that poultry meat is a major source of foodborne diseases. Our study reveals also the importance of storage and packaging in the contamination rates and bacterial loads. Albeit all the samples tested had Staph. aureus loads within the EU regulation limits this loads are dynamic (can increase with time) and also there is a need to test the poultry carcasses for the presence of the enterotoxins excreted by Staph. aureus strains. It is in the interest of all parties involved (authorities, producers, retail sellers, consumers) to improve the processing conditions in the slaughterhouses, and the most important is the use of the individual sealed packaging for the poultry carcasses in order to limit further contamination in the retail shops.

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