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STUDY ON SOME FOOD PRODUCT CONTAMINATION RATE WITH BACTERIA FROM *LISTERIA* GENUS

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Abstract

Included in the large spread high risk group, the Listeria bacteria genus can be diffused throw food products, causing the Listeriosis disease. Listeriosis is a bacterial infection which in serious forms and inadequately treated can reach a 70% mortality. In this study the bacteria were isolated from several categories of samples (poultry and bird organs, raw pork meat, raw beef meat, processed pork meat), which were collected from slaughterhouse houses and department stores. From analysed samples 40% of isolated strains were found in raw pork meat, 25% in raw beef meat, also 25 % in poultry and bird organs and 10% in processed pork meat. The isolation and identification technique was done with an official method, following these steps: pre- enrichment in unselective liquid mediums, enrichment in selective liquid mediums, isolation and identification, identity confirmations.

Key words*: bacteria, contamination, food product, identification, isolation*

INTRODUCTION

Listeria genus belongs to the family *Listeriaceae*, along with the *Brochothrix* genus. It includes 7 species: *Listeria denitrificans* (*Jonesia denitrificans*), *Listeria grayi* (*Listeria murrayi*), *Listeria innocua, Listeria ivanovii* (with two subspecies: *Listeria ivanovii* and *Listeria londoniensis*), *Listeria monocytogenes, Listeria seeligeri* and *Listeria welshimeri*.
Listeria monocytogenes is a polymorphic Gram-positive bacterium (short bacilli, cocobacilli, filamentous forms) (Figure 1).

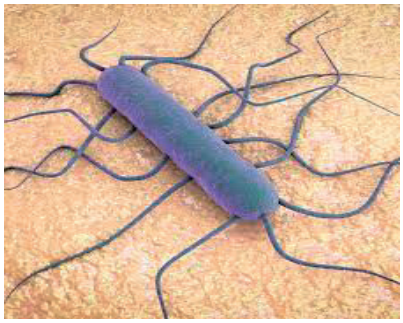

Figure 1. *Listeria monocytogenes*

It is non-capsulogenic, non-sporogenic. In cultures incubated at temperatures below 35°C it is mobile, with 4-5 flagella arranged peritrich, and at 37-46°C it becomes immobile or monoflagellate.
According to the classification made by the International Commission on Microbiological Specifications for Foods which grouped dangerous microorganisms depending on the severity of the risk, *Listeria monocytogenes* is included in the second group, with moderate risks, but with widespread potential (Apostu, 2004).
The natural infection is called listeriosis and affects both humans and many animal species. Man can become infected, either through the digestive tract through food or water (food poisoning), or through the air, genitals or wounds (Ivana, 2002).
Recently, there has been an increase in the percentage of contamination with Listeria of farm animals. In cattle and pigs, the skin and hair are important sources for *Listeria monocytogenes* spreading, with the possibility of its diffusion in carcasses (Tudor, 2002).
Paying attention to hygienic norms can reduce the number of contaminations, but these bacteria cannot be removed, which is why their determination can be a sanitary indicator of the overall hygiene conditions in slaughterhouses. (Dan, 2001).

In general, *Listeria* species are isolated from raw milk, cheese, fresh and frozen meat, chicken, seafood, fruits and vegetables.
Isolation of *Listeria monocytogenes* in food products is difficult and requires selective enrichment of samples before staining them on the surface of selective isolation media (Bărzoi, 2002).

MATERIALS AND METHODS

The study was conducted in 2016-2017 on several categories of meat samples, collected from slaughterhouses and sales units (Bucharest, Ilfov County): 10 samples of poultry and bird organs, 59 samples of raw pork meat, 21 samples raw beef meat, 10 samples processed pork. It is an attempt to highlight the existing interconnections between the quality of the raw meat, the attention paid to respect the hygienic norms on the technological flow of processing, the quality of the finished product and the consumers' safety.

Sample for analysis and initial suspension
For the preparation of the initial suspension, demi-Fraser broth (selective primary enrichment medium) or APT is used as the dilution liquid.
In general, to prepare the initial suspension, there is added 25 g of the test sample to 225 ml of primary enrichment medium, in order to maintain a 1/10 ratio between the test sample and the medium (mass/volume or volume/volume).
1. Primary enrichment
The initial suspension is incubated at 30°C for 25 hours.
2. Secondary enrichment
After incubation of the initial suspension (primary enrichment) for 25 h, 0.1 ml of the culture obtained is transferred to another tube containing 10 ml of Fraser broth (secondary enrichment medium).
The seeded medium is incubated at 37 ± 10°C for 24 ± 2 hours.
3. Stripping and identification
Using a bacteriological loop, the culture obtained at the primary enrichment (Demi-Fraser) will be dispersed on the surface of the first selective isolation medium (Listeria/ Agosti Ottaviani agar), so as to obtain isolated colonies.
In the same way is done with the second selective isolation medium (Palcam agar).
The procedure will be similar in the culture obtained at secondary enrichment (Fraser Broth), incubated for 24 ± 2 h at 37 ± 10°C, by dispersing the two selective media. At ALOA agar, if no signs of microbial development are observed after 24 ± 2 hours of incubation, or colony development is weak, incubation will be extended to 48 hours.
After 24 ± 2 h or 48 hours of incubation, the Petri dishes are examined in order to detect the presence of typical colonies: *Listeria* spp. or *Listeria monocytogenes*.
After incubation, the Petri dishes can be refrigerated at 5°C, before reading, for a maximum of 48 hours.
Is considered to be *Listeria monocytogenes* colonies if there are bluish-green colonies surrounded by an opaque halo (typical colonies). *Listeria ivanovii* colonies are also bluish-green surrounded by an opaque halo.
- *Listeria* spp. are considered likely bluish-green colonies surrounded or not by an opaque halo.
Note: Certain strains of *Listeria monocytogenes* exposed to stress conditions (especially acidity) may have a very weak, even absent, halo. There are also other organisms besides *Listeria* spp. which can produce blue colonies on ALOA agar.
The Petri dishes with Palcam Agar, after incubation at 37 ± 10°C, for 48 hours, are exposed to the air for 1 hour so that the environment regains its red-purple color. After 24 hours *Listeria* spp. forms small or very small olive or gray-green colonies, with a diameter of 1.5-2 mm, sometimes with a black center, but always with a black halo. After 48 hours, the colonies of *Listeria* spp. with a diameter of 1.5-2 mm are green, concave in the center and surrounded by a black halo.

Interpretation of morphological, and physiological properties and biochemical reactions
All species of *Listeria* spp., have the shape of small sticks, Gram-positive, are mobile and catalase positive (Table 1).

Table 1. Confirmation tests for *Listeria* spp.

Tests	***Listeria* spp.**	**Results**
mandatory	GRAM microscopic appearance	Thin and short sticks or coccobacilli form
	Catalase	+
optional	Voges-Proskauer test	+
	Motility at 25^0	+

L. monocytogenes differs from the rest of Listeria species by the characteristics specified in Table 2.

Table 2. Confirmation tests for *Listeria monocytogenes*

Tests	*Listeria monocytogenes*	Results
mandatory	GRAM microscopic appearance Beta-hemolysis L-rhamnose D-xylose	Thin and short sticks or coccobacilli form li + + -
optional	Catalase Motiliy CAMP test	+ + +
the microscopic appearance is optional in the case of the ALOA agar medium and in the case of the second medium (Palcam) if it allows the differentiation between pathogenic and non-pathogenic *Listeria* spp.		

Listeria monocytogenes has a characteristic and intense mobility and performs rolling movements that alternate with short periods of rest. On soft agar, the bacterium mobility is expressed by the culture development in an umbrella shape.

Catalase test: catalase is a hemoporphyrin enzyme that catalyzes the decomposition reaction of hydrogen peroxide. The reaction is evident when a culture loop is put in contact with a drop of hydrogen peroxide. The appearance of gas bubbles is interpreted as a positive reaction, being specific to *Listeria* species (Figure 2).

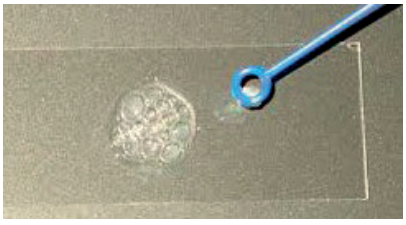

Figure 2. Catalase test

Carbohydrate fermentation: there is used peptone water with red-phenol. *Listeria monocytogenes* ferments glucose and rhamnose, with acid production, without gas production, and does not ferment xylose and mannitol.

Hemolysis test: this test can differentiate two closely related species, namely *Listeria monocytogenes* and *Listeria innocua*. The hemolytic species of *Listeria* species are: *Listeria monocytogenes, Listeria ivanovii* and *Listeria seeligeri.*

The method is as follows: the surface of the sheep's blood agar is stained with the bacterial culture which will be tested. There is an incubation time for 24 hours at 35-37°C. *Listeria monocytogenes* form small colonies with a small, clear halo around, specific to beta hemolysis. *Listeria ivanovii* has a strong hemolytic activity forming around the colonies clear, large areas, and *Listeria seeligeri* produces poor hemolysis.

CAMP test: It can clearly highlight the hemolytic characters and is achieved by seeding *Streptococcus aureus* and *Rhodococcus equi* in the streaks form, in one direction on the plate with blood agar, and the *Listeria* stems perpendicular to their trajectory without touching them. Hemolysis of *Listeria monocytogenes* strains is more exacerbated near the *Streptococcus aureus* stria, and for *Listeria ivanovii* it is intensified only near the *Rhodococcus equi* ridge (Figure 3) (Ivana, 2013).

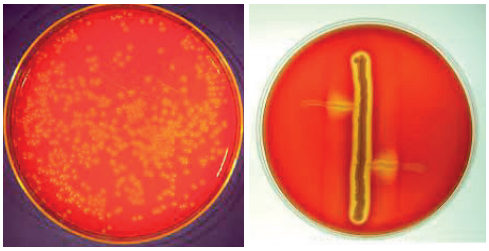

Figure 3. *Listeria* on blood agar, Camp Test

In Table 3, there are presented reactions at diferent tests completed in order to identify the *Listeria* species.

Table 3. Reactions to identify *Listeria* species

Species	Hemolysis	Acid production from:		CAMP test	
		Rhamnose	Xylose	*S. aureus*	*R. equi*
L. monocytogenes	+	+	-	+	-
L. innocua	-	V	-	-	-
L. ivanovii	+	-	+	-	+
L. seeligeri	(+)	-	+	(+)	-
L. welshimeri	-	V	+	-	-
L. grayi subsp. grayi	-	-	-	-	-
L. gravy subsp. murrayi	-	V	-	-	-
V: variable reaction; (+): weak reaction; +: over 90% with positive reactions; -: no reaction.					
NOTE: there are rare strains of *L. monocytogenes*, which do not give β-hemolysis, or CAMP test, under the conditions described in this procedure.					

RESULTS AND DISCUSSIONS

In the present paper, Listeria ssp. were isolated from several categories of samples collected from slaughterhouses and sales units, as previously presented. The results obtained are shown in Table 4 and Figure 4.

Table 4. Incidence of *Listeria* species in the analyzed samples

Sample type	Samples number	Positive results	%	Negative results	%
raw beef meat	21	1	4,76	20	96
poultry and bird organs	10	-	-	10	100
raw pork meat	59	2	3,38	57	97
processed pork	10	-	-	10	100
Total	100	3	3	97	97

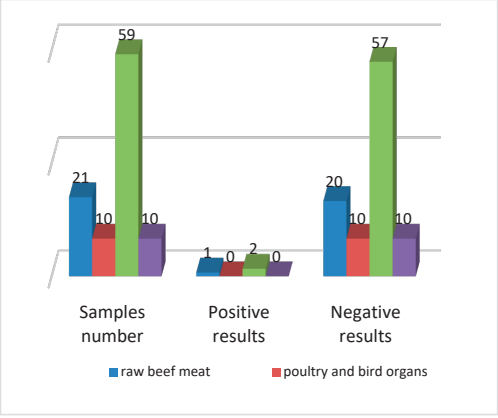

Figure 4. Incidence of *Listeria* species in the analyzed samples

The incidence of *Listeria* ssp. during this study period is shown in Table 5.

Table 5. *Listeria* species frequency results

Specie	Strains number	%
Listeria monocytogenes	1	50
Listeria ivanovii	1	50
Total	2	100

The incidence of *Listeria* species during 2016-2017 period, for the raw beef meat category is shown in Table 6 and Figure 5.

Table 6. Incidence of *Listeria* species in the period 2016-2017, for the raw beef meat category

Period	Samples number	*Listeria* ssp.	Number of isolated stems	%
2016	13	*Listeria monocyto genes*	1	7.69
2017	8	-	-	0
Total	21		1	4.76

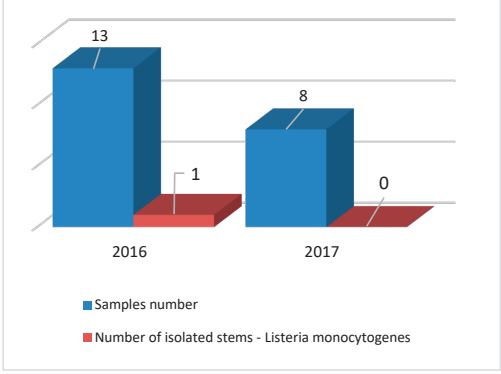

Figure 5. Incidence of *Listeria* species in the period 2016-2017, for the raw beef meat category

The incidence of listeries in 2016, for the category of beef raw material is 7.69%.
The incidence of *Listeria* species in the period 2016-2017, for raw pork meat category is shown in Table 7 and Figure 6.

Table 7. Incidence of *Listeria* species in the period 2016-2017, for raw pork meat category

Period	Samples number	*Listeria* ssp.	Number of isolated stems	%
2016	37	*Listeria monocytogenes*	1	2.7
2017	22	*Listeria ivanovii*	1	4.54
Total	59		2	3.38

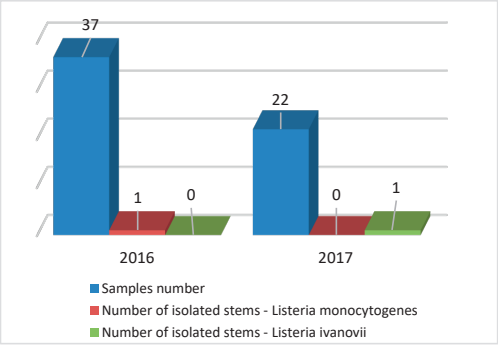

Figure 6. Incidence of *Listeria* species in the period 2016-2017, for the raw pork meat category

The incidence of listeries, for raw pork meat category was 2.7% in 2016 and 4.54% in 2017.

CONCLUSIONS

As a result of the research undertaken regarding the frequency of listeria species in meat samples, some conclusions may result.

There were analyzed 21 samples of beef raw material, 10 samples of poultry and organs, 59 samples of raw pork, 10 samples of processed pork, during 2016-2017 period. *Listeria* ssp was isolated in 3 samples (3%).

In raw beef meat category, in 2016, there was found the presence of 1 contaminated sample, with an incidence of 4.76% for *Listeria monocytogenes*.

In raw pork meat category in 2016, there was found the presence of 1 contaminated samples, with an incidence of 2.7% for *Listeria monocytogenes* and in 2017, there was found also the presence of 1 contaminated samples, with an incidence of 4.54% for *Listeria ivanovii*.

Listeria was not present in the poultry and bird organs or in the processed pork.

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