STUDIES REGARDING THE INCIDENCE OF BACTERIA FROM LISTERIA GENUS IN FISH MEAT

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Abstract: *Listeria is a bacterial genus whose species are Gram positive bacilli and it contains seven representatives. Joseph Lister was an English surgeon who was one of the first in the field of sterile surgery and the name Listeria is given in his honor. Sources of Listeria are soil, contaminated water, animals and vegetables. Because bacteria from Listeria monocytogenes specie are very dangerous for human health, with a mortality rate of 20% for infected patients, a serious attention has to be accorded in consumption of those foods able to transmit microorganisms. This article presents a study about the incidence of Listeria in two species of fish which were tested to prove presence or absence of Listeria monocytogenes, because fish meat is one of the foods susceptible to transmit bacteria and to induce grave intoxication. From fresh water fish Carp was selected and as a representative of ocean fish, Mackerel respectively. Different methods for detection were described and discussed. Those methods are legally accepted in European and Romanian normative for food consumption security as Food Microbiology and Specific Rules for Microbiological Analyzes. OXFORD, PALCAM and CAMP are test used for detection and confirmation of Listeria species of bacteria. Also API-Listeria tests were used for confirmation the presence or absence of different Listeria species in tested samples. Agar with sheep blood medium was inseminated with samples of fish meat because this is the proper medium for bacterial colonies development. The reason is the fact that bacteria colonies formed dark colored rings rounded by a black haloes, because in their metabolic process bacteria produce hydrolysis of aesculin (a glycoside contained in agar medium). The results of analyzes for those two species of fish meat do not confirm the presence of Listeria monocytogenes, but other species of Listeria (Listeria welshimeri and Listeria inocua), fortunately not pathogenic, were present.*

Keywords: *pathogenicity, Listeria monocytogenes, fish meat, infection*

1. Introduction

Bacteria from *Listeria* genus and *Listeria monocytogenes* especially provide a high level of pathogenicity, being responsible by a lot of alimentary toxic-infections and other dangerous diseases, even female abortion. Many sources of *Listeria monocytogenes* exist in nature, like environment, animals, people [1-7].

From environment, *Listeria monocytogenes* was mainly isolated from soil, water and vegetables in decomposition. Weis detected *Listeria monocytogenes* in 21% of samples (from 779 samples of soil and plants). The high capacity of bacteria to survive in difficult conditions permits to explain infections which occur from sources contaminated long time before the reference moment. It was noted that in wastewater dispersed on farm land, bacteria remain active till eight weeks, what could explain human infections in New Scotland – Canada in 1981. The pathogenicity of *Listeria monocytogenes* is proved by the fact that

almost 20% of infected patients died. Even the reported cases of listeriosis diminished in developed countries, researchers found varied modes of transmission of bacteria through diverse foods [1,2,5,7,8].

2. Materials and methods

There were selected two species of fish, a carp representing a fresh water fish and a mackerel representing an ocean fish respectively. The samples were analyzed in The Laboratory for Food Safety of DSVSA Suceava.

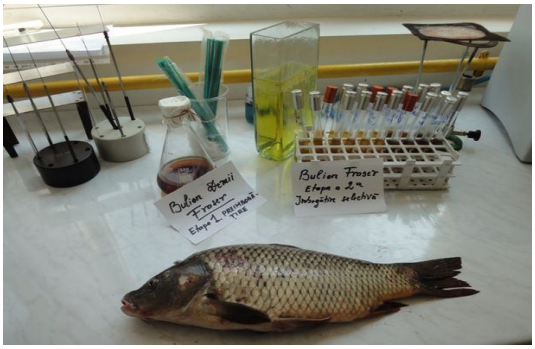

Figure 1. An image of laboratory with a carp fish and culture media for *Listeria monocytogenes*

From fresh water fish Carp was selected and as a representative of ocean fish, Mackerel respectively. Different methods for detection were described and discussed. Those methods are legally accepted in European and Romanian normative for food consumption security as Food Microbiology and Specific Rules for Microbiological Analyzes [9-24].

Figure 2. An image of laboratory with a mackerel fish and culture media for *Listeria monocytogenes*

Preparing of samples followed SR EN ISO 6887-4/2005 regarding Food Microbiology and Specific Rules for Microbiological Analyzes and initial suspensions and decimal dilutions preparing followed SR EN ISO 6887-1/2002.

Fish samples investigation was performed through the method of analyze for detection and confirmation of *Listeria monocytogenes* implemented and actualized in Romania by SR EN ISO 11290-1/2000 with amendment A1/2005 regarding isolation media and hemolysis test. [21-23].

Analyzes evolved following next stages:

- **Primary enrichment**. In 225 mL liquid media with low concentration of selective agents of semi-Fraser bouillon, insemination of 25 mL of sample followed by 24 h incubation at 30°C was performed.
- **Secondary enrichment.** From obtained culture 0.1 mL were transferred in each test tube with 10 mL Fraser bouillon, followed by incubation for 48 h at 35°C or 37°C.

Corrugation and identification. From obtained cultures samples were passed in two isolation media (agar OXFORD and agar PALCAM), with 24 – 48 h incubation at 35°C. In parallel, plates inseminated with positive control tell-tale (reference stem of *Listeria monocytogenes* ATCC 13932) were incubated in similar conditions [3,5,8].

- **Confirmation.**

3. Results and discussions

Examination of final obtained cultures confirmed that OXFORD and PALCAM media were highly selective for inhibition of associated flora [3,5,8].

On control tell-tale *Listeria monocytogenes* formed dark colored colonies, rounded by a black haloes,

because in their metabolic process bacteria produce hydrolysis of aesculin (contained in agar medium) (see figures 3 and 4).

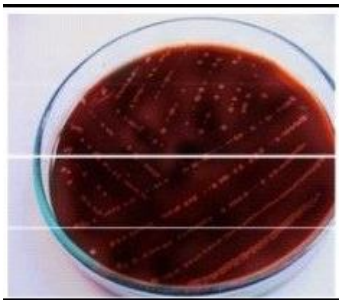

Figure 3. Aspect of colonies of *Listeria monocytogenes* from positive control tell-tale sample on PALCAM selective medium, after 24 hours in thermostatic conditions

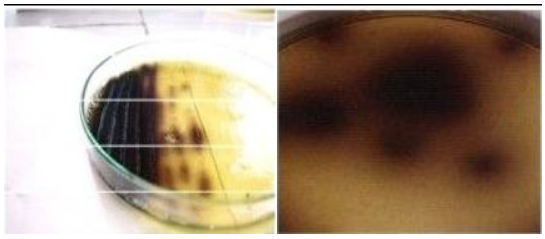

Figure 4. Aspect of colonies of *Listeria monocytogenes* on OXFORD selective medium, after 24 hours in thermostatic conditions

Confirmation for Listeria monocytogenes was realized by hemolysis test and CAMP tests. Because *Listeria* generates hemolysis on agar with sheep blood, this could be used to confirm the presence or absence of bacteria. So, *Listeria monocytogenes* stem from control tell-tale sample produced small colonies rounded by small but clear zone of hemolysis, which is characteristic.

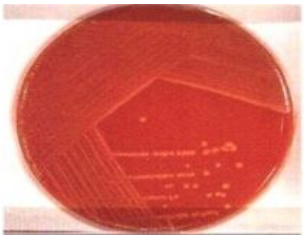

Figure 5. Aspect of colonies of *Listeria monocytogenes* on an agar with sheep blood medium

Another reaction, gentle or accentuated could be highlighted with a CAMP test. This test consists in insemination of agar surface with *Staphylococcus aureus* and *Rhodococcus equi* on vertical parallel lines (knurling). Then were knurled horizontal parallel lines with *Listeria monocytogenes, Listeria innocua and Listeria ivanovii*, but horizontal lines did not intersect vertical lines, a distance of 1 – 2 mm remaining. Incubation time was 24 h at 37°C. Image in figure 6 presents a positive reaction, consisting in an arrow form at virtual intersection of the lines.

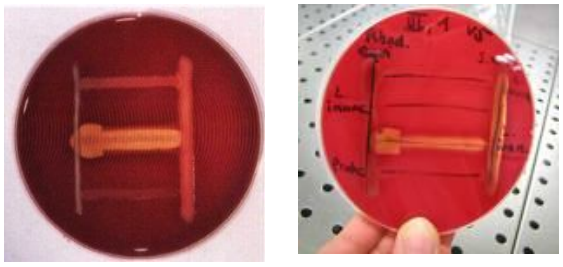

Figure 6. CAMP test – control tell-tale plate (+)

The test results show tha absence of *Listeria monocytogenes and Listeria ivanovii*, but a possible contamination with *Listeria inocua* or other species of *Listeria* genus.

Another confirmation was achieved using Api-Listeria tests, containing dehydrated substrates. Inseminated and incubated for 18 – 24 h at 35 – 37°C, the color change could signalize the presence or absence of bacteria. So, *Listeria inocua* and *Listeria welshimeri* respectively were confirmed for analyzed samples.

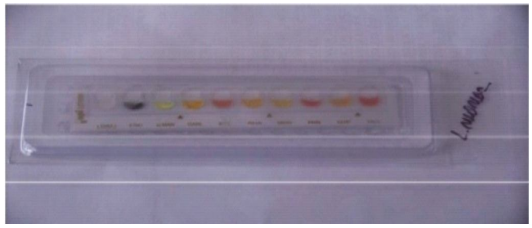

Figure 7. *Listeria monocytogenes* identified on API-LISTERIA galleries

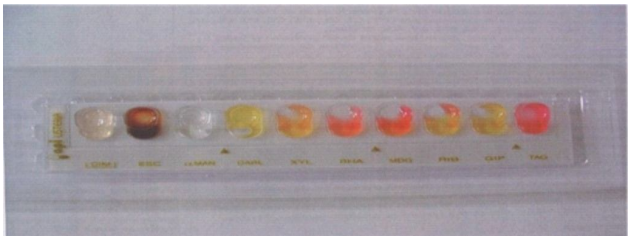

Figure 8. *Listeria welshimeri* identified on API-LISTERIA galleries

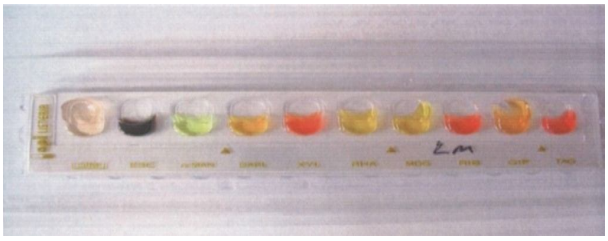

Figure 9. *Listeria inocua* identified on API-LISTERIA galleries

All results of tests are summarized in table 1.

Table 1. Distribution of species of *Listeria* genus bacteria

Examined product	**Number of samples**	***Listeria monocytogenes***	***Listeria inocua***	***Listeria welshimeri***
Probe 1. Fish meat from *Carp* specie	5	absent	absent	2
Probe 2. Fish meat from *Mackerel* specie	5	absent	2	1

4. Conclusions

Analyzes of those two species of fish with five samples each infirm the presence of *Listeria monocytogenes*. Carp fish meat contain *Listeria welshimeri* at two samples from five and Mackerel fish meat contain Listeria inocua at two samples and *Listeria welshimeri* at one sample from five, respectively. Because *Listeria inocua* and *Listeria welshimeri* are not pathogenic, conclusion is that tested fish could be approved for consumption.

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