Listeria monocytogenes in Food Matrix: 
Frequency and Effect of Antagonist Microbial

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Abstract: Listeria monocytogenes causes septicaemia, infections of the central nervous system (meningitis and meningoencephalitis) and abortions. Listeriosis occurs primarily in people at risk, such as the elderly, pregnant women and their newborns or people with a serious weakening the body or whose cellular immunity is impaired. L. monocytogenes is a bacterium widely distributed in the environment. Its ability to persist in the industrial environment was the cause of food contamination from raw animal or plant and was a recurring problem for the food industry, despite the use of the cold chain. L. monocytogenes is a pathogen transmitted through contaminated food. It is responsible for serious infections sometimes fatal, operating mainly as sporadic sometimes epidemic. To assess the presence of L. monocytogenes in region of Rabat and determine their effect on the microbiological quality products from different food matrices, we conducted this study for three years (2009-2011). In total, 2311 samples were collected. Examination of these samples resulted in 58 samples which were positive for L. monocytogenes in: Red meat products 3.7%, white meat products 7.1%, prepared meals 1.25%, the salads 4.19%, dairy 2.34% and pastries 1.3%. However, this pathogen was absent in other analyzed samples (fishery products, creams and ice creams). Although the level of contamination by L. monocytogenes is generally low. In addition our results suggest the intervention of antagonist microbial mechanisms which may affect the survival of this pathogen.

Key words: Antagonist microbial, food matrix, Listeria monocytogenes, listeriosis

INTRODUCTION

The increase in international trade and travels, the adaptation of microorganisms to new environmental conditions, the change in the food systems production and the human demography and behavior make diseases carried through food more threatening. During the last decades, the discovery of microorganisms, the progress realized in the food industry and the fast expansion of international trade of food products made necessary the adoption of various health security measures (Gillespie et al., 2009; Havelaar et al., 2010). In fact, the quarter of the world food supply is lost because of the microbial activity (Varga, 2007).

In order to ensure food safety and to safeguard consumers’ health, innovative prevention strategies are set up. One of these plans is characterized by the analysis of hazards and the mastering of critical points (Jin et al., 2008; Scallan et al., 2011). Besides strategies, consumers themselves should be aware of the importance of food management and consumption conditions (Rodríguez Lázaro et al., 2007; Konteles et al., 2009).

So, the optimization of new control alternatives, the characterization and quantification of food pathogens became, more than ever, essential for the food industry, in order to solve issues linked with the food safety and security (Rodríguez Lázaro et al., 2007; Haavelar et al., 2010). Foodborne epidemics constitute a burden, not only, for the health system but also for the economic activity (Greij and Ravel, 2009).

Listeria monocytogenes is widespread microorganism in the environment. So, it is the cause of various epidemics related with food consumption and the source of invasive human infections (Le Monnier and Leclercq, 2008; Velusamy et al., 2010). Ubiquitous, it’s able to survive in stress conditions, in cold and saline environments. Furthermore, L. monocytogenes is finding in the treatment and transformation of food industry systems (Pal et al., 2008; Naidoo and Lindsay, 2010).

The objectives of this study are to evaluate the existence of L. monocytogenes and its potential safety in food and to show the interest of the laboratory identification of the strain and the prevention from foodborne infections. Furthermore, this study will demonstrate the emergency...
to list the critical points at risk which introduce a microbiologic danger and facilitate its multiplication. Further, another particular and crucial aim would be to give responsibilities all the actors, to elaborate a quality politics and, in particular, by training the producers and the tradespeople in the rules of good hygienic practice.

MATERIALS AND METHODS

Samples: According to the microbiological criteria and the regulations in force 2311 samples requiring L. monocytogenes analysis, were examine, looking. The samples consisted of: raw red meat (n = 669), raw white meat (n = 154), dairy products (n = 171), ice creams (n = 401), seafood (n = 24), cooked meals (n = 399), various salads (n = 262) and pastries (n = 231). L. monocytogenes identification requires multiple steps including two enrichments, two isolations and identification steps (NM EN ISO, 11290-1).

The first enrichment step consists in the revivification of microorganisms that are potentially in food. The elective broth used is the Half Fraser (Oxoid, CM0895) where L. monocytogenes capacity to hydrolyze esculin to esculin, translated by a blackening medium after the appropriate incubation. The antibiotics (Oxoid, SR0169) added to the broth allow to cease the secondary micro flora Gram positive growth (acriflavin) and to block the DNA replication of sensitive microorganisms (nalidixic acid). Thus, 225 mL of Half Fraser broth are added to the 25g of products, thereafter, shacked mechanically during 5 minutes. The mixture constitutes the primary suspension that is then incubated at +36±2°C for 18 to 24 hours period of time.

The second enrichment step contains twice the concentration of nalidixicacid and acriflavin antibiotics using a particular supplement (Oxoid, SR0156) to the medium broth Fraser (Oxoid, CM0895). The 0.1 mL of the pre enriched medium is incorporated to the 10 mL of the selective broth Fraser. The mixture is incubated at +36±2°C, during 24 to 48 hours period of time.

The primary isolation step exploits two agar medium that contain a variety of selective factors. Therefore, one selective Oxford agar plate and one selective Palcam agar plate are seeded using a loopful from the selective broth Fraser, then, incubated at +36±2°C, during 24 to 48 hours. Moreover, within the Oxford medium (Oxoid, CM0856), the negative Gram bacteria are inhibited by the lithium chloride, acriflavin, colistin, ceftolalan, cycloheximide and the fosfomycin contained in the added supplement (Oxoid, SR0206). The typical colonies are surrounded by a black zone due to the formation of iron phenolic compounds derived from aglycone. Concerning the Palcam medium (Oxoid, CM0877), the lithium chloride, cefazolin, polymyxin B and the acriflavin of the supplement added makes it highly selective (Oxoid, SR0150). The typical colonies are grey-green, with a concave center, surrounded by a

RESULTS AND DISCUSSION

Listeria monocytogenes evaluation in food: From three years (2009-2011), 2311 samples, considering all aliment category, were analyzed. Listeria monocytogenes was detected on 59 samples with a recuperation percentage of 2.55% (59/2311). Based on the results obtained by years, the recuperation percentages are of 0.77% (7/905), 3.21% (19/591) and 4.05% (33/815) respectively (Table 1). The results analysis shows that detection of L. monocytogenes frequency in foods is weak. In fact, the recuperation percentage is 2.55% (59/2311) (Table 1). It is important to notice that the detection methods, extremely tedious and lying on various enrichment and isolation phases using selective medium, make the stressed cells lose their cultivable character, their physiological sate playing a crucial role in the detection of L. monocytogenes (Guillier and Augustin, 2005a). The incapacity to multiply that is induced from this lays in the membrane damages of the stressed cells and in the permeability issues that go with them (Gnanou Besse and Coli, 2004).

Although a majority of aliments could be contaminated by L. monocytogenes (Adak et al., 2005), the contamination level principally depends on the nature of the aliment, some supporting growth more than others. If raw and/or transformed aliments and food ready for consumption are considered to be more risky, cooked aliments can be unsafe as well, more often, after an
Table 1: Frequency of *L. monocytogenes* in different matrices by year

<table>
<thead>
<tr>
<th>Alimentary matrices</th>
<th>Total samples analyzed</th>
<th>Samples containing <em>L. monocytogenes</em></th>
<th>Recuperation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red meat product</td>
<td>266</td>
<td>218</td>
<td>185</td>
</tr>
<tr>
<td>Poultry product</td>
<td>20</td>
<td>29</td>
<td>105</td>
</tr>
<tr>
<td>Varied salads</td>
<td>93</td>
<td>70</td>
<td>99</td>
</tr>
<tr>
<td>Cooked meals</td>
<td>145</td>
<td>104</td>
<td>150</td>
</tr>
<tr>
<td>Dairy products</td>
<td>66</td>
<td>32</td>
<td>73</td>
</tr>
<tr>
<td>Pastries</td>
<td>95</td>
<td>40</td>
<td>99</td>
</tr>
<tr>
<td>Creams and ice creams</td>
<td>211</td>
<td>91</td>
<td>90</td>
</tr>
<tr>
<td>Seafood</td>
<td>9</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>All food</td>
<td>605</td>
<td>591</td>
<td>815</td>
</tr>
</tbody>
</table>

insufficient thermal treatment and/or after a post-treatment cross-contamination, especially, subsequent to long periods of conservation at refrigeration temperatures (Pal et al., 2008).

**Frequency of *Listeria monocytogenes* in different alimentary matrices:** During three years of study, *L. monocytogenes* (n = 59) is isolated in 25 raw red meat products (3.73%), 11 raw poultry product (7.14%), 11 varied salads (4.19%), 05 cooked meals (1.25%), 04 dairy products (2.34%) and 03 pastries (1.29%). However, *L. monocytogenes* seems to be inextricable in seafood, creams and ice creams samples analyzed (Table 1). It appears that *L. monocytogenes* is more frequent in meat based products such as red meats, poultry, cooked meals and certain varied salads, since 88% (52/59) of strains are isolated in this category of food (Table 1). As a matter of fact, meats constitute a significant cause of epidemics and, the cases reported are increasing, despite the efforts in terms of hygiene practices by industries (Rhoades et al., 2009).

Besides, the cooking of the meat-based products seems often insufficient, to eliminate totally *L. monocytogenes* (Neves et al., 2008). Big efforts were realized by food industries to limit the contaminations by *L. monocytogenes*, mainly, during the evisceration of animals and treatment of carcasses, however, mechanisms of virulence of *L. monocytogenes* seems to persist, during the food processing (Gebretsadik et al., 2010). As for dairy products, they appear to be protected since *L. monocytogenes* has been detected in 2.34% (4/171) of samples (Table 1). The stabilization effects by physical treatments and the pasteurization mode seem to decrease the presence of *L. monocytogenes* population of 11 logs which eventually minimizes its survival risks (Pres et al., 2009). Among the 231 pastries samples analyzed during three years, *L. monocytogenes* isolated in three samples, corresponding to a recuperation percentage of 1.29% (3/231) (Table 1). A possible explanation of these findings would be an eventual cross-contamination. The absence of *L. monocytogenes* in creams and ice creams (El-Sharefi et al., 2006) (Table 2), would originate from its inability to grow in this food matrices (Newell et al., 2010). This bacterium is able to grow in ice creams' mixtures but not at -18°C. Yet, this pathogen lives through freezing temperatures and develop when the product defreeze (Tasara and Stephan, 2008).

However, the occurrence of *L. monocytogenes* cases, further to the consumption of such products remains rare, this bacterium having already been isolated in only 6% of ice creams (Adak et al., 2005). Despite a frequent contamination of seafood (Leroy et al., 2001), no positive sample is identified (Table 1). This can be explained by the weak number of samples analyzed (24/231), knowing that the Moroccan population favors consuming dairy and meat products rather than seafood.

**The effect of microorganism competition on the *Listeria monocytogenes* survival:** The global analysis of the obtained results indicates that 51.7% (1195/2311) of samples are unsuitable for the human consumption (Table 2), from the viewpoint microbiological, according to the regulations in effect. Based on the annual results obtained, the percentage of samples unfit for consumption is 51.8% (469/905) in 2009, 50.8% (300/591) in 2010 and of 52.2% (426/815) in 2011 (Table 2). The proportion of food unsuitable for consumption, the microbiological point of view, remains identical, year by year, in the region of Rabat (Table 2). The level of contamination by other organisms is important. These results suggest the intervention of antagonist microbial mechanisms such as the production of inhibitory and/or competitive molecules for limited substrates, some microbes populations seem to be the antagonists responsible of the inactivation of *L. monocytogenes*. As a matter of fact, the excessive presence of total aerobic mesophilic flora (FMAT) and of total and fecal coliforms may affect the survival of *L. monocytogenes*, during the treatment's process. For example, the presence of 4 UFC mL⁻¹ of FMAT could decrease the *L. monocytogenes* survival of 2 UFC mL⁻¹ (Nero et al., 2009). Besides, *L. monocytogenes* is also inhibited by acidifying agents, lactates, sodium benzoate, potassium sorbate and lysozyme. The competition phenomena where inhibitor effect of organic
Table 2: Percentage of samples Unit for Consumption (UC%) and percentage of samples containing *Listeria monocytogenes* (CLm%)

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<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>UC (%)</td>
<td>CLm (%)</td>
</tr>
<tr>
<td>RMP</td>
<td>298</td>
<td>77.5 (298/396)</td>
<td>1.50 (4/260)</td>
</tr>
<tr>
<td>WMP</td>
<td>20</td>
<td>80.0 (16/20)</td>
<td>0.0 (0/20)</td>
</tr>
<tr>
<td>VS</td>
<td>93</td>
<td>63.4 (59/93)</td>
<td>2.1 (2/93)</td>
</tr>
<tr>
<td>CM</td>
<td>145</td>
<td>27.6 (40/145)</td>
<td>0.0 (0/145)</td>
</tr>
<tr>
<td>DP</td>
<td>68</td>
<td>34.9 (23/68)</td>
<td>1.6 (1/68)</td>
</tr>
<tr>
<td>P</td>
<td>95</td>
<td>36.9 (35/95)</td>
<td>0.0 (0/95)</td>
</tr>
<tr>
<td>CIC</td>
<td>211</td>
<td>39.9 (84/211)</td>
<td>0.0 (0/211)</td>
</tr>
<tr>
<td>Seafood</td>
<td>9</td>
<td>68.7 (6/9)</td>
<td>0.0 (0/9)</td>
</tr>
<tr>
<td>All food</td>
<td>905</td>
<td>51.8 (469/905)</td>
<td>561 (50.8 (300/591)</td>
</tr>
</tbody>
</table>


Acids coming from fermented sugars or from final pH of products have been described too. More to the point, *Staphylococcus* inhibits *L. monocytogenes* growth (Lemunier et al., 2005). *L. innocua*, seem to have an inhibitory effect on *L. monocytogenes*, by a bacteriocin production or more likely of phages (Lemunier et al., 2005). Probiotic bacteria are able to produce active bacteriocin against *L. monocytogenes* (Oliveir, 2007). Risk assessment related to *L. monocytogenes* allows the evaluation of its presence in foods and its potential dangers. It is necessary to collect enough information on the nature of the aliment, its distribution circuit, its consumption modes and the behavior of the microorganism within the food (Neves et al., 2008).

It is advisable to increase knowledge and comprehension of the phenomena responsible of the growth and/or inhibition of *L. monocytogenes*, during manufacturing processes, maturation and conservation of foods. As a matter of fact, the physiological state largely affects the success or failure of *L. monocytogenes* detection. This point is still unknown but has to be taken into consideration during the microbiological criteria establishment.

In order to prevent the introduction and installation of *L. monocytogenes*, an accrued control and reinforced surveillance of food products is required. Outreach efforts have to be regularly undertaken. In fact, the contamination rates vary in function of the surveillance degree by municipal hygiene services, the number of collected samples and especially the alimentary matrix choice that have to be appropriately targeted.

**Conclusion:** Contamination of food by *L. monocytogenes* remains a major public health problem in industrialized countries and developing countries. This bacterium is an adverse factor for the international food trade, since, even in very small quantities, it poses serious problems in terms of import-export, what must inevitably be taken into account in the development of industry standards. Indeed, *L. monocytogenes* may contaminate any food and even reduced presence requires control at all points of the food chain where it appears to persist, as a health prophylaxis remains the only means of prevention. The contamination rates vary in function of the surveillance degree by municipal hygiene services, the number of collected samples and especially the alimentary matrix choice that have to be appropriately targeted.

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**REFERENCES**


