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

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Abstract: *Listeria monocytogenes* causes septicemia, 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 those 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 59 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 than ever. 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. monocytogene*s 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

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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 exanimate, 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 esculetin, translated by a blackening medium after the appropriate incubation. The antibiotics (Oxoid, SR0166) 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, cefoletan, 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 composants derived from aglycone. Concerning the Palcam medium (Oxoid, CM0877), the lithium chloride, ceftazidin, 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 black zone due to the formation of iron phenolic composants derived from aglycone, in a cherry red background. Listeria monocytogenes does not ferment the mannitol.

The second isolation step uses the nutritive Tryptone Soy Extract Yeast Agar (TSEYA) (Fluka, 93395), from the seeded medium of Tryptone Soy Yeast Extract Broth (TSYEB) (Biolife, 4021672). Both midst contain tryptone, soy and yeast extract as nutritive substances. They contain cycloheximid, nalidixic acid and acriflavin as inhibitrice substances. Thus, 05 typical colonies are introduced in a 5mL tube containing TSYE broth that is incubated at +36±2°C, during 24 hours. Then, an agar TSEYA medium plate is seeded, using one loopful of the TSYE broth, afterwards, nurtured at +36±2°C, during 24 hours. The typical *L. monocytogenes* colonies are convex, colorless, translucent and with smooth edges.

Confirmation tests are necessary. Performed from a typical colony from medium TSAYE, a Gram coloration show *L. monocytogenes* shaped as positive Gram bacillus, thin, short and animated by twirl mobility. The catalase test, performed using one colony suspended in an oxygen peroxide drop, indicates, by the formation of bubbles, that *L. monocytogenes* synthesizes a catalase. Finally, the confirmation of the species is accomplished by the identification of biochemical characteristics using API *Listeria* tests (Api 18R Biomérieux).

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 Colin, 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

	Total samples analyzed			Samples containing *L. monocytogenes*			Recuperation (%)		
Alimentary matrices	2009	2010	2011	2009	2010	2011	2009	2010	2011
Red meat product	266	218	185	4	9	12	1.5	4.1	6.5
Poultry product	20	29	105	0	4	7	0	13.8	6.6
Varied salads	93	70	99	2	3	6	2.1	4.2	6.0
Cooked meals	145	104	150	0	2	3	0	1.9	2.0
Dairy products	66	32	73	1	0	3	1.5	0	4.1
Pastries	95	40	96	0	1	2	0	2.5	2.0
Creams and Ice creams	211	91	90	0	0	0	0	0	0
Seafood	9	7	8	0	0	0	0	0	0
All food	905	591	815	7	19	33	0.77	3.21	4.05

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 inexistent 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 industrials (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 (Gebretsadika *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 (Pires *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, 2006). 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 (Leroi *et al*., 2001), no positive sample is identified (Table 1). This can be explained by the weak number of samples analyzed (24/2311), 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 subtracts, 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 Unfit for Consumption (UC%) and percentage of samples containing *Listeria monocytogenes* (CL.m%)

	GRSAD (2009)			GRSAD (2010)			GRSAD (2011)		
Food matrices	No. (n)	UC (%)	CL.m (%)	No. (n)	UC (%)	CL.m (%)	No. (n)	UC (%)	CL.m (%)
RMP	266	77.5 (206/266)	1.50 (4/266)	218	44.0 (96/218)	4.1 (9/218)	185	65.9 (122/185)	6.5 (12/185)
WMP	20	80.0 (16/20)	0.0 (0/20)	29	44.8 (13/29)	13.8 (4/29)	105	58.1 (61/105)	6.7 (7/105)
VS	93	63.4 (59/93)	2.1 (2/93)	70	78.6 (55/70)	4.2 (3/70)	99	61.6 (61/99)	6.0 (6/99)
CM	145	27.6 (40/145)	0.0 (0/145)	104	39.4 (41/104)	1.9 (2/104)	150	34.0 (51/150)	2.0 (3/150)
DP	66	34.8 (23/66)	1.5 (1/66)	32	62.5 (20/32)	0 (0/32)	73	46.6 (34/73)	4.1 (3/73)
P	95	36.8 (35/95)	0.0 (0/95)	40	50.0 (20/40)	2.5 (1/40)	96	37.5 (36/96)	2.0 (2/96)
CIC	211	39.8 (84/211)	0.0 (0/211)	91	52.7 (48/91)	0 (0/91)	90	61.1 (55/90)	0 (0/90)
Seafood	9	66.7 (6/9)	0.0 (0/9)	7	100 (7/7)	0 (0/7)	8	75.0 (6/8)	0 (0/8)
All food	905	51.8 (469/905)		591	50.8 (300/591)		815	52.2 (426/815)	

GRSAD: Global Results of Samples Analyzed During 2009, RMP: Red meat product, CIC: Creams and ice creams, WMP: White meat product, VS: Varied salads, CM: Cooked meals, DO: Dairy products, P: Pastries

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* (Olivier, 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. monocyto*genes' 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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