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Rapid Monitoring of *Listeria monocytogenes* in Raw Minced Meat Using Real-Time Polymerase Chain Reaction

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Abstract: *Listeria monocytogenes is a pathogenic bacteria of great concern in the food industry due to its wide distribution in the environment, its ability to grow at low temperatures and relatively high salt levels, and the potential severity of the infection with high mortality, mainly in pregnant women, the elderly and immunocompromised patients. Food-borne listeriosis remains a public health threat.*

One hundred forty two minced raw meat samples were collected from various points (processing plants, hotels, restaurants, retail outlets, caterers and wholesalers) and were analyzed for the detection of L. monocytogenes by conventional culture and Real-time polymerase chain reaction (Real-time PCR).

Our results showed frequent contamination of raw minced meats (21%). Our statistical analysis showed a relatively correlation (R^2 = 0.637, p <0.01) between the two methods, while the sensitivity, specificity, and positive and negative PCR method predictive values were respectively 30, 92, 37 and 97%. Continuous environmental monitoring schemes for L. monocytogenes are of major importance to identify potential contamination sources and as an early warning system for meat business operators. Hence, the detection of L. monocytogenes by Real-time PCR method allows good control of the health risk.

Keywords: *Control, Health risk, Listeria monocytogenes, Minced raw meat, Real-time PCR*

I. INTRODUCTION

The quality and safety of foods, both of animal and plant origin, is of outmost importance for governments, industries and consumers. It is well recognized that pathogens, such as *Listeria* spp. particularly *Listeria monocytogenes*, can be transmitted along the food chain and be a source of human illness; they can contaminate foods and multiply under suitable conditions [1]. *L. monocytogenes* is frequently associated with foodborne disease outbreaks that are characterized by widespread distribution and relatively high mortality rates [2]. Furthermore, not all *L. monocytogenes* strains are equally capable of causing disease in humans. Of the 13 serovars of *L. monocytogenes*, only three i.e., 1/2a, 1/2b and 4b, cause more than 90% of the human cases [3].

In recent years there has been a large increase in the production and consumption of meat and meat products, at the same time there is an increasing consumer demand for a health, safe and balance diet [4]. Meat and meat products are excellent substrates for microorganisms growth [5]. In fact, *L. monocytogenes* may also survive for a long time in meat processing facilities [6], which is particularly related to its psychrotrophic nature [7], its ability to attach and form resistant biofilms [8, 9] and its potential for contamination of raw and uncooked meats such as raw minced meat (beef or poultry meat) [10].

The microbiological analysis of meat remains a challenging task for virtually all assays and technologies, especially for particular pathogenic *Listeria* species [11]. The problems may be due to: (i) the complexity of meat and composition, (ii) the heterogeneous distribution of low levels of pathogens, (iii) the stress suffered by the microorganisms during the processing of foods and (iiii) the presence of bacteria from the normal microbiota, especially in raw meats. Thereby, classical microbiological methods for detection of *Listeria* spp. involve the use of pre-enrichment and/or specific enrichment, followed by the isolation of the bacteria in solid media and a final confirmation by biochemical and/or serological tests [12, 13]. However, these procedures are not always effective (e.g. viable but not culturable -VBNC-forms) [12, 13], and are extremely labour intensive, requiring a long time (form days to weeks) to yield a conclusive result. During the past decades, different alternative have been developed to overcome these disadvantages, and a number of molecular methods have been devised to reveal the presence of undesirable microorganisms in different food matrices such as those based on the Polymerase Chain Reaction (PCR) or Real-time PCR [14].

PCR can be superior to culture for detecting the main pathogens in meat samples. The aim to rapidly detect multiple microorganisms in one run is difficult but needed by the food industry and food safety [15, 16]. Real-time PCR has greatly increased the speed and sensitivity of PCR-based detection methods, but the terms “rapid” and “sensitive,” when applied to PCR as a detection method for foodborne pathogens, must be used with

caution [13]. However, Real-time PCR detection of the bacteria in food samples is currently done following the pre-enrichment culture because of the inhibitors in food samples [17, 18].

Additionally other rapid methods using Chromogenic agars, the presumptive identification of *L. monocytogenes* is possible after 24 h, compared with 3-4 days using Oxford and other conventional agars [19]. Chromogenic media is done according to enzymes expressed by the pathogen and production of acids by the fermentation of sugars. Different antimicrobials are added to the media to obtain sufficient selectivity. Chromogenic media is the most used culture confirmation method because of its easy preparation and interpretation [20].

In the present work our aim was to evaluate the basic situation and the potential persistence of *L. monocytogenes* strains in minced raw meat using qualitative methods: ISO 11290-1 standard method, chromogenic and molecular diagnostics (Real-time PCR) and to determine correlation between methods.

II. MATERIALS AND METHODS

1. Food samples and bacterial isolation

In total, 142 minced raw meats samples (Beef or chicken origin) were collected from various local points (processing plants, hotels, restaurants, retail outlets, caterers and wholesalers) in Rabat, Morocco. Samples were transported in clean plastic bags chilled on ice to the laboratory within one hour after sampling. Isolates were recovered using standard laboratory methods for the isolation of *L. monocytogenes* from food (ISO 11290-1:1996) [21]: Examinations for *Listeria* were performed using a two-step enrichment procedure. 25 g of raw minced meat was placed into a bag containing 225 mL of Half Fraser's broth (Oxoid, CM0895) then meat homogenization was incubated for 24 h at 30 °C. A subset of 0.1 ml was then incubated in 10 ml of Fraser broth (Oxoid, CM0895) for 24 h at 37 °C. Subsequently, subsets were plated onto Palcam agar (Oxoid, CM0877), Oxford agar (Oxoid, CM0856), Oxoid Chromogenic Isolation *Listeria* Agar (Oxoid, CM1080) and Brillance *Listeria* Agar (Oxoid, CM1084) which were then incubated for 48 h at 37 °C. Listeria-like colonies were streaked onto Oxoid Chromogenic Identification *Listeria* Agar (Oxoid Ltd.) supplemented with *Listeria* Selective Supplement and *Listeria* Differential Supplement (Oxoid Ltd.) and incubated for 48 h at 37 °C. Presumptive *L. monocytogenes* colonies on the Chromogen agar were streaked onto sheep blood agar (Oxoid Ltd.) for appraisal of hemolysis (CAMP test). To identify other *Listeria* species, the API *Listeria* Identification Kit was used (Api 18R Biomérieux) in accordance with the manufacturer's instructions. The sensitivity of qualitative detection (presence/absence) tests is then defined by the quantity of meats examined. In many cases, the requirement of detection is less than one cell per 25 g of food, as small numbers of some pathogens may be sufficient to cause disease.

2. Detection of *L. monocytogenes* by Real-time PCR

The Real-time PCR approach relies on the optical detection of amplification products by measuring the fluorescent signal generated by either intercalating dyes or specific dual-labeled probes. Further, most of Real-time PCR methods use DNA extraction, followed by Real-time PCR for rapid and sensitive detection, as well as for enumeration of *L. monocytogenes* in meat. Following the TaqMan® *L. monocytogenes* Detection Kit protocol (Applied Biosystems PN 4366102): Perform pre-enrichment consists of growing the specific pathogen from a selected sample, Preparing Samples, DNA extraction and Real-time PCR: Reactions were run on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA). The amplification curves of Real-Time PCR were processed and the concentrations determined using the StepOne software version 2.0 software (Applied Biosystems, Foster City, USA). Specificity and sensitivity of Real-time PCR assays were determined by using cultures of *L. monocytogenes* strain ATCC 19111. Methods were controlled according to the instructions of the following ISO standards: 22119, 20836, 20837 and 20838 [22, 23, 24, 25].

We examined the correlation between the two methods by calculating a Pearson correlation coefficient using the MedCalc software, version 12.6.1.0 (MedCalc Software bvba, Belgium, 2012). As a result we calculated the sensitivity, specificity and predictive values of Real-time PCR method considering the culture method as the reference method for the detection of *L. monocytogenes*.

III. RESULTS

L. monocytogenes was detected in 21% (30/142) samples: Respectively, 14% (20/142) by the classically method and 11% (16/142) by Real-time PCR (Table 1).

Even though it was statistically significant, the correlation between the results by culture and by Real-time PCR was relatively significant, as shown in Figure 1 (R^2= 0.6374, $p < 0.01$), (Fig. 1).

Considering the culture method as the reference method for the detection of *L. monocytogenes*, the sensitivity, specificity, and positive and negative Real-time PCR method predictive values were respectively 30, 92, 37 and 97% (Table 1).

IV. DISCUSSION

In recent years there has been an increase in the number of Real-time PCR assays which are widely used for the detection of *L. monocytogenes* in meat [26, 27, 28].

However, the PCR does not distinguish among viable and dead bacterial cells. DNA from bacterial dead cells can serve as a template for the PCR many days after cell viability has been lost [12]. This inability has impaired the implantation of the PCR for routine monitoring in food microbiology laboratories, where metabolically injured or non-viable cells are generally present after the stress that bacteria suffer during food processing and conservation.

In this study, *L. monocytogenes* was detected in 21% (30/142) samples, likewise, it was detected in 23 of 211 (10.9%) minced beef samples in a retail study in Switzerland in the first half of the year 2000 [29]. A high prevalence of *L. monocytogenes* (52%) was observed in a survey of 100 retail samples of minced beef in Alberta, Canada, in late spring and summer 2001 [30].

Even though, the presence of *L. monocytogenes* can be masked in applied cultural assays by, e.g., *L. innocua* or by the autochthonous microflora of the food matrix and specific antimicrobial components in foods [31, 32]. In fact, of the 10 samples positive by Real-time PCR and negative by culture, four samples 40% (4/10) are positives for *L. innocua*. The detection of *L. monocytogenes* in foods is also hampered by the high population of competitive microflora, the low levels of the pathogen, and the interference of inhibitory food components.

The positive predictive value (PPV) of the Real-time PCR compared to culture is low (PPV=37%) (Table1). This means that for the same sample, a concentration of *L. monocytogenes* determined by Real-time PCR will not necessarily be confirmed by culture. In contrast, the negative predictive value (NPV) is excellent and is 97%. Therefore, it is very likely that nondetectable result by Real-time PCR will be accompanied a nondetectable result in culture. In the current study, 126 samples were negative by Real-time PCR. Of these, only 14 were culture positive, indeed various studies have reported the presence of Real-time PCR inhibitors in meat samples [17, 18]. The Real-time PCR method could, thanks to its high VPN in the raw minced meat, be used in an optical screening for ensure rapidly of the absence of *L. monocytogenes* without having to resort to culture. In contrast, the low PPV of the Real-time PCR method involves, in the case of a positive result, the need for culture that is currently the reference method. In our study, this occurs for 63% of the samples, which limits the usefulness of the Real-time PCR method for the microbiological monitoring of raw minced meat [33, 34].

Table 1: Comparison of the detection of *L. monocytogenes* by Real-time PCR and Culture (Standard method) in raw minced meat (n = 142)

	Detection by Culture			
	Positive	Negative	**Total**	**predictive Value**
Detection by Real-time PCR				
Positive	6	10	16	PPV[c] = 0,37
Negative	14	112	126	NPV[d] = 0,97
Total	20	122	142	
Sensitivity and Specificity	Sensitivity[a] = 0.30	Specificity[b] = 0.92		

a Sensibility = true positive/ (true positive + false negative)
b Specificity = true negative/ (true negative + false positive)
c PPV (Positive Predictive Value) = true positive/ (true positive + false positive)
d NPV (Negative Predictive Value) = true negative/ (true negative + false negative)

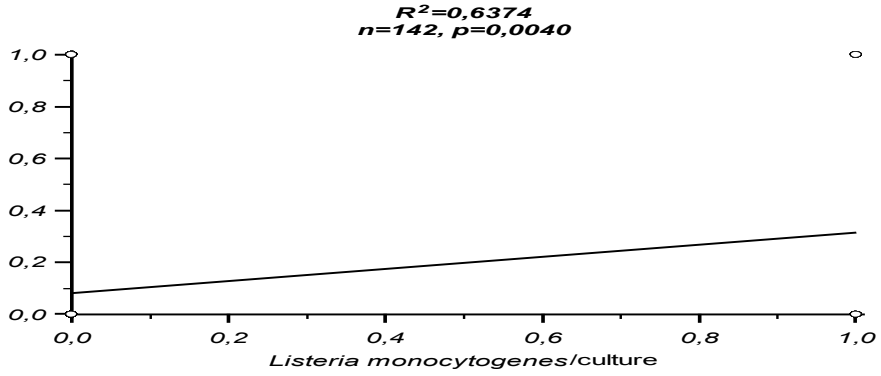

Figure 1: Correlation between *L. monocytogenes* obtained by Real-time PCR with those obtained by culture in raw minced meat

V. CONCLUSION

The specificity and sensitivity of Taq Man real-time PCR technology for detecting *L. monocytogenes* in raw minced meat were high, and the assay could be completed within 2 h. This method could be used to detect other food samples contaminated by *L. monocytogenes* and identify the cause of food-borne Listeriosis outbreaks.

Production of meats free from *L. monocytogenes* contamination remains a challenge for the meat industry. Proactive approaches beyond "plant floor inspection" and "test and hold" protocols are needed in order to effectively protect consumers. Furthermore, continuous environmental monitoring schemes for *L. monocytogenes* are of major importance to identify potential contamination sources and as an early warning system for meat business operators.

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