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Research Paper

Prevalence of *Listeria* spp. in Dairy Products With a Focus on Raw Milk Cheeses in Algeria

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ABSTRACT

Introduction: Raw milk and its derived cheeses are frequently implicated in foodborne outbreaks worldwide. This study aimed to assess the contamination by *Listeria* spp., particularly *Listeria monocytogenes*, in three types of cheeses made from raw cow's milk at various stages of production in three units located in the Algiers region. This work provides a synthesis of available data on the prevalence of *Listeria* spp. in dairy products, especially cheeses, in Algeria and at the international level. It highlights critical contamination points, the influence of production conditions on bacterial proliferation, and the main challenges associated with controlling this pathogenic bacterium within the dairy sector.

Materials & Methods: Samples were analyzed according to EN ISO 11290-1 and EN ISO 11290-2, covering both the qualitative detection and quantitative enumeration of *L. monocytogenes* and other *Listeria* species.

Results: Out of a total of 385 samples analyzed, 52(13.5%) tested positive for a *Listeria* species. Contamination was higher in the processing unit, with a prevalence of 18%, compared to 11.9% in the production units. Four *Listeria* species were identified. *L. monocytogenes*, the major pathogenic species, had an overall prevalence of 5.2%, with higher contamination in processed products (12%) than in locally produced cheeses (2.8%). *Listeria innocua* was the most frequently detected species, with an overall prevalence of 6.5% (6.7% in local products and 6% in processed ones). *Listeria grayi* (1.3%) and *Listeria welshimeri* (0.5%) were isolated exclusively from locally produced cheeses.

Conclusion: These results demonstrate the diversity of *Listeria* species present in the studied cheeses and reveal a higher prevalence of *L. monocytogenes* in processed products.

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1. Introduction

The food industry is continuously exposed to the risk of contamination by pathogenic agents, such as the *Listeria* genus, which is ubiquitous in the environment (soil, water, plants), as well as in animals and humans [1]. Among them, *Listeria monocytogenes* is the most virulent and is responsible for listeriosis, a serious zoonotic disease [2].

The ingestion of contaminated food remains the main route of transmission [3]. *L. monocytogenes* can proliferate at all stages of the food chain, making its control particularly challenging [4]. Most foodborne outbreaks are associated with ready-to-eat products, which are often contaminated after processing [5].

In Algeria, the reported prevalence of *L. monocytogenes* ranges from 0.19% to 2.61% [6-8]. Although these levels are sometimes low, they pose a latent risk due to the high pathogenicity of the bacterium, a risk further exacerbated by inadequate hygiene practices and, in some cases, insufficient pasteurization.

From a regulatory perspective, Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs requires the absence of *L. monocytogenes* in 1 g or 25 g of product, depending on the nature and shelf life of the food. This requirement, which is stricter than the 1994 directive, reflects the severity of the risk posed by this pathogen. In Algeria, the Interministerial Decree of September 25, 2005, also mandates the testing of *L. monocytogenes* in milk and dairy products, underscoring its public health importance.

Within this context, the present study was conducted to evaluate the health risk associated with the presence of *L. monocytogenes* in raw milk cheeses sold for human consumption in Algeria. This work is original in that it offers a comprehensive evaluation of the presence of *L. monocytogenes* in three types of raw milk cheeses monitored throughout all stages of their production chain,from milk collection to the final product. Unlike classical studies that focus solely on the final product, this approach allows for the identification of potential critical contamination points throughout the manufacturing process.

To date, few studies have undertaken a systematic and comparative analysis of multiple types of raw milk cheeses produced in Algeria, particularly within the framework of integrated microbiological surveillance. This study therefore helps fill the gap in recent data concerning the prevalence of *L. monocytogenes* at various stages of dairy processing while highlighting deficiencies in hygiene, quality control, and microbial risk management.

2. Materials and Methods

The study was conducted between February 2014 and May 2016 in three raw milk cheese production facilities located in the wilayas of Boumerdes, Blida, and Algiers. These sites were selected to represent different cheese-making regions in central Algeria. Five visits were carried out at each facility, covering multiple production batches.

Facility 1, located in Boumerdes, produces a semi-hard, uncooked pressed cheese similar to Edam. Facility 2, based in Blida, manufactures a soft-ripened cheese of the Camembert type. Facility 3, situated in Algiers, specializes in the processing and packaging of an imported hard cheese resembling Maasdam. Samples were collected at various stages of the production process, transported on ice at +4 °C, and analyzed within a maximum of two hours at the laboratory of the Higher National Veterinary School of Algiers (Algeria).

A total of 285 samples were collected from Facilities 1 and 2. Among these, 135 were raw milk samples (75 from Facility 1 and 60 from Facility 2), 10 were pasteurized milk samples (five per facility), 20 were curd samples (ten per facility), 20 were samples collected during the ripening process, 50 were finished product samples (25 per facility), and 50 were surface swabs (25 per facility), taken from equipment and workers' hands. In Facility 3, 100 samples were collected, including 25 raw material, 25 sliced cheese, 25 grated cheese, and 25 surface swabs. This comprehensive sampling strategy allowed an in-depth assessment of microbiological risks at each stage of the production and processing chain, with a particular focus on the detection of *L. monocytogenes*.

Microbiological analyses were conducted in accordance with ISO 11290-1 and ISO 11290-2 (2017), which define the qualitative detection and quantitative enumeration of *L. monocytogenes* and other *Listeria* species. Sample preparation and general microbiological procedures followed ISO 6887-1 and ISO 7218 standards.

For quantitative analysis, the ISO 11290-2 method was applied. Characteristic colonies were counted on plates containing at least 15 colonies, and a weighted average concentration was calculated from two successive dilutions. Results were expressed as colony-forming units

per gram (CFU/g) or per milliliter (CFU/mL), depending on the sample type. For qualitative analysis, Listeria detection followed the ISO 11290-1 protocol, and suspected colonies were subjected to confirmatory and identification tests.

The isolated strains were purified and stored on slanted agar at +4 °C and in glycerolized brain–heart infusion broth at –20 °C. Characterization of isolates was performed using a series of biochemical and enzymatic tests, including Gram staining (to identify gram-positive bacilli), catalase testing, umbrella-shaped motility testing at 25 °C, detection of β-hemolysis on blood agar, and the CAMP test to evaluate enhanced hemolysis in the presence of *Staphylococcus aureus* or *Rhodococcus equi*. A conventional biochemical panel, including TSI, VP, MR, oxidase, esculin hydrolysis, urease, and indole tests, was also performed. Specific identification was completed using the API Listeria® test strip, which is based on ten enzymatic and fermentative reactions to generate a numeric identification profile. Finally, the DIM test was used to differentiate *L. monocytogenes* (DIM–) from *L. innocua* (DIM+).

3. Results

3.1. Overall prevalence of Listeria

The overall prevalence of *Listeria* spp. and the different *Listeria* species identified in locally produced and imported samples are presented in Table 1.

Out of the 385 samples analyzed, 52(13.5%) tested positive for at least one species of the genus Listeria. Among these, 34 samples originated from the local production units (out of 285), corresponding to a prevalence of 11.9%, whereas 18 out of 100 samples from the imported product processing unit tested positive, indicating a higher prevalence of 18%. Four *Listeria* species were identified: *L. monocytogenes*, *Listeria innocua*, *Listeria grayi*, and *Listeria welshimeri*. *L. monocytogenes*, the major pathogenic species of concern, was isolated from 20 samples (5.2%), with a prevalence of 2.8% in locally produced cheeses (8/285) and 12% in imported products (12/100). *L. innocua* was the most frequently detected species, with 25 isolates (6.5%), occurring in both the local units (19/285, i.e. 6.7%) and the imported product processing unit (6/100, i.e., 6%). *L. grayi* was detected exclusively in locally produced cheeses, with 5 positive samples (1.3%), as was *L. welshimeri*, which was found in 2 samples (0.5%). Neither of these two species was identified in imported products. These findings highlight both the diversity of *Listeria* species present in raw milk cheeses and a higher contamination rate with *L. monocytogenes* in products processed from imported cheeses.

3.2. Prevalence and distribution of *Listeria* Species in production units 1 and 2

The prevalence and distribution of the different *Listeria* species identified in the two production units are presented in Tables 2 and 3.

3.3. Prevalence of *Listeria* in the processing unit

The prevalence and distribution of the different *Listeria* species identified in the processing unit are presented in Table 4.

3.4. Enumeration results of *Listeria*

All samples tested using the enumeration methods were negative for *Listeria* spp. However, following the enrichment step, the detection method identified *Listeria* spp. in positive samples at levels of less than 100 CFU/g. The results of the positive and negative samples after enrichment are presented in Table 5.

4. Discussion

Various dairy products frequently consumed worldwide, including in Algeria, can serve as transmission vectors for *L. monocytogenes*. Several reports have revealed contamination by *L. monocytogenes* in milk and

Table 1. Overall prevalence of *Listeria* in the analyzed samples

Sampling Type	No. (%)				
	Listeria spp.	*L. monocytogenes*	*L. innocua*	*L. grayi*	*L. welshimeri*
Locally produced cheeses (n=285)	34(11.9)	8(2.8)	19(6.7)	5(1.7)	2(0.7)
Imported and processed cheese (n=100)	18(18)	12(12)	6(6)	0(0)	0(0)
Total (n=385)	52(13.5)	20(5.2)	25(6.5)	5(1.3)	2(0.5)

Table 2. Prevalence and distribution of samples contaminated with *Listeria* in production unit 1

Production Step (number of samples)	No. (%)				
	***Listeria* spp.**	***L. monocytogenes***	***L. innocua***	***L. grayi***	***L. welshimeri***
Raw milk (n=75)	19(12.7)	6(4)	11(7.3)	2(1.3)	0(0)
Pasteurized milk (n=5)	0(0)	0(0)	0(0)	0(0)	0(0)
Curd (n=10)	1(0.7)	0(0)	0(0)	1(0.1)	0(0)
Ripening stage (n=10)	5(3.3)	1(0.7)	2(1.3)	2(1.3)	0(0)
Final product (n=25)	1(0.7)	0(0)	1(0.7)	0(0)	0(0)
Environmental swabs (n=25)	2(1.3)	0(0)	2(1.3)	0(0)	0(0)
Total (n=150)	28(18.7)	7(4.7)	16(10.7)	5(3.3)	0(0)

dairy products [9]. This study was conducted to estimate the prevalence of different *Listeria* species in three types of cheese throughout the production process, with particular focus on *L. monocytogenes* and its bacteriological characterization.

The overall prevalence of *Listeria* spp. recorded in our study was 13.5%. This result is comparable to those reported by other authors, such as Farber et al. in 1988 in Canada (12%) [10]. However, it is higher than the prevalence rates recorded in Algeria by Bouayad et al. in 2012 (9.3%) [11].

The highest prevalence was recorded for *L. innocua* (6.7%), followed by *L. monocytogenes* (5.2%), *L. grayi* (1.7%), and *L. welshimeri* (0.7%).

Our study revealed a prevalence of 5.2% for *L. monocytogenes*. Several studies have investigated the presence of *L. monocytogenes* in milk and dairy products. In Algeria, the prevalence of *L. monocytogenes* recorded in raw milk ranges from 1.9% to 3.2% [6, 8].

In both production units, the highest prevalence of *Listeria* spp., particularly *L. monocytogenes*, was observed in raw milk collected from collectors' tanks, with rates of 12.7% and 5.9% in units 1 and 2, respectively. This contamination mainly originates from the animals' gastrointestinal tract, the environment, and the skin of the teats [12]. Other contributing factors include poor hygiene of the collection tanks and the persistence of certain strains in the production environment [8].

Recent studies have reported similar rates of *L. monocytogenes* in raw milk. In North Africa, a meta-analysis highlighted an average prevalence of 4.67% across the region, including Algeria, with ranges between 0 and 2.61 % in certain areas [13]. In Italy, official surveillance of raw milk reported prevalence rates ranging from 0.1% to 1.4% [14]. These results collectively suggest that raw

Table 3. Prevalence and distribution of samples contaminated with *Listeria* in production unit 2

Sampling Step (number of samples)	No. (%)				
	No. (%)	***L. monocytogenes***	***L. innocua***	***L. grayi***	***L. welshimeri***
Raw milk (n=60)	8(5.9)	1(0.7)	3(2.2)	2(1.5)	2(1.5)
Pasteurized milk (n=5)	0(0)	0(0)	0(0)	0(0)	0(0)
Curd (n=10)	0(0)	0(0)	0(0)	1(0.7)	0(0)
Ripening stage (n=10)	0(0)	0(0)	0(0)	0(0)	0(0)
Final product (n=25)	0(0)	0(0)	0(0)	0(0)	0(0)
Environmental swabs (n=25)	0(0)	0(0)	0(0)	0(0)	0(0)
Total (n=135)	8(5.9%)	1(0.7%)	3(2.2%)	3(2.2%)	2(1.5%)

Table 4. Prevalence and distribution of samples contaminated with *Listeria* in the processing unit

Sampling Stage: Number of Samples	No. (%)				
	No. (%)	*L. monocytogenes*	*L. innocua*	*L. grayi*	*L. welshimeri*
Bulk cheese: 25	0(0)	0(0)	0(0)	0(0)	0(0)
Sliced cheese: 25	0(0)	0(0)	0(0)	0(0)	0(0)
Grated cheese: 25	15(15)	11(11)	4(4)	0(0)	0(0)
Swabs : 25	3(3)	1(1)	2(2)	0(0)	0(0)
Total: 100	18(18)	12(12)	6(6)	0(0)	0(0)

milk contamination of raw milk by *L. monocytogenes* is variable and generally low to moderate, yet sufficiently frequent to warrant strict monitoring due to its potential public health risks.

More recent studies corroborate these observations. A 2022 study on "quesillo" (a traditional Honduran cheese) demonstrated that prolonged heating at 65 °C for approximately 35 minutes achieved a 7-log reduction of *L. monocytogenes* cells, rendering the product safe for consumption.

Similarly,in buffalo mozzarella, Márquez-González et al. (2021) [15] observed significant variability in thermal resistance between strains; however, kneading at 77–80 °C resulted in a reduction of over 5 log units, confirming the effectiveness of heating during the final steps of manufacturing. These findings confirm that, during the production of Edam-type cheese, the curdling stage may allows fluctuations in bacterial population, whereas the high-temperature kneading step remains essential for ensuring product safety.

During the ripening process in unit 1, the prevalence of *Listeria* spp. reached 3.3%, while *L. monocytogenes* was present at a rate of 0.7%.

Microbial enzymes play a major role in modifying texture and developing cheese flavors, giving them their specific organoleptic properties.

Although soft cheeses provide a favorable environment for *L. monocytogenes* growth, the prevalence observed in this study remains low (0.7%), with only one strain isolated from raw milk. This low rate highlights the critical importance of implementing a HACCP (hazard analysis and critical control points) system throughout the production chain to minimize the presence of this pathogen at critical points.

The HACCP system is a systematic and structured approach designed to enhance food safety by identifying stages requiring intervention to maintain product safety at an acceptable level [16].

In the final product, the prevalence of *Listeria* spp. was 0.7%, corresponding to a strain of *L. innocua* isolated from an uncooked pressed cheese (Edam type). The presence of a *Listeria* species other than *L. monocytogenes* does not automatically guarantee product safety, as it suggests that *L. monocytogenes* could also be present [17].

Table 5. Enumeration results of *Listeria* spp. after enrichment

Sample Type	No. (%)	
	Negative Samples after Enrichment	Positive Samples after Enrichment
Locally produced cheeses (n=285)	251(88.1)	34(11.9)
Imported cheese (n=100)	82(82)	18(18)
Total (n=385)	333(86.5)	52(13.5)

In the hard cheese (grated) processing unit, the prevalence of *Listeria* spp. reached 15%, including 11% for *L. monocytogenes*, despite no contamination being detected in the raw material. These findings indicate an environmental origin of contamination. This hypothesis is supported by the presence of *Listeria* spp. on surface swabs: 3% for the *Listeria* genus and 1% for *L. monocytogenes*. These organisms, which are readily isolated from the environment, can persist by forming biofilms that are resistant to conventional cleaning protocols [18].

More recent research confirms that *L. monocytogenes* forms resilient biofilms on equipment surfaces (such as conveyors, drains, and joints), especially in hard-to-reach "niches" that escape standard cleaning and ensure its persistence and cyclical recontamination [19].

The enumeration method applied to samples from the three production units yielded 100% negative results. However, after enrichment, the presence of *Listeria* spp. was detected in 13.5% of cases, and *L. monocytogenes* in 5.2%, always at levels below 100 CFU/g. This suggests that these bacteria were initially present in very low quantities, undetectable without prior concentration, and that the enrichment process revives stressed strains and increases their number to detectable levels. According to the Codex Alimentarius Guidelines on the application of general principles of food hygiene to the control of *L. monocytogenes* in foods (CAC/GL 61-2007), foods that do not support the growth of *L. monocytogenes* may contain up to 100 CFU/g throughout their shelf life [20]. The detected contamination levels, although low, remained below the microbiological criterion of 100 CFU/g established for ready-to-eat foods that do not support the growth of *L. monocytogenes* throughout their shelf life [20].

Thus, the contamination levels detected in this study, although low, may represent a real risk, especially considering the scenario proposed by Lyytikäinen et al. (2001) [20], suggesting that repeated ingestion of low doses can lead to infection. Dairy products, particularly raw milk cheeses, have been implicated in several listeriosis outbreaks worldwide. *L. monocytogenes* is the etiological agent of listeriosis, an infection associated with significant health risks and economic impact. Although the incidence of this pathogen is relatively low, the mortality rate remains high, ranging from 20% and 30%.

In this study, 385 samples collected at various levels of production and processing of three types of raw milk cheeses were tested. *Listeria* spp. and *L. monocytogenes* were isolated from all three production and processing units at different manufacturing stages, with varying prevalence rates. The overall prevalence of *Listeria* spp. was 13.5%, and that of *L. monocytogenes* was 5.19%.

The high prevalence of *L. monocytogenes* recorded in grated cheese (12%), along with the absence of contamination in the raw material, suggests that *Listeria* spp. contamination occurs during post-processing stages, reflecting inadequate hygiene during manufacturing. The isolation of the bacterium from a surface swab further highlights the role of the processing environment as a source of food contamination.

The low prevalence observed in raw milk (3%) at the soft cheese production unit underscores the importance of implementing a food safety control system, such as HACCP, as an effective means of controlling this contaminant. The absence of *Listeria* in heat-treated milk indicates that pasteurization is sufficient to eliminate this pathogen.

Quantitative analysis revealed contamination levels below 100 CFU/g. However, the psychrotrophic nature of *Listeria* does not rule out the possibility of bacterial growth during cheese storage. Therefore, these products may still pose a risk to consumers since they undergo no further thermal treatment before consumption.

Overall, the results of this study support the notion of diversity of *L. monocytogenes* across different production stages and highlight the need to identify and characterize *Listeria* spp. contaminants in the food industry to determine their pathogenic potential and sensitivity to chemotherapeutic agents. This research also provided, for the first time, insights into the phenotypic characteristics and pathogenic potential of *L. monocytogenes* isolated from three types of cheeses marketed in Algeria.

The detection of *L. monocytogenes* in raw milk cheeses, even at low levels, has significant public health implications. Consumers of unpasteurized dairy products are particularly vulnerable, as these foods are often consumed without further heat treatment, allowing psychrotrophic pathogens such as *L. monocytogenes* to survive and potentially multiply during storage. This pathogen poses a serious risk to susceptible populations, including pregnant women, newborns, and the elderly, and immunocompromised individuals, in whom listeriosis can lead to severe outcomes such as meningitis, septicemia, or miscarriage. Given the increasing demand for artisanal and raw milk cheeses in Algeria and worldwide, reinforcing hygiene practices, implementing continuous environmental monitoring, and maintaining strict temperature control throughout the production and distribu-

tion chain are essential to safeguard public health. Future research should focus on the molecular characterization and antimicrobial resistance profiling of *L. monocytogenes* isolates from dairy environments. Several recent studies have reported the emergence of antimicrobial-resistant and hypervirulent clones, such as sequence types ST1, ST2, and ST6, which have been implicated in foodborne outbreaks globally [22]. Whole-genome sequencing (WGS) and multilocus sequence typing (MLST) approaches could provide valuable insights into the genetic diversity, virulence determinants, and persistence mechanisms of *Listeria* strains circulating in Algerian dairy plants. Such genomic surveillance would not only improve understanding of contamination sources and transmission routes but also support the design of more effective control measures and guide the prudent use of antimicrobials in food production systems.

5. Conclusion

In conclusion, this study underscores the need for continuous microbiological surveillance and the integration of molecular tools in food safety monitoring programs to better control *Listeria* contamination and protect consumers of raw milk dairy products in Algeria.

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Compliance with ethical guidelines

This study did not involve experiments on live animals or human participants. Therefore, ethical approval was not required. Sampling was carried out exclusively on dairy products (raw milk and cheeses) obtained from cheese production units in the Algiers region, with the consent and collaboration of the unit managers. All procedures complied with national and international standards for food safety research.

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Authors' contributions

Conceptualization, data curation, and investigation: Lynda Abdellaoui; Data analysis: Nassim Ouchene and Nadjet Amina Khelifi Touhami; Writing the original draft: Lynda Abdellaoui and Dalila Tarzaali; Review and editing: Leila Bouayad; Supervision and validation: Taha Moussadak Hamdi.

Conflict of interest

The authors declared no conflict of interest.

Data availability

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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