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Research article

Occurrence, antimicrobial resistance, serotyping and virulence genes of *Listeria monocytogenes* isolated from foods

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ABSTRACT

Listeria monocytogenes is a pathogen contaminated food, it is the cause of listeriosis worldwide. The aims of this study were to investigate the occurrence, antimicrobial resistance, serotyping and virulence genes of *L. monocytogenes* isolated from foods in Meknes city of Morocco. From June 2017 to May 2018, 520 food samples were randomly collected from a traditional market and two overcrowded popular neighborhoods (Lahdim and Hamria) and subjected to the detection of *L. monocytogenes*. Then, the antimicrobial susceptibility of the isolated strains were evaluated using the standard disk diffusion method and the determination of serotypes and virulence genes was performed by PCR. The results showed the detection of *L. monocytogenes* in fifteen (2.9%) of 520 samples, including three (5.7%) isolates in traditional whey, raw minced meat and raw sausage, two (3.8%) in raw milk and one (1.9%) in smen (traditional butter), raw bovine meat, raw poultry meat and raw fish, while salads and rayeb (traditional coagulated milk) were not contaminated. Among the fifteen isolated *L. monocytogenes*, nine (60%) belonged to the serogroup (1/2a, 1/2c, 3a and 3c), two (13.3%) belonged to the serogroup (1/2b, 3b, 4b and 4d) and four (26.6%) do not belong to any studied serogroup. Furthermore, fifteen (100%) isolates showed the presence of *actA* gene, fourteen (93.3%) harbored *hlyA*, *prfA* and *plcB* genes, thirteen (86.7%) carried *inlA* and *inlC* genes and twelve (80%) showed *inlJ* gene. The antimicrobial susceptibility analysis showed that the isolated strains were more resistant to amoxicillin/clavulanic acid (67.0%), erythromycin (60.0%), sulphamethoxazole (40.0%), ampicillin and sulphamethoxazole/trimethoprim (33.0%) and tetracycline (20.0%). Furthermore, 66.7% (10/15) were multidrug-resistant. From this study, we can conclude that foods marketed in Meknes city were contaminated by multidrug-resistant strains of *L. monocytogenes* harboring virulence genes, which may cause a serious risk to public health.

1. Introduction

Listeria monocytogenes is a Gram-positive bacilli, facultative anaerobic, and can grow in a wide range of pH (4.3–9.4), temperatures (from 0 to 45 °C), at a high salt concentration (of up 14%) and water activity (higher than 0.92) [1,2,3]. These particular physicochemical factors are in favor of the survival and proliferation of *L. monocytogenes* in a wide variety of foodstuffs, including seafood, meat and meat products, milk and dairy products, and vegetables [4].

Invasion of host cells by *L. monocytogenes* involves many virulence factors. The *hly* gene encodes an extracellular listeriolysin O (LLO) which has a role in the regulation of the host cell by *L. monocytogenes*. The ActA protein is essential for actin polymerization and intracytoplasmic movement of *L. monocytogenes* [5], *plcA* and *plcB* are involved in the lysis of the double membrane vacuole formed during cell-to-cell propagation [6]. PrfA is a positive regulatory factor for *hly, plcA, mpl, actA and plcB*, it regulates the expression of factors necessary for cell invasion (*InlA and InlB*) and intracellular proliferation (Hpt) [6]. *InlA* is implicated in the invasion of *L. monocytogenes* into intestinal epithelial cells by expressing

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the E-cadherin receptor. The *InlB* gene induces hepatocyte invasion via the c-Met receptor [6]. For somatic (O) and flagellar (H) antigens, they are used as monoclonal and polyclonal antibodies. There are 15 somatic (O) (I-XV) and 4 flagellar (H) (A-D) antigens [7]. Based on the characteristics of somatic (O) and flagellar (H) antigens agglutination, thirteen serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7) have been identified in *L. monocytogenes* [7]. Five other serogroups have been determined in *L. monocytogenes* such as IIa (1/2a-3a), IIb (1/2b-3b-7), IIc (1/2c-3c), IVa (4a-4c) and IVb (4ab- 4b, 4d-4e) [8], the serotypes (1/2a, 1/2b and 4b) are responsible for human listeriosis at a rate of almost 95%, of which 1/2a, 1/2b are mainly isolated from food and 4b from clinical cases [7].

L. monocytogenes is responsible for listeriosis with a high fatality rate of 20%–30% [7]. This pathogen can cause, meningoencephalitis, cerebral abscesses, cerebritis, bacteremia, meningitis, and sepsis, especially in the immunocompromised individuals and pregnant women [9,10]. On the other hand, antimicrobial resistance spread rapidly worldwide, which cause a health threat and economic burden, owing to the excessive use of antibiotic in human and veterinary medicine [11,12]. The resistance of *L. monocytogenes* to antibiotics which are commonly used in the treatment of human and animal diseases is worrisome [8].

Listeriosis causes severe damages to public health. In the European Union, the incidence of listeriosis is about 0.47 cases per 100, 000 population [4] and 0.24 cases per 100, 000 population in the United States [13]. In Morocco, the actual incidence of listeriosis remains unknown due to the lack of epidemiological surveillance, in fact only one case of neonatal listeriosis has been reported [14]. However, several studies showed the prevalence of *L. monocytogenes* in raw and processing foods [15,16].

The objectives of this study were (i) to evaluate the occurrence of *L. monocytogenes* in food samples collected from Meknes city of Morocco, (ii) to determine their susceptibility profiles of antibiotics and serotypes (iii) and to study their virulence by the amplification of the targeted virulence genes.

2. Material and methods

2.1. Sample collections

From June 2017 to May 2018, a total of 520 samples including raw milk, whey, rayeb, smen, raw bovine meat, raw poultry meat, raw minced meat, raw sausage, raw fish and salads (52 samples per each) were randomly collected from street traders, butcheries and restaurants with forty-three samples per month. Sampling was carried out in a traditional market and two overcrowded neighborhoods popular (Lahdim and Hamria). Then, samples were transported to the Laboratory of Microbiology at the Faculty of Science in Meknes. The microbiological analyses were performed on the same day of sampling.

2.2. Isolation and identification of L. monocytogenes

The protocol was made according to the Moroccan standard method [17]. Briefly, 10 g of each sample was aseptically homogenized with 90 mL of half Fraser broth in a stomacher 400 Circulator (Seward, West Sussex, UK) for 3 min at 260 rpm and incubated at 30 °C for 24 h. After incubation, 0.1 mL was transferred to the tube containing 10 mL of Fraser broth (Biokar, Beauvais, France) and incubated at 37 °C for 48 h. From it, a streak culture was performed on Agar Listeria acc. to Ottaviani & Agosti (ALOA, Biolife, Milan, Italy) and incubated at 37 °C for 48 h. A maximum of 5 colonies presumed to be *L. monocytogenes* were purified onto tryptone soya yeast extract agar (Biokar, Beauvais, France) and incubated at 30 °C for 24 h. Gram staining, catalase, oxidase, β-hemolysis, CAMP test and Listeria api have been used to confirme *L. monocytogenes* strains.

2.3. PCR-serorogroups analysis and virulence genes determination

DNA extraction of *L. monocytogenes* isolates was performed using heating method. Multiplex PCR was used for the determination of serotypes [18] and the amplification of virulence genes of the isolated strins [19], using the specific amorces described in Tables 1 and 2. PCR assays were performed in final volume of 25 μL, which containing 13.9 μL of ddH_2O, 2.5 μL of buffer (10×), 2 μL of 25 mM $MgCl_2$, 2.5 μL of 1 μM dNTP mix (KAPA Biosystems), 0.1 μL of Taq DNA polymerase (1 U/μL KAPA Biosystems), 2 μL of template DNA, 1 μL of each 10 μM primer. The PCR program was set as follows for all studied primers: initial denaturation at 5 min for 95 °C, 35 cycles of denaturation at 94 °C for 80 s, annealing at 58 °C for 90 s, elongation at 72 °C for 1 min and final elongation at 72 °C for 5 min primer. The amplified PCR products were visualized by ethidium bromide in 2 % agarose gel under UV light. *L. monocytogenes* strain ATCC19112 considered as positif control.

2.4. Antimicrobial susceptibility

The determination of antimicrobial susceptibility profile was carried out by disk diffusion test with reference to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [18], and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [20]. Fifteen antimicrobials were selected for this study on basis of their uses in the treatment of diseases in humans and veterinary medicine [8]: amoxicillin/clavulanic acid (30 μg), ampicillin (10 μg), penicillin (10 μg), amikacin (30 μg), gentamicin (30 μg), streptomycin (10 μg), imipenem (10 μg), erythromycin (15 μg), tetracycline (30 μg), vancomycin (30 μg), chloramphenicol (30 μg), sulphamethoxazole/trimethoprim (25 μg), sulphamethoxazole (200 μg), ciprofloxacin (5 μg), and kanamycin (30 μg). In this study, the isolated strains showing a decrease in susceptibility (intermediate) were considered as resistant, and *L. monocytogenes* ATCC19112 was used as a reference strain. Afterward, the Multiple Antibiotic Resistance (MAR) index was assessed as, the number of antimicrobials to which the isolated strains were resistant divided by the total number of antimicrobials to which the isolated strains were tested [21,22].

2.5. Statistical analysis

Statistical analysis was performed using SPSS software (SPSS version 20, IBM Corp, Armonk, NY, USA), The Chi-squared test was performed to assess the relationship between the variables of interest. *P*-value ˂ 0.05 was used in testing the statistical significance of all experimental data.

3. Results

3.1. Isolation and identification of L. monocytogenes

From a total of 520 analyzed food samples, 15 (2.9%) were positive for *L. monocytogenes* (Table 3). The highest value was detected in traditional whey, raw minced and raw sausage with 5.7%, followed by raw milk (3.8%), and finally smen, raw bovine meat, poultry meat and raw fish with 1.9%. However, *L. monocytogenes* was not detected in salads and rayeb (Table 3). The statistical analysis showed that the occurrence of *L. monocytogenes* do not depends on the food matrice ($p = 0.47$), seasons ($p = 0.52$) and sites ($p = 0.82$).

Table 1. Primer used for the amplification of virulence genes of *L. monocytogenes*.

Gene	Sequences (5′-3′)	Length (bp)
inlA	F-CCTAGCAGGTCTAACCGCAC R-TCGCTAATTTGGTTATGCCC	256
inlC	F-AATTCCCACAGGACACAACC R-CGGGAATGCAATTTTTCACTA	517
inlJ	F-TGTAACCCCGCTTACACAGTT R-AGCGGCTTGGCAGTCTAATA	238
actA	F-CCAAGCGAGGTAAATACGGGA R-GTCCGAAGCATTTACCTCTTC	650
prfA	F-ACCAATGGGATCCACAAGA R-CAGCTGAGCTATGTGCGAT	467
hlyA	F-ATCATCGACGGCAACCTCGGAGAC R-CACCATTCCCAAGCTAAACCAGTGC	404
plcB	F-AATATTTCAATCAATCGGTGGCTGA R-GGGTAGTCCGCTTTCGCTCTT	289

Table 2. Primer used for serotype determination of *L. monocytogenes*.

Primer	Sequences (5′-3′)	Product size (bp)	Serotype specificity
lmo0737	F-AGGGCTTCAAGGACTTACCC R-ACGATTTCTGCTTGCCATTC	691	1/2a, 1/2c, 3a and 3c
ORF2819	F-AGCAAAATGCCAAAACTCGT R-CATCACTAAAGCCTCCCATTG	471	1/2b, 3b, 4b and 4d

3.2. PCR analysis of virulence genes and serotyping of L. monocytogenes

The serotyping analysis showed that among the 15 tested *L. monocytogenes* strains, nine isolates (60%) belonged to serogroup (1/2a, 1/2c, 3a and 3c) and two (13.3%) belonged to serogroup (1/2b, 3b, 4b and 4d), while four (26.6%) did not belong to any of the studied serogroup (Figure 1 and Table 4). Seven virulence-associated genes (*inlA*, *inlC*, *inlJ*, *prfA*, *plcB*, *hlyA* and *actA*) were identified. All strains carried *actA* gene. Whereas, *hlyA*, *prfA* and *plcB* were detected in 14 (93.3%) strains, *inlA* and *inlC* in 13 (86.7%) strains and *inlJ* in 12 (80%) strains. Furthermore, the genes (*actA, inlA* and *inlC*), (*actA, hlyA, plcB* and *prfA)* and (*actA, hlyA, plcB* and *prfA*) were detected in *L. monocytogenes* strains from raw milk (strain MK9), raw bovine meat (B510) and raw minced meat (M23), respectively (Figure. 2 and Table 4).

3.3. Antimicrobial susceptibility

The results of this study showed that *L. monocytogenes* isolated from food present a high resistance to amoxicillin/clavulanic acid (67.0%), followed by erythromycin (60.0%), sulphamethoxazole (40.0%), ampicillin and sulphamethoxazole/trimethoprim (33.0%), tetracycline (20.0%), chloramphenicol, gentamicin, ciprofloxacin, amikacin, streptomycin, and vancomycin (17.0%). However, they were susceptible to penicillin and imipenem (Table 5). In addition, the MAR index value of the isolated *L. monocytogenes* ranged from 0.00 to 0.73 (Table 4).

Table 3. Rate of occurrence of *L. monocytogenes* in different food products.

Food type	No. of samples	No. of positive isolates (%)
Raw milk	52	2 (3.8)
Traditional whey	52	3 (5.7)
Rayeb	52	0 (0)
Smen	52	1 (1.9)
Raw bovine meat	52	1 (1.9)
Raw poultry meat	52	1 (1.9)
Raw minced meat	52	3 (5.7)
raw sausage	52	3 (5.7)
raw fish	52	1 (1.9)
Salads	52	0 (0)
Total	**520**	**15 (2.9)**

The bold indicates the total number of samples studied and the percentage of positive isolates.

4. Discussion

The present study showed that the rate of occurrence of *L. monocytogenes* in foods consumed in Meknes city of Morocco was 2.9%. This value is in agreement with those reported in Iran (2.99%) [18], Estonia (2.6%) [1] and Algeria (2.6%) [23]. However, it's higher than that found previously in Tetouan city of Morocco (1.5%) [24], in Japan (1.7%) [25] and India (1.5%) [26], and lower than that reported previously in Casablanca city of Morocco (23.3%) [27], Ireland (5.8 %) [28], Uruguay (11.2%) [29], China (21.7%) [30], Chile (25%) [31], Greece (14.3%) [32] and Spain (6.2%) [33]. Furthermore, traditional whey, minced mead, and raw sausage were the most contaminated foods (5.7%), followed by raw milk (3.8%), smen, bovine meat, poultry meat and raw fish (1.9%). These results were higher than those reported in other regions of Morocco for poultry meat (6.6%), red meat products (6.5%), salads (6%) and seafoods (0%) [34], ground meat and sausage (3.3%) and raw poultry (1.3%) [27], raw milk (0.83%) [35], dairy products (0.74%) and poultry meat (0%) [24], and lower than those reported in poultry and bovine meat products (0% and 2.7%, respectively) [24], chiken meat (3.66%) [36], dairy products (4.1%) [34] and raw milk (8.33%) [37]. However, our findings are comparable to those reported in salads (0%) [24] and traditional whey (5.20%) [37]. The difference in the occurrence of *L. monocytogenes* in food products may be due to the foods, sampling strategy, geographical differences and hygienic conditions of preparation and storage. Indeed, food samples collected from street vendors, restaurants and butchers do not meet food safety standards. In some countries, street foods represent a significant proportion of the food consumed by the urban population, and their distribution is relatively related to socio-economic and cultural factors [38,39]. In addition, many studies have reported that foodstuffs promote the growth of *L. monocytogenes* through their nutrient values and physicochemical properties [40,41].

The present study indicated the highest occurrence of serogroup (1/2a, 1/2c, 3a, and 3c), followed by serogroup (1/2b, 3b, 4b, and 4d). In Ireland, a study performed by Leong et al. showed that *L. monocytogenes* strains isolated from dairy, meat, seafood and vegetable are of serogroup (1/2a, 3a), (1/2b, 3b, 7), (1/2c, 3c), (4b, 4d, 4e), (1/2a), (1/2b), (1/2c) and (4b/4e) [42]. A study carried out in Iran reported that *L. monocytogenes* isolated from seafood products, market and processing environments belonged to serotype 1/2a (45.7%), followed by 4b (40.3%), 1/2c (5.39%), 1/2b(4.68%), and 4c (3.96%) [15]. Another study performed in Poland by Skowron and their colleagues, showed that *L. monocytogenes* strains isolated from fish processing plant belonged to the serogroups 1/2a-3a (38.6%) and 1/2b, 3b (32.8%) [16]. In China, Su et al. reported the distribution of serogroups 1/2c, 3c (39.1%), 1/2a, 3a (36.7%) and 1/2b, 3b, 7 (24.2%) in *L. monocytogenes* isolated from foods and humans samples [43]. However, in Spain, the serogroups distributed

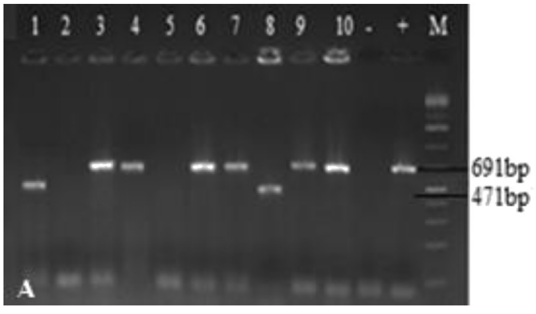

Figure 1. Serotypes identified in *L. monocytogenes*. A: *ORF2819* (471 bp), *lmo0737* (691bp), M: Size marker (1 kb), +: *L. monocytogenes* strain ATCC19112, -: Negative control, From one to ten: *L. monocytogenes* isolates tested.

Table 4. Source, antimicrobial resistance profiles, serotypes and virulence genes of *L. monocytogenes* isolated from foods.

Source	*L. monocytogenes* isolate code	Antimicrobial resistance profile	MAR index	Virulence genes	Serotypes
Traditional whey	W500	AMC,AMP, E, SMX, SXT, VA, AK, C, CN, K, S	0.73	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c
Smen	S220	AMC, AMP, E, SMX, SXT, CIP	0.40	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2b, 3b, 4b and 4d
Raw poultry meat	P2	AMC, E, SMX, TE	0.26	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c
Raw minced meat	M80	AMC, E, SMX, TE	0.26	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	I1/2a, 1/2c, 3a and 3c
Traditional whey	W77	AMC, SMX, SXT	0.20	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c
Raw sausage	Sg 90	AMC, SMX, SXT	0.20	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c
Raw milk	Mk 60	AMC, AMP, E	0.20	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2b, 3b, 4b and 4d
Raw milk	Mk 9	AMC, AMP, E	0.20	*actA, inlA, inlC,*	-
Raw fish	F300	E, SMX, SXT	0.20	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c
Traditional whey	W5	AMC, E, TE	0.20	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c
Raw bovine meat	B510	AMC, AMP	0.13	*actA, hlyA, plcB, prfA*	-
Raw sausage	Sg44	AMC, SMX	0.13	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c
Raw minced meat	M23	AMC, E,	0.13	*actA, hlyA, plcB, prfA*	-
Raw minced meat	M140	E, TE	0.13	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	-
Raw sausage	Sg310	-	0.00	*actA, hlyA, inlJ, inlA, inlC, plcB, prfA*	1/2a, 1/2c, 3a and 3c

AK: amikacin; AMC: amoxicillin/clavulanic acid; AMP: ampicillin; C: chloramphenicol; CIP: ciprofloxacin; CN: gentamycin; E: erythromycin; IPM: imipenem; K: kanamycin; MAR: multiple antimicrobial resistance; P: penicillin; S: streptomycin; SMX: sulphamethoxazole; SXT: sulphamethoxazole/trimethoprim; TE: tetracycline; VA: vancomycin; MAR: Multiple Antibiotic Resistance.

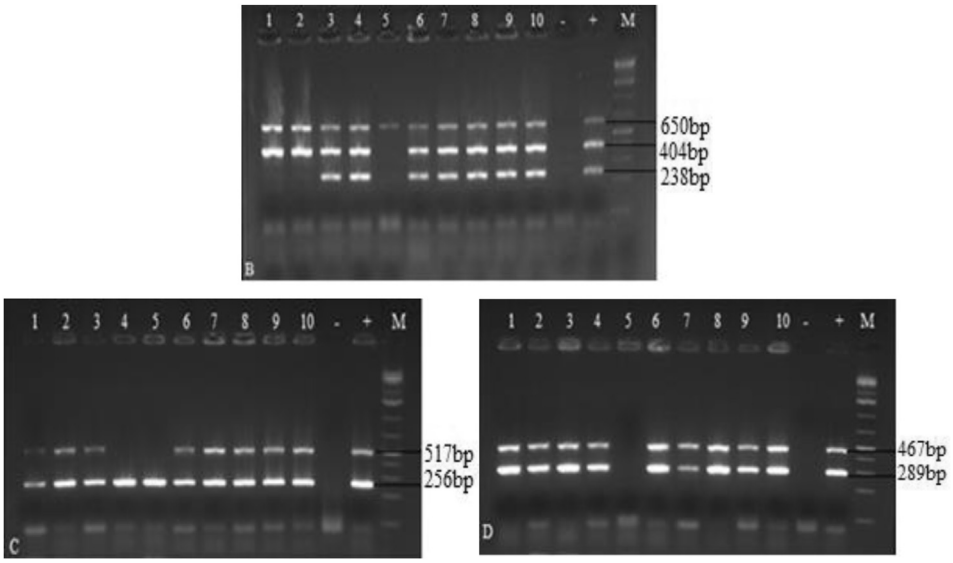

Figure 2. Virulence genes of *L. monocytogenes*. B: *actA* (650 bp), *hlyA* (404 bp), *inlJ* (238 bp), C: *inlA* (256 bp), *inlC* (517 bp), D: *plcB* (289 bp), *prfA* (467 bp), M: Size marker (1 kb), +: *L. monocytogenes* strain ATCC19112, -: Negative control, From one to ten: *L. monocytogenes* strains tested.

in *L. monocytogenes* isolated from the environment of dairy processing were 1/2a, 3a (72.73%) followed by 1/2b, 3b, 7 (11.36%), 4b, 4d, 4e (11.36%) and 1/2c, 3c (4.55%) [44]. Previous studies reported that the serotypes 1/2a, 1/2b, 1/2c, and 4b are involved for approximately 95% of all described cases of human listeriosis in the world [45,46,47]. The presence of these serotypes in food products, especially ready-to-eat foods, is a potential risk to public health and can cause severe cases of human listeriosis.

The presence of virulence genes in *L. monocytogenes* strains may have had a significative effect on their degree of pathogenicity. In fact, the results obtained in this study were similar to those decribed previously for *actA* gene in *L. monocytogenes* isolated from fish and fish processing plant [16], retail raw foods [48] and fresh seafoods [49,50]. Furthermore, *hlyA*, *inlA*, *inlC* and *inlJ* genes were reported in all *L. monocytogenes* strains isolated from ready-to-eat food in Malaysia and China [51,52]. A study performed in Italy showed the presence of *prfA*, *hlyA*, *actA*, *inlA*, *plcB* genes only in one strain of *L. monocytogenes* isolated from Ricotta Salata cheese [53]. Another study performed by Jamali and his group in open-air fish market environments, showed that 100% of isolated *L. monocytogenes* were positive for *hlyA*, *inlA* and *inlC*, and 97.7% were positive for *inlJ* and *prfA* genes [54]. However, *hlyA, prfA* and *inlA* genes were detected in 60.8% of *L. monocytogenes* strains isolated from raw milk in Egypt [9]. On the other hand, *inlA*, *inlC* and *inlJ* genes were detected in *L. monocytogenes* isolated from human, animals and vegetables [43,47,55,56]. Therefore, the isolation of *L. monocytogenes* showing a high rate of virulence genes is very harmful to public health, and the consumption of ready to eat food contaminated by theses strains is considered a major risk for humans and can cause severe cases of morbidity and fatality.

L. monocytogenes acquired natural resistance to many β-lactams and cephalosporins [20,57]. Moreover, penicillin, ampicillin, amoxicillin with or without gentamicin or trimethoprim-sulfamethoxazole were commonly used as the first solution for the therapeutic treatment of listeriosis, while ciprofloxacin, tetracycline and chloramphenicol were

Table 5. Antimicrobial resistance percentages of *L. monocytogenes* isolated from foods.

Antimicrobial agent	No. of *L. monocytogenes* isolates (n = 15)	
	S	R
Amoxicillin/clavulanic acid (30μg)	5 (33)	10 (67)
Erythromycin (15 μg)	6 (40)	9 (60)
Sulphamethoxazole (200 μg)	9 (60)	6 (40)
Ampicillin (10 μg)	10 (67)	5 (33)
Sulphamethoxazole/trimethoprim(25 μg)	10 (67)	5 (33)
Tetracycline (30 μg)	12 (80)	3 (20)
Chloramphenicol (30 μg)	14 (93)	1 (7)
Gentamicin (30 μg)	14 (93)	1 (7)
Ciprofloxacin (5 μg)	14 (93)	1 (7)
Amikacine (30 μg)	14 (93)	1 (7)
Streptomycin (10 μg)	14 (93)	1 (7)
Vancomycin (30 μg)	14 (93)	1 (7)
Kanamycin (30 μg)	14 (93)	1 (7)
Penicillin G (10 μg)	15 (100)	0 (0)
Imipenem (10 μg)	15 (100)	0 (0)

used as a second solution [4,8,24]. In the present study, two strains of *L. monocytogenes* isolated from traditional whey (W500) and smen (S220) showed a high resistance to many antibiotics, including ampicillin, trimethoprim-sulfamethoxazole, gentamicin and chloramphenicol for strain W500 and ampicillin, trimethoprim-sulfamethoxazol and ciprofloxacin for strain S220. Thus, resistance to tetracycline was observed in *L. monocytogenes* isolated from raw minced meat (M80 and M140), traditional whey (W5) and raw poultry meat (P2). Also, *L. monocytogenes* strains from traditional whey (W33), raw sausage (Sg310) and raw fish (F300) were found to be resistant to trimethoprim-sulfamethoxazole. The resistance to ampicillin is also detected in two strains from raw milk (Mk9 and Mk100) and one strain from raw bovine meat (B510).

Resistance of *L. monocytogenes* to these antibiotics may be a result of their overuses in livestock to promote the growth and for the treatment of bacterial infections. A study carried out by Maćkiw and his group in Poland showed that the isolated strains of *L. monocytogenes* were sensitive to chloramphenicol, gentamicin, ciprofloxacin, while 9.5% of them were resistant to ampicillin [8]. Another study performed in Iran, in seafoods showed a high resistance of penicillin (57%) and ampicillin (100%) [58]. Moreover, our study revealed that all the isolated strains were sensitive to penicillin, which is in agreement with that of Gómez et al. [57]. However, a study performed in Malaysia in chicken carcasses showed that the islolated strains were resistant to penicillin (17.2%), ampicillin (6.9%) and erythromycin (6.9%) [59]. In Egypte, Tahoun et al. showed that *L. monocytogenes* present a high resistance for tetracycline (81%) and ciprofloxacin (66.7%), and a susceptibility for ampicillin, erythromycin and trimethoprim-sulfamethoxazole [9].

The results of this study showed that MAR index varies between 0.00 and 0.73, with the highest value detected in strains W500 (0.73) and S220 (0.40) isolated from traditional whey and smen, respectively. It should be noted that traditional whey and smen were consumed without any treatment to eliminate the pathogenic bacteria. In other studies, MAR values of 0.38–0.63 and 0.5 were recorded in chicken, meat products and raw milk, respectively [9,60]. Morover, 66.7% of isolated *L. monocytogenes* strains were resistant to three or more than three class of antibiotics which is a serious risk for public health.

5. Conclusion

The present study provided the data about occurrence, antimicrobial resistance, serotype distribution and virulence genes of *L. monocytogenes* isolated from foods in Meknes city of Morocco. This study highlighted that the rate of presence of *L. monocytogenes* strains is 2.9% from 520 food samples. These isolates belonged to serogroups (1/2a, 1/2c, 3a and 3c) and (1/2b, 3b, 4b and 4d), and harbored several virulence genes (*inlA, inlC*, *inlJ*, *prfA, plcB*, *hlyA* and *actA*) in addition to their high resistance to antimicrobial agents. However, the presence of these strains in food products presents a major risk for consumers and public health. This study provides baseline information to Moroccan regulatory authorities to allow the application of guidance for controlling *L.monocytogenes* and to improve the microbiological safety of foods.

Declarations

Author contribution statement

Aziz Bouymajane, Fouzia Rhazi Filali, Aboulkacem Amal, Bouchra Ouhmidou, Mohieddine Moumni: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Said Oulghazi, Nada Lafkih, Abdelaziz Ed-Dra, Abdallah El Allaoui: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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