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Tarbiat Modares University

Association among Biofilm Formation, Serogroups, and Virulence Factors in *Listeria monocytogenes* Isolated from Food, Clinical, and Livestock Sources

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Authors
Sara Parvin, *MSc*[1]
Farzaneh Broumand Sorkhabi, *MD*[2]
Elnaz ohadi, *PhD*[3]
Lida Lotfollahi, *PhD*[1*]

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[1]Department of Microbiology, faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.
[2]Department of Obstetrics and Gynecology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran.
[3]Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

*** Correspondence***
Address: Department of Microbiology, faculty of Medicine, Urmia University of Medical Sciences, 11km of Serow Road, Urmia, Iran.
Email : lotfollahi.l@umsu.ac.ir

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ABSTRACT

Backgrounds: *Listeria monocytogenes* is an opportunistic pathogen causing listeriosis, its pathogenicity is due to the presence of virulence factors including InlA, InlB, PlcA, PlcB, ActA, Iap, and Hly. The purpose of this study was to evaluate the formation of biofilm and its association with serotypes and virulence factors in *L. monocytogenes* isolates.

Materials & Methods: In this study, 51 *L. monocytogenes* isolates were collected from blood, urine, feces, placenta, rectum, and vagina samples as well as livestock and food samples. Biofilm production was measured using microtiter plate assay, and virulence genes were identified by PCR method.

Findings: Out of 51 isolates, 27 (52.9%) were non-biofilm producers, 17 (33.3%) were weak biofilm producers, four (7.8%) were medium biofilm producers, and three (5.9%) were strong biofilm producers. According to this study results, different *L. monocytogenes* strains could form biofilm with various intensities. The *actA*, *flaA*, *inlJ*, *inlA*, and *plcB* genes were observed in all the isolates. The frequency of the *hlyA, plcA, iap, inlB*, and *inlC* genes among the isolates was 90.2, 94.1, 98, 88.2, and 82.4%, respectively. There was no significant correlation between the presence/absence of virulence genes in biofilm producing and non-biofilm forming isolates, except for the *inlC* and *iap* genes, which showed a significant correlation with the ability to form biofilm.

Conclusions: Due to the high prevalence rate of biofilm formation among the isolates and the importance of biofilm production in medical surfaces and food industries, eradication of biofilm-forming isolates is important.

Keywords: Biofilms, Serogroup, *Listeria monocytogenes*, Virulence factors

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Introduction

Listeria monocytogenes is a rod-shaped, catalase positive, and Gram positive bacterium which is considered as the major agent of listeriosis. This bacterium is isolated from environments such as soil, water, vegetables, domestic and wild animals, livestock , poultry, and seafood [1]. Listeriosis is a food-borne disease that could specifically affect patients with compromised immunity or AIDS, neonates, pregnant women, and the elderly [2, 3].

According to the Food and Drug Administration (FDA), listeriosis accounted for 30% of food-borne deaths between 1996 and 2005. The manifestation of this disease varies from a mild and non-invasive form to an aggressive/severe disease. The aggressive form could lead to sudden neonatal deaths, abortion, and preterm delivery. In newborns, listeriosis could cause septicemia, meningitis, and even death, whereas in individuals with immune deficiencies, it could cause meningitis, encephalitis, and meningeal septicemia [4]. Conversely, mild symptoms of food poisoning include fever, headache, and diarrhea, which are usually referred to as gastroenteritis [5].

It has been well documented that different proteins, including internalins (encoded by *int* genes), hemolysin (encoded by *hly* gene), phosphatidyl inositol (PI-PLC) (encoded by *plcA* gene), and ActA (coded by *actA* gene), play a key role in mediating the infection cycle of *L. monocytogenes* [6-8].

L. monocytogenes is able to grow in a wide pH range, tolerate salt, grow at low temperatures, and survive various stress conditions [4, 9]. Consumption of ready-to-eat (RTE) food, undergoing industrial processings and requiring storage at low temperatures, is often considered as a source of listerial infections [10]. Contamination of these foods is mostly attributed to the persistence of *L. monocytogenes* in food processing environments [11, 12]. Although this bacterium is susceptible to pasteurization, bacterial contamination usually occurs in packaging and final preparations (after pasteurization). This bacterium also has the ability to bind to surfaces and form biofilms, leading to higher resistance rates and continuous contamination of workplaces and food products [13].

The term "biofilm" was first used to describe the sticky form of microbial life, in which the adherence of microorganisms to viable and non-viable surfaces is evident and is contributed to the production of extracellular polymeric materials. Nowadays, it is known that many bacteria have the ability to form biofilms [14]. Biofilms are especially formed under unfavorable conditions by most microorganisms, including food-borne bacteria such as *L. monocytogenes*. The formation of biofilms on medical devices causes contamination and consequently transmission of hospital infections [15]. Persistance and resistant to cleaning, UV light, desiccation, and disinfectants in *L. monocytogenes* have been attributed to its ability to form biofilm [16, 17].

The association between biofilm formation ability and various serotypes of *L. monocytogenes* has been reported in several studies; however, the findings remain contradictory or inconclusive [18-20]. Although there are about 13 serotypes, only a few (1/2a, 1/2b and 4b) have been reported to be predominantly related to epidemic cases due to their greater pathogenicity and ability to survive in severe and critical environmental conditions [21].

The biofilm formation ability of *L.monocytogenes* varies depending on the growth temperature and growth surfaces [22]. *L. monocytogenes* could form biofilms on different surfaces, and its adherence to steel, glass, polypropylene, plastic, stainless steel surfaces reportedly occurs in a short period

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of 20 minutes [23]. Although many biofilms have been isolated from food equipment, there is no direct evidence indicating a link between the presence of biofilms and the disease outbreaks. The growth of this bacterium on different devices could increase the total contamination level, which highlights the need to use more appropriate cleaning methods [24].

Objectives: As the data overly vary, and no obvious associations have been established between serotypes/lineages and the ability to form biofilm, this study was designed to figure out the potential link between *L. monocytogenes* isolates with different genetics from different sources and the ability to form biofilm.

Materials and Methods

Sample collection and bacterial isolation: Sampling was performed from workplaces (using sterile cotton swabs under sterile conditions from meat and fish supply centers) and food sources (including cabbage, lettuce, eggplant, tomatoes, and dairy products) between 2012 to 2015. These specimens were transferred to a microbiological laboratory (Urmia, Iran) for primary isolation of *L. monocytogenes* strains. Isolated bacteria were identified using standard microbiological and biochemical methods, including oxidase, catalase, beta hemolysis, urease, motility, and Gram staining tests. The preparation of bacterial culture was performed according to a method previously described by Pan et al. (2006) [25] using tryptic soy broth (TSB) supplemented with 0.7% yeast extract (TSBYE; Difco Laboratories, Detroit, MI). A dilution (1:100) of bacterial cultures with overnight incubation was prepared in TSBYE and incubated again either at 37 °C for 12 hrs or at 30 °C for 18 hrs in order to prepare cells for biofilm formation as follows. Sterile saline (0.85% NaCl) was used to wash each culture using centrifugation (3,500 × g for 10 min at 10 °C). Cultures were then resuspended in saline or in a 1:10 dilution of TSBYE in sterile water. Each strain was adjusted to a concentration of 10^8 CFU/mL by calculating the optical density at 600 nm. Trypticase soy broth supplemented with 0.6% yeast extract and 5% glycerol was used to store stock cultures at −75 °C. The obtained cultures were placed on trypticase soy agar (TSA) (Difco, Detroit, Mich.) slants at 4 °C for 30 days. Before each experiment, a loopful of bacteria was cultured in 10 mL of TSBYE and incubated at 37 °C for 18 hrs.

Motility test: The freshly cultured colony was picked up with a sterile loop from the surface of the TSA medium, cultured in SIM (Sulfide, Indole, Motility) medium, and incubated at 37 and 25 °C for 24 hrs. *L. monocytogenes* is non-motile at 37 °C, grows at 25 °C in the medium, and has the appearance of an upside-down umbrella.

Microtiter plate biofilm production assay: To evaluate the biofilm formation ability of bacteria, the isolates were first cultured in yeast-containing TSA medium for 24 hrs, transferred to a TSB culture medium by a sterile swab, and incubated at 37 °C for 24 hrs. Next, a dilution of 1: 100 was prepared from 24-h bacterial culturs. Then 200 μL of each suspension was transferred into a U-shaped 96-well microplate, and eight replicates were used for each isolate. The microplates were incubated at 37 °C for 24 hrs while shaking at 150 rpm. After the removal of liquid, each well was rinsed three times with 250 μL of sterile water to remove any remaining unattached cells. The plates were then left to dry in an inverted position for 30 min. Next, 250 μL of 0.1% crystal violet (CV) solution was added to each well to stain biofilms, and parafilm was used to seal the plates. Subsequently, we carried out incubation at normal temperature (room) for 15-20 min. Unbound dye was removed

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Table 1) Primers used for detection of virulence genes of *L. monocytogenes*

Gene	Primer Sequence (5'→3')	Product Size(bp)	Annealing	Reference
iap	F: ACA AGCTGCACC TGTTGCAG R: TGACAGCGTGTGTAGTAGCA	131	55°C for 2min	[27]
hlyA	F: ATGAAA AAAATAATGCTAG R: TTA TTC GATTGGATT ATC T	1590	50°C for 30s	[28]
inlA	F: ACGAGTAACGGGACA AATGC R: CCCGAC AGTGGTGCTAGATT	800	55°C for 20s	[29]
inlB	F: TGGGAGAGTAAC CCAACC AC R: GTTGACCTTCGATGGTTGCT	884	55°C for 20s	[29]
inlC	F: AATTCCCACAGGACACAACC R: CGGGAATGCAATTTTTCACAT	517	55°C for 20s	[29]
inlJ	F: TGTAAC CCC GCTTACACAGTT R: AGCGGCTTGGCAGTCTAATA	238	55°C for 20s	[29]
actA	F: TGAAGA GGT AAATGCTTCGGACTT R: CGCTTATTTTCGGTA CCTTTG GA	Type I:623 Type II:518	45°C for 10s	[30]
plcB	F: GGG AAA TTTGACACAGCGTT R: ATTTTCGGGTAGTCCGCTTT	261	60°C for 2min	[31]
plcA	F: TTAGTTGAATTTATTGTTTTTTATG R: TTGTATAAGAATTATTTGC	954	45°C for 30s	[28]
flaA	F: AGCTCTTAGCTCCATGAGTT R:ACATTGTAGCTAAGGCGACT	450	94°C for 30s	[27]

by washing three times with 250 μL of sterile water. The microplate was incubated for 24 hrs to completely dry, and then 200 μL of ethanol was poured into the 96-well plate at room temperature for 15-20 min. At last, the contents of the wells were transferred to a sterile polystyrene microtiter plate, and OD595 of each well was measured by a microplate reader. Final OD was measured after the subtraction of the OD of the control wells from the average OD of seven test wells. To control the quality of biofilms, the standard *L. monocytogenes* ATCC7644 strain was used. The results were verified using a previously reported formula [26] as follows: OD<ODc = poor biofilm producing isolate; ODc< OD< 2×ODc= weak biofilm producing isolate; 2×ODc< OD< 4×ODc= moderate biofilm producing isolate; and OD> 4×ODc= strong biofilm producing isolate. In order to fulfill statistical analysis, we used ANOVA test (one-way).

Polymerase Chain Reaction for the detection of virulence genes: Bacterial genomic DNA was extracted by a DNA extraction kit (Yekta tajhiz Azma, Tehran, Iran) according to the manufacturer's protocols. The quality and quantity of DNA were figured out using a Nano-drop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA), and the integrity of the extracted DNA was assessed via electrophoresis on 1% agarose gel. In order to identify virulence genes, PCR reaction was performed using the specific primers shown in Table 1. The PCR amplification of the target genes was performed in a 0.2 mL micro-tube containing 12.5 μL of Amplicon Mastermix, 4 μl of genomic DNA (50 ng/μL), 4.5 μL d.d H_2O, and 2 μL of each primer (10 pmol/μL) according to the following thermocycling program: a primary denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 50 s, annealing (mentioned in Table 1), extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. After the

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Table 2) Results of biofilm formation by microtiter plate method

Isolates		Mean	Standard Deviation	Isolates		Mean	Standard Deviation
Strong	1	1.201125	0.288209	Nonformer	1	0.044125	0.022869
	2	1.703625	0.642171		2	0.043875	0.009672
	3	0.109125	0.046348		3	0.058875	0.01556
Moderate	1	0.159286	0.172273		4	0.025875	0.023247
	2	0.14475	0.072594		5	0.0425	0.01526
	3	0.14025	0.063796		6	0.055625	0.028264
	4	0.082375	0.060912		7	0.051	0.015703
Weak	1	0.073	0.032133		8	0.038375	0.018647
	2	0.056875	0.016991		9	0.044125	0.009433
	3	0.049	0.018205		10	0.03725	0.010152
	4	0.07	0.059639		11	0.039	0.011058
	5	0.079125	0.107679		12	0.124125	0.034361
	6	0.079375	0.046325		13	0.063125	0.01109
	7	0.056625	0.032133		14	0.08825	0.031079
	8	0.14475	0.072594		15	0.09825	0.030747
	9	0.08675	0.035748		16	0.076	0.047896
	10	0.229	0.222592		17	0.07175	0.040749
	11	0.0375	0.021374		18	0.0285	0.026333
	12	0.028	0.004276		19	0.041143	0.029384
	13	0.0345	0.007483		20	0.012333	0.006154
	14	0.034125	0.011993		21	0.02825	0.026164
	15	0.020625	0.024065		22	0.114625	0.146984
					23	0.040625	0.011211
	16	0.116625	0.020819		24	0.039875	0.015394
					25	0.040375	0.0351
	17	0.0485	0.0199		26	0.027625	0.012972
					27	0.3285	0.278745

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experiment, PCR products were separately electrophoresed to assess the presence or absence of the target genes.

Statistical analysis: Using Sigma Stat statistical software (SPSS, Inc.), we employed Spearman rank order correlation, paired comparative tests, and Tukey's method. The results of these tests indicated that with a *p* value of <.050, there were meaningful differences among the strains.

Results

Bacterial isolation: A total of 51 *L. monocytogenes* were isolated from 1214 clinical (blood, urine, feces, placenta, rectum, and the vagina), food, and livestock samples.

Evaluation of biofilm production: In order to identify the ability of the isolates to form biofilm, a total of 51 *L. monocytogenes* isolates were examined using the microtiter plate assay. Of 51 isolates, 27 (52.9%) were non-biofilm producers, 17 (33.3%) were weak biofilm producers, four (7.8%) were moderate biofilm formers, and three (5.9%) were strong biofilm producers (Figure 1) (Table 2).

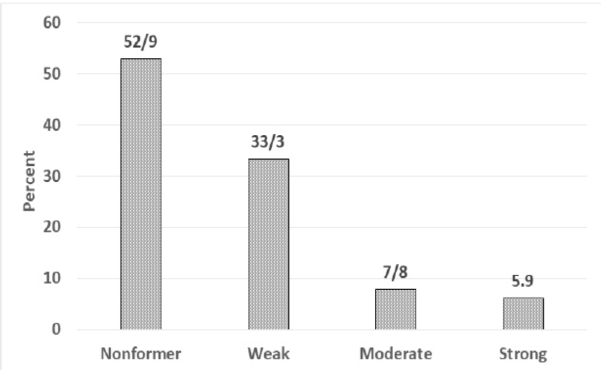

Figure 1) Capability of biofilm production in *L.monocytogenes* strains isolated from clinical, food, and livestock samples

Frequency of virulence genes based on PCR results: The *actA*, *flaA*, *inlJ*, *inlA*, and *plcB* genes were observed in all the isolates as shown in Table 3. The isolates belonging to the serotypes 1/2c and 4b were not positive for *flaA* gene, and only one livestock-isolated strain belonging to the serotype 1/2a was reported to be positive for *flaA*. In examining the presence of *actA* gene using a specific primer (*actA* typing), 47 (97.9%) out of 51 isolates were classified as type II, and four (7.8%) were classified as type I. The frequency of the *hlyA* and *plcA* genes among the isolates was 90.2 and 94.1%, respectively. According to the results, most isolates lacking these two genes were from clinical sources, and only one sheep-isolated strain with serotype 1/2a was reported to be negative for the *hlyA*, *iap*, and *plcA* genes. Noticeably, most isolates negative for internalin genes were from clinical sources.

Table 3) Prevalence of virulence genes in *L. monocytogenes* isolates

Virulence Gene	Frequency	Percent (%)
hly	46	90.2
iap	50	98
plcA	48	94.1
plcB	51	100
inlA	51	100
inlB	45	88.2
inlC	43	82.4
inlJ	51	100
flaA	51	100
actA	51	100

The relationship between the presence of virulence genes and biofilm production: Out of 24 biofilm producing isolates, 21 (87.5%), 23 (95.8%), 24 (100%), 24 (100%), 24 (100%), 21 (87.5%), 24 (100%), and 24 (100%) isolates were positive for *hly*, *iap*, *inlA*, *inlB*, *inlJ*, *inlC*, *flaA*, and *actA* genes, respectively. Whereas out of 27 non-biofilm forming isolates, 25 (89.2%), 27 (100%),

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85.7%, 27 (100%), 27 (100%), 25 (89.2%), 27 (100%), and 27 (100%) isolates harbored the *hly*, *iap*, *inlB*, *inlA*, *inlJ*, *inlC*, *flaA*, and *actA* genes, respectively. In the present study, (35.9%) were poor biofilm producers, 18 (48.7%) were non-biofilm formers, three (7.7%) were medium biofilm producers, and three (7.7%) were strong biofilm producers.

Table 4) Relationship of biofilm production and the presence of virulence genes in *L. monocytogenes* isolates

Virulence Genes	Non-Biofilm Producers (27)		Biofilm Producer (24)		P-Value
	Frequency	Percent	Frequency	Percent	
hly	25	(89.2)	21	(87.5)	.4
iap	27	(100)	23	(95.8)	.007
plcA	26	(92.9)	22	(91.7)	.4
plcB	27	(100)	24	(100)	-
inlA	27	(100)	24	(100)	-
inlB	24	(85.7)	24	(100)	.1
inlC	25	(89.3)	21	(87.5)	.02
inlJ	27	(100)	24	(100)	-
flaA	27	(100)	24	(100)	-
actA	27	(100)	24	(100)	-

there was no significant correlation between the presence/absence of virulence genes in two groups of biofilm producing and non-biofilm forming isolates, except for the *inlC* and *iap* genes, which showed a significant correlation with the ability to form biofilm (Table 4).

Frequency of different serotypes: Out of 51 isolates, 38 clinical isolates belonged to serotype 1/2c, eight isolates belonged to serotype 3c, one isolate from sheep belonged to serotype 1/2a, two animal isolates and one clinical (fecal) isolate belonged to serotype 4b, and one clinical (fecal) isolate belonged to serotype 4c.

Relationship between serological groups and biofilm production capability: Out of 38 isolates belonging to serotype 1/2c, 14 Of eight isolates belonging to serotypes 3c, five (62.5%) were non-biofilm producers, and three (37.5%) were weak biofilm producers. Of the three isolates belonging to serotype 4b and one isolate belonging to serotype 4c, none were able to produce biofilms, and one isolate belonging to serotype 1/2a was a moderate biofilm producer. In general, according to the results, the production of biofilm was more significant in clinical isolates. Regarding the relationship between serotypes and biofilm production, given that most of the isolates in the present study belonged to serotype 1/2c, it could not be conclusively stated that a specific serotype has a moderate or strong ability to form biofilm. However, regarding the inability to form biofilm, two livestockisolates and one

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clinical isolate belonging to serotype 4b were unable to produce biofilms. Based on the findings, no meaningful relationship was found between serotypes and the ability to form biofilm.

Discussion

As a foodborne pathogen, *L. monocytogenes* causes a very significant health concern due to the contamination of food during the production and packaging procedures in the food industry. In the present study, after sampling from food sources (fruits, corrosive vegetables, ready-to-eat foods, dairy products, meat, and meat preparation environments), only food samples were found to be contaminated. The frequency of *Listeria* was 8.7% among food samples. The rate of listerial contamination in various studies has been reported to vary from 9 to 20% depending on the sample type and sampling conditions [32-34]. Fewer incidences have been reported in Iran in comparison to other countries, especially developed countries, probably due to the lack of specific culture medium and instruments for sample preparation and bacterial isolation. *L. ivanovii* is the most common *Listeria* species found in meat and related samples. *L. innocua* has frequently been reported in dairy products and ready-made foods in most parts of the world. Variable reports on the isolation of different *Listeria* species highlight the need for paying attention to species with less clinical significance.

Several studies have eveluated the biofilm formation ability of *L. monocytogenes* with different serotypes; however, no consistent trends have emerged yet. This could be owing to differences in environmental conditions during biofilm formation, strains, and media [35]. In the current study, biofilm formation ability of *L. monocytogenes* isolates was such that 27 (52.9%) isolates were non-biofilm producers, 17 (33.3%) isolates were weak biofilm producers, four (7.8%) isolates were moderate biofilm formers, and three (5.9%) isolates were strong biofilm producers. The data obtained by microtiter plate assay revealed that the majority of the isolates were weak or moderate biofilm producers, which is consistent with the results of previous studies indicating that listerial isolates were generally weak to moderate biofilm producers [21, 36-38].

Serotyping often plays an essential role in determining species and subspecies. Since serotypes are considered as one of the major strain-differentiating factors and helpful assets in epidemiological studies, it has been massively documented that serotypes are correlated with different traits of *L. monocytogenes*. With regard to biofilm formation in *Listeria*, previous available data linking phylogenetic division, serotype, and biofilm formation have remained unclear [18, 20, 21]. Although some studies have shown a correlation between particular serotypes of *L. monocytogenes* and the ability to form biofilm, some have failed to find such correlation [21]. The findings of the present work showed no association between *L. monocytogenes* serotypes and their biofilm-production capacities. These observations revealed that there might be no relation between specific serotypes and the ability to form biofilm, while the observed correlations could be due to random strong biofilm-producing isolates in independent studies. The biofilm formation of *L. monocytogenes* was apparently dependent on the studied strains. In the present study, of 51 isolates, two belonged to serotype 4b, which were either weak or non-biofilm producers. In line with this finding, Doijad et al. (2015) reported that none of the isolates belonging to serotype 4b had the ability to produce strong biofilms, 69.57% produced a weak biofilm, and 30.43% produced moderate biofilms [38]. In the study of Folosm et al.

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(2006), isolates belonging to serotype 1/2a showed greater ability to form biofilm compared to those belonging to serotype 4b, which is probably due to their greater isolation rate from the food industry as well as their greater ability to adhere to food surfaces [39].

In the present study, an isolate belonging to serogroup 1/2a was moderate in terms of its ability to produce biofilm. Doijad and colleagues (2015) reported that 18.75% of the isolates belonging to serotype 1/2a were moderate biofilm-formers, and that most serotypes were weak biofilm-producers [38]. Consistent with our findings, Kadam et al. (2013) showed that serotypes 1/2a and 1/2b were stronger biofilm-formers than serotype 4b [18]. As serotype 1/2a and 4b strains are often isolated from food-processing environments and foodborne listeriosis outbreaks (respectively), assessing the biofilm formation ability of strains belong to serotypes 1/2a and 4b could be very interesting [40-44]. Consistent with our findings, serotype 1/2a has been reported in other studies to be as the most common serotype observed in *L. monocytogenes* isolated from various sources [20, 45].

Confirmation of the pathogenicity could be explained by various methods/ mechanisms including: in vivo inoculation of pathogenic bacteria in mouse, in vitro testings, use of cell lines and finally, PCR in terms of the examination of the presence/ absence of virulence genes [29]. Among the aforementioned methods, given its high availability and ease of use, PCR could be the first step in evaluating bacterial virulence. In order to increase the accuracy and efficiency of this method, scientists have always sought to find genes that are only found in pathogenic strains. In the follow up of these efforts, Liu et al. (2007) suggested the presence of *inlJ* gene as a criterion for differentiating virulent strains from non-virulent ones; however, pathogenic IIIB strains lacking the *inlJ* gene have been identified, in which three *inlA*, *inlJ*, and *inlC* genes are targeted using multiplex PCR for the molecular determination of virulent strains [29]. In the current study, *inlJ* and *inlA* were observed in 100% of the isolated strains, and *inlB* and *inlC* were present in 88.2 and 82.4% of the strains, respectively, which are in line with previous reports [32, 46, 47]. Based on our findings, the frequency of *hlyA*, *plcA*, *plcB*, and *iap* genes was 90.2, 94.1, 100, and 98%, respectively, which are similar and consistent with the results of previous studies [27, 34, 48]. The isolates lacking the *hlyA* gene in the present samples all belonged to serotype 1/2c, and only one animal isolate belonged to serotype 1/2a. The isolates lacking the *plcA* and *inlB* genes were all isolated from clinical samples and belonged to serotype 1/2c; however, the strains lacking *inlC* were isolated from clinical sources and belonged to serotype 1/2c, except for one strain isolated from food sources. It could be concluded that there is a significant relationship between the source of isolates and the prevalence of virulence genes. In terms of the association between virulence genes and the ability to form biofilm, a significant correlation was found only for the presence of *iap* and *inlc* genes. It should be noted that the livestock strains (isolated from sheep), which were negative for the presence of *hlyA*, *iap,* and *plcA* genes, belonged to serotypes 1/2a and were moderate biofilm formers. Out of three strong biofilm producers, one isolate harbored all virulence genes, and the other two harbored all genes, except for *plcA* and *inlB*. In the present study, only one animal isolate had the *flaA* gene, which belonged to serotype 1/2a. Taken together, according to the present study results, *L. monocytogenes* strains have the ability to form biofilm on important industrial surfaces. Although we observed

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a correlation between biofilm formation and serotype, this link was inconclusive and dependant on individual strains. Although most *L. monocytogenes* isolates formed moderate to weak biofilms, the food industry environment might carry multicellular biofilms and elevate *L. monocytogenes* prevalence. It is necessary to note that the formation of biofilm in the food chain could be highly problematic. Furthermore, our findings showed that lineage II isolates had a greater ability to form biofilm compared to the lineage I isolates. The present study revealed that the presence of *inlA, flaA, actA, plcB*, and *inlA* genes was 100%. The high frequency of virulence genes among the isolates is a warning that this bacterium is highly contagious and could be transmitted through food and the environment.

Conclusion

In conclusion, the results indicated that i) there was no association between *L. monocytogenes* serotypes and their biofilm-production capacities, ii) serotype 1/2a was the most common serotype observed in *L. monocytogenes* isolated from various sources, iii) there was a significant correlation between the source of isolates and the prevalence of virulence genes, and iv) there was a significant correlation between the presence of *iap* and *inlc* genes and the ability to form biofilm.

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