

Supplemental: Prevalence, pathogenicity, virulence, antibiotic resistance and phylogenetic analysis of biofilm producing *Listeria monocytogenes* isolated from different ecological niches in Egypt: Food, humans, animals and environment

Table S1. Pathogenicity profiles of *Listeria monocytogenes* recovered from different samples

Source of samples	n=, of recovered isolates	Serotype	Pathogenicity profile																		
			CAM P (+/-) with S/R	PI-PL C	Anton's eye test	Mice lethality	Chick-embryo lethality	Vero cell ingestion assay	Biofilm		Virulence genes										
									CT	MPA (O.D.)	<i>prfA</i>	<i>hlyA</i>	<i>PlcA</i>	<i>pLcB</i>	<i>Iap</i>	<i>actA</i>	<i>flaA</i>	<i>InlA</i>	<i>InlB</i>	<i>InlC</i>	<i>InlJ</i>
Reference strain (ATCC 7494)	4	4	+/-	+	+	+	+	++	Strong	Strong (0.16)	+	+	+	+	+	+	+	+	+	+	+
She-camel milk	1	4	+/-	+	+	+	+	++	Strong	Very Strong (0.56)	+	+	+	+	+	+	+	+	+	+	+
Buffalo milk	1	1	+/-	+	+	+	+	++	Moderate	Strong (0.15)	+	+	+	+	+	+	+	+	+	+	+
Cow milk	2	1	+/-	+	+	+	+	++	Strong	Strong (0.13)	+	+	+	+	+	+	+	+	+	+	+
		4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	+	+	+	+	+
Goat milk	2	1	+/-	+	+	+	+	++	Moderate	Very Strong (0.21)	+	+	+	+	+	+	+	+	+	+	ND
		4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	+	+	+	+	+
Ewe milk	1	4	+/-	+	+	+	+	++	Strong	Strong (0.12)	+	+	+	+	+	+	+	+	+	+	ND
Silage	3	4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	+	+	+	+	+
		4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	ND	+	+	+	+
		4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	ND	+	+	+	+
Rabbits brain	1	4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	+	ND	ND	+	ND

CAMP: Christie, Atkins, Munch-Petersen test; S/R: *Staphylococcus aureus*/*Rhodococcusequi*; PI-PLC: phosphatidylinositol-specific phospholipase C; O.D.: Optical Density; O.D.₅₉₅<0.1= Weak; O.D.₅₉₅≤0.1= Strong; O.D.₅₉₅>1= Very Strong; CT, Christensen's tube; MPA, Microtiter plate assay; ND, not detected

Table S1 cont . Pathogenicity profiles of *Listeria monocytogenes* recovered from different samples

Source of samples	n= of recovered isolates	Serotype	Pathogenicity profile																			
			CAMP (+/-) with S/R	PI- PLC	Anton's eye test	Mice lethality	Chick-embryo lethality	Vero cell ingestion assay	Biofilm		Virulence genes											
									CT	MPA (O.D.)	<i>prfA</i>	<i>hlyA</i>	<i>PLcA</i>	<i>pLcB</i>	<i>lap</i>	<i>actA</i>	<i>flaA</i>	<i>InlA</i>	<i>InlB</i>	<i>InlC</i>	<i>InlJ</i>	
Kariesh cheese	1	4	+/-	+	+	+	+	++	Strong	Strong (0.16)	+	+	+	+	+	+	+	ND	+	+	+	
Hamburger	1	4	+/-	+	+	+	+	++	Strong	Very Strong (0.56)	+	+	+	+	+	+	+	ND	+	+	+	
Broilers	3	1	+/-	+	+	+	+	++	Strong	Strong (0.13)	+	+	+	+	+	+	+	ND	ND	ND	ND	+
		4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	ND	+	+	+	+	+	+	ND	ND	ND	ND
		4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	+	+	+	+	ND	+
Layers	3	1	+/-	+	+	+	+	++	Moderate	Very Strong (0.21)	+	+	+	+	+	+	+	+	+	+	ND	+
		4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	+	+	+	+	ND	ND
		4	+/-	+	+	+	+	++	Strong	Strong (0.17)	+	+	+	+	+	+	+	+	ND	ND	ND	ND
Table eggs		1	+/-	+	+	+	+	++	Moderate	Strong (0.15)	+	+	+	+	+	+	+	+	+	+	+	ND
Ducks	1	1	+/-	+	+	+	+	++	Moderate	Strong (0.15)	+	+	+	+	+	+	+	+	+	+	+	+
Aborted Goat fetal liver	1	1	+/-	+	+	+	+	++	Strong	Strong (0.17)	+	ND	+	+	+	+	+	ND	+	+	+	+
Septicemic ewe	1	4	+/-	+	+	+	+	++	Strong	Strong (0.17)	+	+	+	+	+	+	+	+	+	+	+	+
Septicemic women	1	4	+/-	+	+	+	+	++	Strong	Strong (0.17)	+	+	+	+	+	+	+	+	+	+	+	+
Frozen fish	1	4	+/-	+	+	+	+	++	Strong	Strong (0.14)	+	+	+	+	+	+	+	+	ND	ND	ND	+
		4	+/-	+	+	+	+	++	Strong	Strong (0.17)	+	+	+	+	+	+	+	+	+	+	ND	ND
Herring	1	1	+/-	+	+	+	+	++	Moderate	Strong (0.15)	+	+	+	+	+	+	+	+	+	+	+	ND

CAMP: Christie, Atkins, Munch-Petersen test; S/R: *Staphylococcus aureus/Rhodococcusequi*; PI-PLC: phosphatidylinositol-specific phospholipase C; DLABN: DL-alanine-b-naphthylamide HCl; DAPN: D-alanine-p-nitroanilide; O.D.: Optical Density; O.D.₅₉₅<0.1= Weak; O.D.₅₉₅≤0.1= Strong; O.D.₅₉₅>1= Very Strong; CT, Christensen's tube; MPA, Microtiter plate assay; ND, not detected

Table S2. Antibigram for *L. monocytogenes* isolates

n=	Source	Ampicillin (25 µg)	Amoxicillin/clavulanic (10 µg)	Amoxicillin (25 µg)	Penicillin G (10 IU)	Cloxacillin (5 µg)	Oxacillin (1 µg)	Ofloxacin (10 µg)	Enrofloxacin (10 µg)	Ciprofloxacin (5 µg)	Flumequine (30 µg)	Pefloxacin (30 µg)	Amikacin (30 µg)	Gentamicin (10 µg)	Kanamycin (30 µg)	Neomycin (10 µg)	Streptomycin (10 µg)	Chloramphenicol (30 µg)	Tetracycline (30 µg)	Sulpha-trimethoprine 1:19 (25 µg)	Cefotaxime (30 µg)	Cephalothin (30 µg)	Lincomycin (2 µg)	Clindamycin (2 µg)	Bacitracin (10 units)	Vancomycin (30 µg)	Erythromycin (15 µg)	Spiramycin (100 µg)	Rifamycin (5 µg)	MAR _{index}
1	Cow milk	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	S	S	R	R	R	R	R	S	S	S	S	0.3
2	Cow milk	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	S	S	R	R	R	R	R	S	S	S	S	0.3
3	Buffalo milk	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	S	S	R	R	R	R	R	S	S	S	S	0.3
4	She-camel milk	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	S	S	R	R	R	R	R	S	S	S	S	0.3
5	Ewe milk	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	S	S	R	R	R	R	R	S	S	S	S	0.3
6	Goat milk	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	S	R	R	R	R	R	S	S	S	S	0.3
7	Goat milk	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	S	R	R	R	R	R	S	S	S	S	0.3
8	Kariesh cheese	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	S	R	R	R	R	R	S	S	S	S	0.3
9	Hamburger	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	S	R	R	R	R	R	S	S	S	S	0.3
10	Broilers	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	S	R	R	R	R	R	S	S	S	S	0.3
11	Broilers	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
12	Broilers	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
13	Layers	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
14	Layers	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
15	Layers	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
16	Table eggs	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	R	S	R	R	R	R	R	R	S	S	S	S	0.4
17	Duck spleen	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	R	S	S	R	R	R	R	R	S	S	S	S	0.3
18	Silage	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	R	S	S	R	R	R	R	R	S	S	S	S	0.3
19	Silage	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	R	R	R	S	S	S	S	0.4
20	Silage	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	S	0.4
21	Goat fetal liver	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
22	Sheep blood (Septicemia)	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
23	Human blood (Septicemia)	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	R	R	R	R	R	R	R	R	S	R	S	S	0.4
24	Frozen fish	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	R	S	R	R	R	R	R	R	S	R	S	S	0.4
25	Frozen fish	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	R	R	R	R	R	R	R	R	S	S	S	S	0.4
26	Herring	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	S	R	R	R	R	R	S	S	S	S	0.3
27	Rabbit brain tissue	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	S	R	R	R	R	R	S	S	S	S	0.3

Table S3: PCR Primers and references for 16S rRNA of *Listeria* and virulence genes

Name	Amplicon Size (bp)	Primer Sequence	References
16S rRNA	938	F: 5'-CAG-CMG-CCG-CGG-TAA-TWC-3' R: 5'-CTC-CAT-AAA-GGT-GAC-CCT-3'	Vazquez-Boland et al. [1]
<i>prfA</i>	571	F: 5'-CCCCAAGTAGCAGGACATGCTAA-3' R: 5'-GTATCACAAAGCTCACGAG-3'	Cooray et al. [2]
<i>hlyA</i>	702	F: 5'-CCTAAGACGCCAATCGAA-3' R: 5'-AAGCGCTTGCAACTGCTC-3'	Mengaud et al. [3]
<i>inlA</i>	800	F: 5'-ATTATGCCAAGTGGTTACGGA-3' R: 5'-ATCTGTTTGCAGACCGTGTC-3'	Liu et al. [4]
<i>inlB</i>	500	F: 5'-CATGGGAGAGTAACCCAACC-3' R: 5'-GCGGTAACCCCTTTGTCATA-3'	Zhang and Knabel [5]
<i>InlC</i>	400	F: 5'-CCCACAATCAAATAAGTGACCTT-3' R: 5'-CTGGGTCTTTGACAGTATTTGTT-3'	
<i>InlJ</i>	238	F: 5'-TGTAACCCCGCTTACACAGTT-3' R: 5'-AGCGGCTTGGCAGTCTAATA-3'	Liu et al. [4]
<i>plcA</i>	1484	F: 5'-CTGCTTGAGCGTTCATGTCTCATCCCCC-3' R: 5'-ATGGGTTTCACTCTCCTTCTAC-3'	Notermans et al. [6]
<i>plcB</i>	795	F: 5'-GCAAGTGTCTAGTCTTTCCGG-3' R: 5'-ACCTGCCAAAGTTTGCTGTGA-3'	Cooray et al. [2]
<i>actA</i>	839	F: 5'-CGCCGCGGAAATTAAGAAAAAGA-3' R: 5'-ACGAAGGAACCGGGCTGCTAG-3'	Suarez et al. [7]
<i>Iap</i>	131	F: 5'-ACAAGCTGCACCTGTTGCAG-3' R: 5'-TGACAGCGTGTGTAGTAGCA-3'	Furrer et al. [8]
<i>flaA</i>	420	F: 5'-AGCTCTTAGCTCCATGAGTT-3' R: 5'-AGTAGCAGCACCTGTAGCAGT-3'	Gray and Kroll [9]

Data S1: Fasta formatted alignments of sequences obtained from MEGA7

>NH1

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>Goat_Milk_KP271938

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>Hamburger_KP271941

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>Broilers_KP271942

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>Broilers_KP271943

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