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Genetic Determinants for Cadmium and Arsenic Resistance among *Listeria monocytogenes* Serotype 4b Isolates from Sporadic Human Listeriosis Patients

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In *Listeria monocytogenes* serotype 4b isolates from sporadic listeriosis, heavy metal resistance was primarily encountered in certain clonal groups (ECI, ECII, and ECIa). All arsenic-resistant isolates harbored the arsenic resistance cassette previously identified in pLI100; ECIa harbored additional arsenic resistance genes and a novel cadmium resistance determinant in a conserved chromosomal locus.

Listeria monocytogenes is the etiological agent of listeriosis, which occurs rarely but exhibits a relatively high fatality rate (approximately 16%) (1, 2). Listeriosis outcomes include sepsis, meningitis, stillbirths, and abortions. At high risk are neonates, the elderly, pregnant women, and immunocompromised individuals (1). Strains of certain serotypes (1/2a, 1/2b, and 4b) are responsible for the majority (over 95%) of human clinical cases (3, 4). Serotype 4b strains have been implicated in numerous outbreaks and in a significant portion of sporadic cases (4, 5). Three serotype 4b clonal groups (ECI, ECII, and ECIa [also designated ECIV]) have been responsible for multiple outbreaks (6). Members of these clonal groups have also been frequently isolated from food processing environments and foods (7–13).

While conducting a longitudinal study of 136 serotype 4b isolates obtained from sporadic human listeriosis in the United States between 2003 and 2008 (to be presented separately), we identified 45 isolates with resistance to cadmium and/or arsenic. Resistance of *L. monocytogenes* to these heavy metals has long been recognized and has also even been utilized as a subtyping tool (14, 15), but limited information is available on the genetic determinants mediating such resistance in strains from human listeriosis. The few available reports have investigated the distribution of cadmium resistance determinants among isolates of environmental or food origin (11, 16); determinants associated with arsenic resistance have remained elusive.

The panel of 45 heavy metal-resistant clinical isolates investigated here consisted primarily of the three previously recognized clonal groups: ECI ($n = 26$), ECII ($n = 7$), and ECIa ($n = 8$) (Table 1). DNA hybridizations and PCR were employed as previously described (17) with the DNA probes and primers listed in Table 2.

We assessed the presence of three cadmium resistance determinants previously employed in the analysis of environmental and food isolates: *cadA1*, associated with the plasmid-borne transposon Tn*5422* (14, 18); *cadA2*, harbored by large plasmids such as pLM80 (19, 20); and *cadA3*, associated with an integrative conjugative element on the chromosome of *L. monocytogenes* EGDe (21). We also included a novel putative cadmium resistance determinant, *cadA4*, recently identified on the chromosome of the ECIa strain *L. monocytogenes* Scott A (22). A putative arsenic resistance cassette (*arsR1D2R2A2B1B2* in Fig. 1) was identified on pLI100, harbored by *L. innocua* CLIP 11262 (19, 21). An extended arsenic resistance cassette, which includes *arsR1D2R2A2B1B2* and two additional upstream genes (*arsD1A1*), was recently identified on the Scott A chromosome, where it is part of a 35-kb genomic island (with a GC content of 34%—lower than the *L. monocytogenes* average of 38%) (20, 22). This island harbors several additional genes, including the putative cadmium resistance determinant *cadA4* mentioned previously (Fig. 1) (22).

Association of cadmium resistance determinants with clonal groups and lower cadmium tolerance levels in strains harboring the novel determinant *cadA4*. The determinants *cadA1*, *cadA2*, and *cadA4* were frequently detected among the 45 cadmium-resistant clinical isolates, while only one strain (J4685) was found to harbor *cadA3* (Table 1). Multiple determinants were also only detected in one strain (J4503), which harbored both *cadA2* and *cadA4*. Several isolates ($n = 15$) lacked any of the four cadmium resistance determinants (Table 1), suggesting the presence of one or more as-yet-unidentified cadmium resistance determinants.

Associations were noted between resistance determinants and clonal groups: *cadA1* was only encountered in ECI, accounting for 8 (ca. 33%) of the cadmium-resistant ECI isolates, while none of the ECI isolates harbored *cadA2*; *cadA2* and *cadA4* were primarily encountered in ECII and ECIa, respectively ($P < 0.0001$ for both when analyzed with Fisher's exact test using SAS [SAS Institute, Inc., Cary, NC]) (Fig. 2). All 15 isolates negative for *cadA1* to *cadA4*, and presumably harboring as-yet-unidentified determinants, were ECI, constituting >50% of this clonal group (Fig. 2 and Table 1).

The absence of *cadA2* among the clinical ECI isolates was in contrast to findings for serotype 4b isolates from foods and food processing plants, where ECI isolates were found to be equally likely to harbor *cadA1* or *cadA2* (11). The reasons for this discrepancy are unknown but may reflect differential pathogenicity: ECI strains harboring *cadA1* or those with an unknown cadmium re-

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TABLE 1 *L. monocytogenes* strains used in this study

Name	Isolation year	State, Country	EC[a]	Cd[b]	Ars[b]	*cadA1*[c]	*cadA2*[c]	*cadA3*[c]	*cadA4*[c]	*arsA1*[c]	*arsA2*[c]	*LMOf2365_2257*[d]	MLGT[e]	MIC			Reference
														Cd[f]	Ars[f]	Arsenate[f]	
J2275	2003	PA, USA	ECI	■	□	□	□	□	□	□	□	■	1.13_4b_Sw87_EC1	>180	500	1,250	(26)
J2985	2004	IL, USA	ECI	■	□	□	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J3106	2004	NY, USA	ECI	■	□	□	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J3592	2005	ME, USA	ECI	■	□	□	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J4116	2006	ME, USA	ECI	■	□	□	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J4297	2006	PA, USA	ECI	■	□	□	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J2213	2003	AZ, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1				(26)
J2302	2003	CA, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1	140	2,500	30,000	(26)
J2353	2003	IL, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1				(26)
J3232	2004	OK, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1				
J3916	2006	NM, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1				
J4274	2006	NH, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1				
J5080	2008	NM, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1				
J5095	2008	MD, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1				
J5136	2008	SC, USA	ECI	■	■	□	□	□	□	□	■	■	1.13_4b_Sw87_EC1	140	1,250	30,000	
J4685	2007	MO, USA	ECI	■	□	□	□	■	□	□	□	■	1.13_4b_Sw87_EC1	>180	500	1,250	
J2269	2003	GA, USA	ECI	■	□	■	□	□	□	□	□	■	1.13_4b_Sw87_EC1				(26)
J2854	2004	AZ, USA	ECI	■	□	■	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J3082	2004	GA, USA	ECI	■	□	■	□	□	□	□	□	■	1.13_4b_Sw87_EC1	140	250	2,500	
J3133	2004	TX, USA	ECI	■	□	■	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J4001	2006	TX, USA	ECI	■	□	■	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J4977	2008	NC, USA	ECI	■	□	■	□	□	□	□	□	■	1.13_4b_Sw87_EC1				
J4979	2008	TX, USA	ECI	■	□	■	□	□	□	□	□	■	1.13_4b_Sw87_EC1	140	250	750	
J2584	2003	VT, USA	ECI	■	■	■	□	□	□	□	■	■	1.13_4b_Sw87_EC1				(26)
J4187	2006	WI, USA	ECI	▒	■	□	□	□	■	■	■	□	1.13_4b_Sw87_EC1	70	2,500	30,000	
J4600	2007	OK, USA	ECI	▒	■	□	□	□	■	■	■	□	1.13_4b_Sw87_EC1	70	2,500	30,000	
J2446	2003	OH, USA	ECII	■	□	□	■	□	□	□	□	■	1.9_4b_US98_EC2	>180	250	750	(26, 27)
J2685	2004	NY, USA	ECII	■	□	□	■	□	□	□	□	■	1.8_4b_US98_US02_EC2				(26)
J3033	2004	IL, USA	ECII	■	□	□	■	□	□	□	□	■	1.8_4b_US98_US02_EC2				(26)
J3170	2004	MI, USA	ECII	■	□	□	■	□	□	□	□	■	1.8_4b_US98_US02_EC2				
J3200	2004	CT, USA	ECII	■	□	□	■	□	□	□	□	■	1.8_4b_US98_US02_EC2				(26)
J3881	2006	CO, USA	ECII	■	□	□	■	□	□	□	□	■	1.8_4b_US98_US02_EC2				
J4485	2007	MA, USA	ECII	■	□	□	■	□	□	□	□	■	1.8_4b_US98_US02_EC2	>180	250	750	
J2967	2004	CA, USA	ECIa	■	□	□	■	□	□	□	□	■	1.2_4b_UK88_EC1a	>180	250	750	
J4503	2007	NYC, NY, USA	ECIa	■	■	□	■	□	■	■	■	□	1.2_4b_UK88_EC1a	>180	2,500	30,000	
J3290	2004	ME, USA	ECIa	▒	■	□	□	□	■	■	■	□	1.2_4b_UK88_EC1a	70	1,250	30,000	
J3419	2005	CA, USA	ECIa	▒	■	□	□	□	■	■	■	□	1.2_4b_UK88_EC1a	70	1,250	30,000	
J3768	2005	CO, USA	ECIa	▒	■	□	□	□	■	■	■	□	1.2_4b_UK88_EC1a	70	2,500	30,000	
J3921	2006	CT, USA	ECIa	▒	■	□	□	□	■	■	■	□	1.2_4b_UK88_EC1a	70	2,500	30,000	
J4948	2008	GA, USA	ECIa	▒	■	□	□	□	■	■	■	□	1.2_4b_UK88_EC1a	70	2,500	30,000	
J4954	2008	CT, USA	ECIa	▒	■	□	□	□	■	■	■	□	1.2_4b_UK88_EC1a	70	1,250	30,000	
J3422	2005	LA, USA		▒	■	□	□	□	■	■	■	■	1.16_4b	70	2,500	30,000	
J3618	2005	NJ, USA		▒	■	□	□	□	■	■	■	■	1.7_4b	70	2,500	30,000	
J4434	2007	TN, USA		▒	■	□	□	□	■	■	■	■	1.7_4b	70	2,500	30,000	
J2422	2003	RI, USA		▒	■	□	□	□	■	■	■	■	1.7_4b	70	2,500	30,000	(26)

[a] The EC designation was determined with the DNA-DNA hybridization results for previously described genetic markers (30) and confirmed by multilocus genotyping (MLGT) (28).

[b] For cadmium (Cd), black and gray boxes indicate MICs of ≥140 μg/ml (growth at 70 μg/ml) and 70 μg/ml (growth at 35 μg/ml but not at 70 μg/ml), respectively. Tolerance levels were determined based on growth in the presence of the indicated amount of cadmium chloride on Iso-Sensitest agar, as described previously (23). For arsenic (Ars), black and white boxes indicate resistance and susceptibility, respectively, based on the presence or absence of growth in the presence of sodium (meta)arsenite (500 μg/ml) on Iso-Sensitest agar, as described previously (23).

[c] Black and white squares represent the presence and absence, respectively, of signal in the hybridization experiment using the probe targeting the designated resistance gene or of the amplicon derived from the primers targeting the relevant DNA probe.

[d] Black and white squares signify the presence and absence, respectively, of the amplicon when PCR was conducted with primers P4F and P4R (Fig. 1 and Table 2). The extension time was set at 1 min.

[e] MLGT was conducted as described previously (28).

[f] MICs for cadmium (Cd), arsenic (Ars, arsenite), and arsenate (another chemical form of arsenic, which is reduced to arsenite and then extruded by arsenic resistance cassettes [25]) were determined as previously described (23), using Iso-Sensitest agar plates containing different heavy metal concentrations: 10, 35, 70, 140, and 180 μg/ml of cadmium chloride (Fischer Scientific, Pittsburg, PA); 50, 125, 250, 500, 750, 1,250, 2,500, and 5,000 μg/ml of sodium (meta)arsenite (Sigma, St. Louis, MO); and 50, 125, 250, 500, 750, 1,250, 2,500, 5,000, 10,000, 12,000, 15,000, and 30,000 μg/ml of sodium arsenate dibasic heptahydrate (Sigma).

sistance determinant(s) may constitute subsets of ECI more likely to be involved in human illness than those with *cadA2*. Further studies of ECI strains from different sources are needed to identify possible relationships between determinant types and source.

The presence of *cadA4* was found to be associated with a lower level of resistance to cadmium regardless of the clonal group. With the exception of strain J4503, which harbored both *cadA2* and *cadA4* and had a cadmium MIC of >180 μg/ml, the cadmium

TABLE 2 Probes and primers employed in this study

Probe	Primer (alternative name)	Sequence (5′ to 3′)	Target	GenBank accession no.	Reference
cadA1	cadA1-Tn*5422*F	CAGAGCATTTACTGACCATCAATCGTT	Tn*5422*-associated *cadA* (*cadA1*)	L28104	16
	cadA1-Tn*5422*R	TCTTCTTCATTTAACGTTCCAGCAAAAA	Tn*5422*-associated *cadA* (*cadA1*)	L28104	16
cadA2	cadA2-pLM80F	ACAAGTTAGATCAAAAGAGTCTTTTATT	*cadA* (*LMOh7858_pLM80_0083*) on pLM80 (*cadA2*)	AADR01000058	16
	cadA2-pLM80R	ATCTTCTTCATTTAGTGTTCCTGCAAAT	*cadA* (*LMOh7858_pLM80_0083*) on pLM80 (*cadA2*)	AADR01000058	16
cadA3	cadA3-EGDeF	TGGTAATTTCTTTAAGTCATCTCCCATT	*cadA* (*lmo1100*) in EGD-e (*cadA3*)	AL591977	16
	cadA3-EGDeR	GCGATGATTGATAATGTCGATTACAAAT	*cadA* (*lmo1100*) in EGD-e (*cadA3*)	AL591977	16
	LMOSA_2330F (P1F)	GCATACGTACGAACCAGAAG	*cadA* (*LMOSA_2330*) on the chromosome of Scott A (*cadA4*)	AFGI01000005.1	This study
	LMOSA_2330R (P1R)	CAGTGTTTCTGCTTTTGCTCC	*cadA* (*LMOSA_2330*) on the chromosome of Scott A (*cadA4*)	AFGI01000005.1	This study
	LMOSA_2220F (P2F)	CAACTTTGACCCTGTGGAG	*arsA* (*LMOSA_2220*) on the chromosome of Scott A (*arsA1*)	AFGI01000005.1	This study
	LMOSA_2220R (P2R)	CTTTCCATTCAATCACTGCG	*arsA* (*LMOSA_2220*) on the chromosome of Scott A (*arsA1*)	AFGI01000005.1	This study
	pLI37_F (P3F)	CAACCAGATCAGTTACCATTAAC	*arsA* on pLI100 (*pli0037*) and the chromosome of Scott A (*LMOSA_2260*) (*arsA2*)	NC_003383	This study
	pLI37_R (P3R)	TGCTTCTCCAGAGATTTCTTCTG	*arsA* on pLI100 (*pli0037*) and the chromosome of Scott A (*LMOSA_2260*) (*arsA2*)	NC_003383	This study
	F2365_2257F (P4F)	ACATTGCGAGAACACCTTGG	*LMOf2365_2257*	NC_002973.6	This study
	F2365_2257R (P4R)	GATTTATCGGCGCAATGACG	*LMOf2365_2257*	NC_002973.6	This study

MIC for all other *cadA4*-harboring isolates was 70 μg/ml regardless of their clonal group (Table 1). Even though these isolates grew poorly or not at all at the previous resistance threshold of 70 μg/ml (11, 15, 23), they grew well at 35 μg/ml. In contrast, other cadmium-susceptible isolates of *L. monocytogenes* typically have a cadmium MIC of 10 μg/ml (24). All other cadmium-resistant isolates had MICs of ≥140 μg/ml, regardless of their clonal group or determinant type (Table 1). The reasons for the association of *cadA4* with relatively reduced cadmium resistance remain to be determined; however, we suspect that this finding could be related to the fact that the protein encoded by *cadA4* is more divergent from those encoded by *cadA1* to *cadA3*, although highly conserved regions were still observed (data not shown). To illustrate, in the pairwise comparisons of CadA1 to CadA3, identities ranged from 68 to 74%, whereas the identities between CadA4 and other CadA proteins were 35 to 36%.

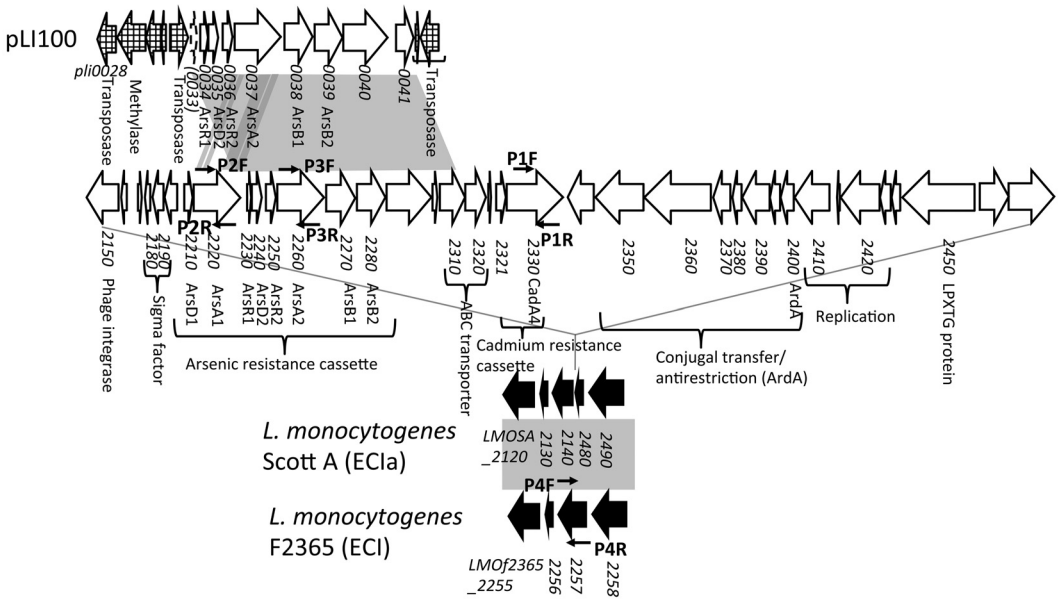

FIG 1 Genomic organization of the ca. 35-kb island harboring arsenic and cadmium resistance cassettes in ECIa strain Scott A. The DNA sequence of Scott A genome contig 5 (accession no. AFGI01000005.1) was retrieved from the NCBI database, annotated with the xBASE bacterial genome annotation service (http://www.xbase.ac.uk/annotation/) using F2365 as the reference genome (29–34), and analyzed with DNA and protein BLAST (35) and NCBI's Conserved Domain Search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (36). This annotation was later updated based on that available in NCBI (accession no. CM001159.1). The Scott A genomic region was compared with pLI100 and F2365 using the online Artemis Comparison Tool (WebACT; http://www.webact.org/WebACT/home) (37). Genes and pseudogenes are shown as arrows and stippled arrows, respectively. The conserved flanking genes in *L. monocytogenes* are shown in black, and other genes in the 35-kb insertion are in white. Flanking open reading frames (ORFs) on pLI100 are in a grid pattern. Homologous regions are represented in gray. Arrows above and below selected ORFs indicate the locations and orientations of primers (Table 2).

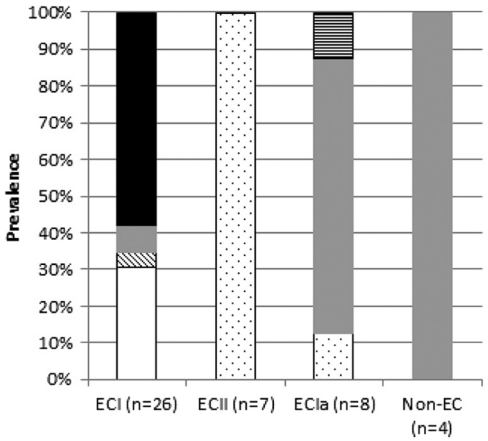

FIG 2 Prevalence of the different cadmium determinants within epidemic clones. Different cadmium resistance determinants are indicated as follows: white, *cadA1*; dots, *cadA2*; diagonal lines, *cadA3*; gray, *cadA4*; horizontal lines, copresence of *cadA2* and *cadA4*; and black, unidentified determinants.

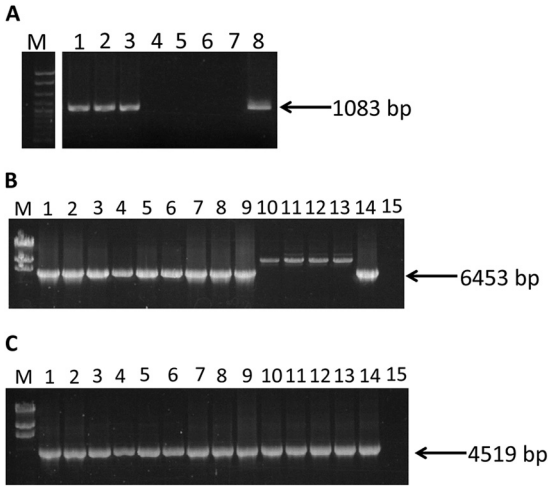

FIG 3 Locations of the arsenic resistance cassette in arsenic-resistant isolates. (A) PCR with primers P4F and P4R. Lanes: 1 to 3, J5080, J5095, and J5136, respectively, harboring only *arsA2*; 4 to 7, ECIa isolates J3290, J3419, J3768, and J3921, respectively; 8, F2365 (positive control); and M, exACTGene cloning DNA ladder (Fisher Scientific). (B) PCR with primers P4F and P2R. Lanes: 1 and 2, J4187 and J4600, respectively (ECI isolates positive for both *arsA1* and *arsA2*); 3 to 9, ECIa isolates J3290, J3419, J3768, J3921, J4503, J4948, and J4954, respectively; 10 to 13, non-EC isolates J3422, J2422, J3618, and J4434, respectively; 14, Scott A (positive control); 15, negative control; and M, DNA molecular mass marker II (Roche Diagnostics, Indianapolis, IN). The larger bands in lanes 10 to 13 represent unspecific PCR products. (C) PCR with primers P2F and P3R. Lanes are as in panel B. For negative controls, sterile water was used as the template. The size of the intended amplicon is indicated by an arrow.

All arsenic-resistant strains harbor pLI100-associated *arsA2*, while a subset additionally harbor *arsA1* in a conserved chromosomal locus. As previously mentioned, a putative arsenic resistance cassette along with *cadA4* and several other genes was part of a 35-kb island in the ECIa strain Scott A (22) (Fig. 1). In Scott A, this genomic island appears to be inserted in a gene (homolog of *LMOf2365_2257* in F2365) that is uninterrupted in F2365 and other strains with sequenced genomes (Fig. 1).

Two *arsA* genes, *arsA1* and *arsA2*, were selected as genetic markers representing arsenic resistance genes either unique to Scott A (*arsA1*) or shared between Scott A and pLI100 (*arsA2*) (Fig. 1 and Table 2). PCR employing primers derived from *arsA1* and *arsA2* revealed that all 23 arsenic-resistant isolates were positive for *arsA2*. However, a subset (13/23) of the *arsA2*-positive isolates also harbored *arsA1*. None of the arsenic-susceptible isolates was positive for either *arsA1* or *arsA2* (Table 1). All arsenic-resistant ECIa isolates harbored both *arsA1* and *arsA2*, as did two of the 12 ECI arsenic-resistant isolates and all four non-ECI, -ECII, or -ECIa isolates. All isolates harboring both *arsA1* and *arsA2* also harbored *cadA4* (Table 1). None of the isolates harbored *arsA1* in the absence of *arsA2* (Table 1), suggesting that the additional arsenic resistance genes found in Scott A were acquired by a genetic element that already harbored the arsenic resistance cassette previously detected in pLI100.

As mentioned earlier, the *LMOf2365_2257* homolog in the genome of Scott A appears to have been interrupted by the insertion of the 35-kb genomic island harboring the arsenic resistance cassette (including both *arsA1* and *arsA2*) and *cadA4* (Fig. 1). PCR with primers P4F and P4R annealing to the flanking region (Fig. 1 and Table 2) revealed that this gene (i.e., the *LMOf2365_2257* homolog) was intact in all arsenic-susceptible isolates as well as in those resistant isolates that only harbored *arsA2* (Table 1 and Fig. 3A). Of the 13 resistant isolates that harbored both *arsA1* and *arsA2*, the expected PCR product (1,083 bp) was not obtained from the two ECI isolates or the seven ECIa isolates, suggesting that the gene was interrupted by a large insertion; however, the expected PCR product was obtained from the four remaining isolates (all four outside ECI, ECII, or ECIa), suggesting that they possessed the arsenic resistance determinants in a locus different from the *LMOf2365_2257* homolog (Table 1 and Fig. 3A). The locations of the resistance determinants in these isolates or in those only harboring *arsA2* remain to be identified.

The locations of arsenic resistance cassettes were further examined by PCR using primers P4F and P2R, annealing to *LMOf2365_2257* and *arsA1*, respectively (Fig. 1 and Table 2). The expected PCR product (6,453 bp) was obtained with ECI and ECIa isolates positive for *arsA1* and *arsA2*, confirming that, like Scott A, these isolates harbored the arsenic resistance cassette in the *LMOf2365_2257* homolog (Table 1 and Fig. 3B). In contrast, the four non-EC isolates positive for both *arsA* genes failed to yield the expected amplicon, in agreement with the presence of an uninterrupted *LMOf2365_2257* homolog and suggesting the presence of the resistance genes in other, currently unidentified locations (Table 1 and Fig. 3B). PCR of isolates harboring both *arsA1* and *arsA2* using primers P2F and P3R, annealing to *arsA1* and *arsA2*, respectively (Fig. 1 and Table 2), revealed that, regardless of genomic location, the two arsenic resistance genes were close to each other, with the PCR product having the size expected (4,519 bp) based on the gene arrangement in Scott A (Fig. 3C).

To examine whether the presence of different arsenic resistance genes was associated with different levels of tolerance to arsenic, MICs of selected isolates were determined for arsenite (also initially employed to determine resistance to arsenic) and arsenate (another chemical form of arsenic which is reduced to arsenite and pumped out by ArsB transporters in the arsenic resistance cassettes) (25) (Table 1). As expected, all tested arsenic-resistant isolates showed a higher arsenite MIC (1,250 to 2,500 μg/ml) compared to the susceptible isolates, whose MICs ranged from 250 to 500 μg/ml (Table 1). A similar trend was observed for

arsenate (30,000 μg/ml for arsenic-resistant isolates versus 750 to 2,500 μg/ml for those susceptible to arsenic) (Table 1). Arsenite or arsenate MICs were similar, regardless of whether an isolate harbored *arsA2* only or both *arsA1* and *arsA2* (Table 1).

In conclusion, we have described unexpected associations between heavy metal resistance determinants and clonal groups of serotype 4b *L. monocytogenes* from sporadic human listeriosis in the United States. Further studies are warranted regarding novel cadmium resistance determinants, most likely to be found among cadmium-resistant ECI isolates. The identification of a chromosomal island harboring the arsenic resistance cassette in several arsenic-resistant isolates, including all those of ECIa, agrees with earlier conclusions (based on plasmid curing outcomes) that arsenic resistance in *L. monocytogenes* was not associated with plasmids (15).

Our findings reflect a complex and diverse repertoire of heavy metal resistance genes within serotype 4b *L. monocytogenes* from human listeriosis that are likely to be acquired horizontally from various gene pools. The arsenic and cadmium resistance determinants examined here were predominantly found among isolates of clonal groups that have been repeatedly implicated in outbreaks. We can hypothesize that the association of epidemic clones with human illness may reflect the acquisition by these strains of a complex combination of accessory genes that facilitate their proliferation in different environments and may enhance their virulence potential. The complex repertoire of heavy metal resistance genes (and the complicated evolutionary history suggested by their distribution) is consistent with this hypothesis. Further studies on cadmium and arsenic resistance genes will enhance our understanding of the evolution and function of these accessory genes and are needed to elucidate the possible contributions of such genes to the frequent involvement of epidemic clones in food contamination and human food-borne disease.

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