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Heavy Metal and Disinfectant Resistance of *Listeria monocytogenes* from Foods and Food Processing Plants

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The persistence of *Listeria monocytogenes* in food processing plants and other ecosystems reflects its ability to adapt to numerous stresses. In this study, we investigated 138 isolates from foods and food processing plants for resistance to the quaternary ammonium disinfectant benzalkonium chloride (BC) and to heavy metals (cadmium and arsenic). We also determined the prevalence of distinct cadmium resistance determinants (*cadA1*, *cadA2*, and *cadA3*) among cadmium-resistant isolates. Most BC-resistant isolates were resistant to cadmium as well. Arsenic resistance was encountered primarily in serotype 4b and was an attribute of most isolates of the serotype 4b epidemic clonal group ECIa. Prevalence of the known cadmium resistance determinants was serotype associated: *cadA1* was more common in isolates of serotypes 1/2a and 1/2b than 4b, while *cadA2* was more common in those of serotype 4b. A subset (15/77 [19%]) of the cadmium-resistant isolates lacked the known cadmium resistance determinants. Most of these isolates were of serotype 4b and were also resistant to arsenic, suggesting novel determinants that may confer resistance to both cadmium and arsenic in these serotype 4b strains. The findings may reflect previously unrecognized components of the ecological history of different serotypes and clonal groups of *L. monocytogenes*, including exposures to heavy metals and disinfectants.

Listeriosis is a rare but severe food-borne disease caused by *Listeria monocytogenes*, a Gram-positive facultative intracellular bacterium ubiquitous in the environment. The illness has a fatality rate of 16% and affects primarily the elderly, pregnant women, and their fetuses as well as individuals with compromised immune systems (27, 28). Processed, ready-to-eat foods such as deli meats, soft cheeses, and produce have been implicated in major outbreaks of listeriosis. The ability of *L. monocytogenes* to colonize the food processing plant environment is considered to be a major contributor to contamination of such foods (2, 8, 17).

Environmental persistence of *L. monocytogenes* is complex and mediated by a number of mechanisms, including the ability to grow in the cold, biofilm formation, resistance to disinfectants and to *Listeria*-specific phage (2, 8, 16, 17). Certain major outbreaks (including the 1998–1999 hot dog outbreak and the 2001 turkey deli meat outbreak) involved strains resistant to the quaternary ammonium disinfectant benzalkonium chloride (BC) (7, 18, 26). Strains of epidemic clone II (ECII) (including those from the 1998–1999 hot dog outbreak) also exhibit temperature-dependent resistance to phage (16).

Some of the longest known environmental adaptations of *L. monocytogenes* include those associated with resistance to heavy metals, specifically cadmium and arsenic. Such resistance occurs with sufficient frequency to allow its use in subtyping applications (1, 23, 31). It is currently not clear whether or how such resistance may contribute to overall fitness of *L. monocytogenes* in the processing plant environment and in foods. However, it is worthy of note that several major outbreaks of listeriosis have involved cadmium-resistant strains (7, 9, 14, 18, 26). Furthermore, analysis of *L. monocytogenes* from the environment of turkey processing plants revealed that, without exception, BC-resistant isolates were also resistant to cadmium (24). Another study showed that cadmium resistance was more common among strains repeatedly isolated from foods than among those recovered sporadically (12). The prevalence of resistance to these heavy metals varies among different serotypes of *L. monocytogenes* (19, 23, 24).

Arsenic resistance in *L. monocytogenes* appears to be chromosomally encoded (23), but the specific genes have not yet been identified. Significantly more information is available about resistance to cadmium, for which three distinct *cadAC* energy-dependent efflux systems have been identified. These *cadAC* cassettes are related (68 to 71% identity at the level of the deduced polypeptide sequences), and their relative prevalence can be monitored by PCR and DNA-DNA hybridizations (25). The first to be identified and characterized, *cadA1*, is associated with a transposon (Tn*5422*) harbored on plasmids (18, 20, 21). The second, *cadA2*, was identified from the genome sequencing of strain H7858, implicated in the 1998–1999 hot dog outbreak, and is harbored on pLM80, a large (ca. 80-kb) plasmid. This pLM80-associated *cadAC* (*cadA2*) is part of a putative composite transposon that also harbors genes for resistance to BC (7, 18, 26). The third determinant, *cadA3*, is a component of an integrative conjugative element (ICE) on the chromosome of *L. monocytogenes* EGDe (10).

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TABLE 1 Serotype 1/2a and 1/2c *L. monocytogenes* strains used in this study

Strain	Isolation date (mo-yr)	Source	Serotype	Resistance[a]			*cadA* determinant[b]		
				Cd	BC	As	*cadA1*	*cadA2*	*cadA3*
LW-A11	10-01	Avocado	1/2a	□	□	□			
LW-A19	03-02	Avocado	1/2a	□	□	□			
LW-A29	08-02	Avocado	1/2a	□	□	□			
LW-A3	02-01	Avocado	1/2a	□	□	□			
LW-A30	10-02	Avocado	1/2a	□	□	□			
LW-A33	02-03	Avocado	1/2a	□	□	□			
LW-A36	03-03	Guacamole	1/2a	□	□	□			
LW-A37	04-03	Guacamole	1/2a	□	□	□			
LW-A43	10-01	Avocado, paste	1/2a	□	□	□			
LW-A48	09-01	Frozen avocado halves	1/2a	□	□	□			
LW-A5	02-01	Avocado	1/2a	□	□	□			
LW-A57	09-01	Avocado, pulp	1/2a	□	□	□			
LW-A76	NK[c]	NK	1/2a	□	□	□			
LW-A95	07-04	Avocado, pulp	1/2a	□	□	□			
LW-A52	05-01	Frozen avocado pulps	1/2a	□	□	□			
LW-A7	12-00	Guacamole	1/2a	□	□	□			
LW-A8	11-00	Avocado	1/2a	□	□	□			
LW-A108	03-06	Environmental swab	1/2a	□	□	□			
LW-A118	05-05	Halibut fillet	1/2a	□	□	□			
LW-A120	05-05	Environmental swab	1/2a	□	□	□			
LW-A121	05-05	Albacore tuna	1/2a	□	□	□			
LW-A122	05-05	Environmental swab	1/2a	□	□	□			
LW-A123	05-05	Environmental swab	1/2a	□	□	□			
LW-A132	08-06	Cooked crawfish tail meat	1/2a	□	□	□			
AT-01	01-86	Cheese, Brie	1/2a	■	□	□	■	□	□
LW-A14	01-02	Environmental swab	1/2a	■	□	□	■	□	□
LW-A38	04-03	Frozen snow crab clusters	1/2a	■	□	□	■	□	□
LW-A62	03-02	Environmental swab	1/2a	■	□	□	■	□	□
LW-A63	03-02	Seasoned flying fish roe	1/2a	■	□	□	■	□	□
LW-A66	07-02	Environmental swab	1/2a	■	□	□	■	□	□
LW-A67	09-01	Frozen capelin roe	1/2a	■	□	□	■	□	□
LW-A78	11-03	Soft Mexican style cheese	1/2a	■	□	□	■	□	□
LW-A81	08-03	Soft Mexican style cheese	1/2a	■	□	□	■	□	□
LW-A96	11-04	Breaded catfish	1/2a	■	□	□	■	□	□
LW-A75	03-03	Cold-smoked salmon	1/2a	■	■	□	■	□	□
LW-A97	11-04	Avocado, puree	1/2a	■	■	□	■	□	□
LW-A107	07-06	Cooked king crab legs	1/2a	■	■	□	■	□	□
LW-A113	01-06	Smoked salmon fillet	1/2a	■	■	□	■	□	□
LW-A114	01-06	Smoked salmon fillet	1/2a	■	■	□	■	□	□
LW-A116	11-05	Smoked salmon fillet	1/2a	■	■	□	■	□	□
LW-A117	09-05	Sliced smoked salmon	1/2a	■	■	□	■	□	□
LW-A131	07-06	Soft ripened cheese	1/2a	■	■	□	■	□	□
LW-A10	10-00	Frozen roasted eel	1/2a	■	■	□	□	■	□
LW-A18	03-02	Biscuit for sandwich	1/2a	■	□	□	□	■	□
LW-A115	01-06	Frozen scallops	1/2a	■	□	□	□	□	□
LW-A119	05-05	Cooked shrimp	1/2a	■	□	□	□	□	□
AT-22	06-05	Flounder, stuffed	1/2c	□	□	□			
FDA 87	05-86	Cheese, soft	1/2c	□	□	□			
FDA 88	05-87	Milk, low fat, pasteurized	1/2c	□	□	□			
BS-09	NK	Cheese, soft	1/2c	■	□	□	■	□	□
LS-025	NK	Cheese, soft	1/2c	■	□	□	■	□	□
LW-A15	03-02	Sandwich	1/2c	■	□	□	□	■	□
LW-A124	04-05	Guacamole	1/2c	■	■	■	□	■	□

[a] White and black boxes represent susceptibility and resistance, respectively, for cadmium (Cd), benzalkonium chloride (BC), and arsenic (As), determined as described in Materials and Methods.

[b] White and black boxes represent negative and positive PCR results, respectively, for *cadA1*, *cadA2*, and *cadA3*, as described in Materials and Methods. Blank spaces represent isolates that were cadmium susceptible and not tested.

[c] NK, not known.

TABLE 2 Serotype 1/2b *L. monocytogenes* strains used in this study

Strain	Isolation date (mo-yr)	Source	Serotype	Resistance[a]			*cadA* determinant[b]		
				Cd	BC	As	*cadA1*	*cadA2*	*cadA3*
LW-A2	11-00	Avocado	1/2b	□	□	□			
LW-A39	06-03	Baby clam meat	1/2b	□	□	□			
LW-A44	04-01	Frozen avocado pulps	1/2b	□	□	□			
LW-A51	05-01	Frozen avocado pulps	1/2b	□	□	□			
LW-A55	06-01	Environmental swab	1/2b	□	□	□			
LW-A56	02-01	Avocado, chunky	1/2b	□	□	□			
LW-A59	02-01	Avocado, chunky	1/2b	□	□	□			
LW-A9	12-00	Avocado, pulp	1/2b	□	□	□			
BA-11	NK[c]	Ice cream, bar	1/2b	□	□	□			
BS-018	NK	Lobster meat	1/2b	□	□	□			
LW-A22	06-01	Sliced turkey	1/2b	□	■	□			
BA-36	01-87	Crabmeat	1/2b	■	□	□	□	■	□
LW-A12	10-01	Frozen prepared eel	1/2b	■	□	□	□	■	□
LW-A50	06-01	Sandwich	1/2b	■	□	□	□	■	□
LW-A53	06-01	Sliced cheddar cheese	1/2b	■	□	□	□	■	□
LW-A54	06-01	Sliced provolone cheese	1/2b	■	□	□	□	■	□
LW-A60	06-01	Sandwich	1/2b	■	□	□	□	■	□
FDA 101	12-86	Ice cream plant	1/2b	■	□	□	□	■	□
AT-05	06-05	Milk raw	1/2b	■	□	□	■	□	□
AT-06	06-05	Environment	1/2b	■	□	□	■	□	□
AT-26	06-05	Shrimp, cooked peeled	1/2b	■	□	□	■	□	□
BA-05	06-05	Environment	1/2b	■	□	□	■	□	□
LW-A34	02-03	Cuttlefish	1/2b	■	□	□	■	□	□
LW-A42	07-01	Whole milk mozzarella	1/2b	■	□	□	■	□	□
LW-A49	07-01	Mozzarella cheese	1/2b	■	□	□	■	□	□
LW-A79	04-02	Environmental swab	1/2b	■	□	□	■	□	□
LW-A80	04-02	Environmental swab	1/2b	■	□	□	■	□	□
LW-A82	08-03	Soft Mexican style cheese	1/2b	■	□	□	■	□	□
LW-A83	03-04	Imitation crab ball	1/2b	■	□	□	■	□	□
LS-085	NK	Cheese, ricotta	1/2b	■	□	□	■	□	□
LW-A68	12-01	Environmental swab	1/2b	■	□	□	■	□	□
LW-A127	03-06	Cheese	1/2b	■	□	□	■	□	□
AT-24	06-05	Crabmeat	1/2b	■	■	□	■	□	□
LW-A41	05-01	Grilled eel	1/2b	■	■	□	■	□	□
LW-A94	07-04	Cheese, chile relleno	1/2b	■	■	□	■	□	□
LW-A106	07-06	Cold-smoked salmon	1/2b	■	■	□	■	□	□
LW-A111	02-06	Cuttlefish	1/2b	■	■	□	■	□	□

[a] White and black boxes represent susceptibility and resistance, respectively, for cadmium (Cd), benzalkonium chloride (BC), and arsenic (As), determined as described in Materials and Methods.
[b] White and black boxes represent negative and positive PCR results, respectively, for *cadA1*, *cadA2*, and *cadA3*, as described in Materials and Methods. Blank spaces represent isolates that were cadmium susceptible and not tested.
[c] NK, not known.

Characterization of cadmium-resistant isolates from turkey processing plants revealed the presence of *cadA1* and *cadA2* (alone or together), while *cadA3* was not identified among any of the isolates (25). However, *L. monocytogenes* strains from other processing plant environments or foods have not been characterized with regard to the prevalence of these different cadmium resistance determinants.

In this study, we characterized a panel of *L. monocytogenes* strains from foods and food processing plants in terms of their resistance to cadmium, arsenic, and BC. We also investigated the prevalence of the three known cadmium resistance determinants among cadmium-resistant isolates from these sources.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and resistance determinations. The 138 *L. monocytogenes* isolates used in this study are grouped by serotype and listed in Tables 1, 2, and 3. They were isolated from a wide range of processed foods and from processing plant environments between 1986 and 2006 (Tables 1 to 3). Of the 138 isolates, 46 were serotype 1/2a (or 3a), 7 were 1/2c (or 3c), 37 were 1/2b (or 3b), and 48 were of serotype 4b. Bacteria were routinely grown in brain heart infusion (BHI) (Becton, Dickinson & Co, Sparks, MD) at 37°C. Serotype designations were determined by multiplex PCR as described previously (5). Resistance to cadmium, arsenic, and BC was assessed as described previously (23, 24). Isolates were considered resistant to cadmium and arsenic if they yielded confluent growth on Iso-Sensitest agar (ISA) (Oxoid, Hampshire, England) supplemented with 70 μg/ml cadmium chloride anhydrous (Sigma, St. Louis, MO) or 500 μg/ml sodium arsenite (Fluka, Steinheim, Germany), respectively, following incubation at 37°C for 48 h. BC resistance was assessed on Mueller-Hinton agar (MHA) (Mueller-Hinton broth with 1.2% Bacto agar [Becton, Dickinson and Co.]) supplemented with 10 μg/ml of benzalkonium chloride (Acros, Morris Plains, NJ) and 2% sheep blood (BBL, Sparks, MD). The plates were incubated at 37°C for 48 h.

TABLE 3 Serotype 4b *L. monocytogenes* strains used in this study

Strain	Isolation date (mo-yr)	Source	Serotype	MLGT[a]	Clonal group	Resistance[b]			*cadA* determinant[c]		
						Cd	BC	As	*cadA1*	*cadA2*	*cadA3*
LW-A4	02-01	Avocado	4b		ECI	□	□	□			
SK-1343	01-92	Frankfurters (beef)	4b		ECII	□	□	□			
LW-A32	01-03	Guacamole	4b		ECI	□	□	□			
LW-A6	02-01	Avocado	4b		ECI	□	□	□			
LW-A110	02-06	Frozen seafood mix	4b	1.13_4b_Sw87_EC1	ECI	□	□	□			
LW-A125	03-06	Cheese	4b	1.59_4b	ND[e]	□	□	□			
LW-A130	03-06	Cheese	4b		ECIa	□	□	□			
LW-A77	NK[d]	NK	4b		ECI	□	□	□			
LW-A85	04-04	Cheese mix	4b		ECIa	□	□	■			
LW-A87	04-04	Environmental swab	4b		ECIa	□	□	■			
LW-A88	04-04	Environmental swab	4b		ECIa	□	□	■			
LW-A89	04-04	Sliced turkey	4b		ECIa	□	□	■			
LW-A90	04-04	Sliced turkey	4b		ECIa	□	□	■			
LW-A91	04-04	Environmental swab	4b		ECIa	□	□	■			
LW-A92	04-04	Environmental swab	4b		ECIa	□	□	■			
LW-A98	01-05	Environmental swab	4b		ECIa	□	□	■			
LW-A99	01-05	Environmental swab	4b		ECIa	□	□	■			
FDA 34	07-88	Shrimp fresh cooked	4b		ECI	□	□	■			
FDA 35	08-88	Environmental swab	4b		ECIa	□	□	■			
FDA 96	10-88	Lobster meat, frozen	4b		ECI	□	□	■			
FDA 10	02-88	Cheese	4b		ECIa	□	□	■			
LW-A112	02-06	Seasoned cuttlefish	4b		ECIa	□	■	□			
LW-A58	06-01	Virginia ham	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
LW-A84	04-04	Diced ham	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
BS-26	NK	Environment	4b	1.16_4b	ND	■	□	■	□	□	□
FDA 100	12-86	Environmental swab	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
FDA 11	02-87	Ice cream	4b	1.4_4b_EC1a	ECIa	■	□	■	□	□	□
LW-A1	11-00	Avocado	4b	1.13_4b_Sw87_EC1	ECI	■	□	■	□	□	□
LW-A100	01-05	Environmental swab	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
LW-A101	01-05	Vegetable food plate	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
LW-A102	01-05	Cut ham	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
LW-A103	01-05	Environmental swab	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
LW-A104	01-05	Environmental swab	4b	1.2_4b_UK88_EC1a	ECIa	■	□	■	□	□	□
LW-A45	04-01	Frozen avocado pulps	4b	1.13_4b_Sw87_EC1	ECI	■	□	■	□	□	□
LW-A46	08-01	Smoked salmon	4b	1.13_4b_Sw87_EC1	ECI	■	□	■	□	□	□
AT-16	01-88	Fish, red snapper	4b	1.13_4b_Sw87_EC1	ECI	■	□	■	□	■	□
LW-A69	12-01	Environmental swab	4b		ECI	■	□	■	□	■	□
LW-A61	02-03	Cooked crab	4b		ECII	■	□	□	□	■	□
LW-A105	04-04	Environmental swab	4b		ECII	■	□	□	□	■	□
LW-A86	04-04	Environmental swab	4b		ECII	■	□	□	□	■	□
FDA 5	08-86	Ice cream, bar	4b		ECI	■	□	□	□	■	□
FDA 97	07-86	Ice cream, bar	4b		ECI	■	□	□	□	■	□
LW-A109	02-06	Seasoned octopus	4b		ND	■	□	□	□	■	□
FDA 93	07-86	Environmental, dairy	4b		ECI	■	■	□	□	■	□
LW-A13	12-01	Frozen raw shrimp	4b	1.46_4b	ND	■	■	□	□	■	□
LW-A126	03-06	Cheese	4b	1.13_4b_Sw87_EC1	ECI	■	□	□	■	□	□
LW-A128	03-06	Cheese	4b	1.13_4b_Sw87_EC1	ECI	■	□	□	■	□	□
LW-A129	03-06	Cheese	4b	1.13_4b_Sw87_EC1	ECI	■	□	□	■	□	□

[a] MLGT haplotypes for selected isolates were determined as described in reference 6.
[b] White and black boxes represent susceptibility and resistance, respectively, for cadmium (Cd), benzalkonium chloride (BC), and arsenic (As), determined as described in Materials and Methods.
[c] White and black boxes represent negative and positive PCR results, respectively, for *cadA1*, *cadA2*, and *cadA3*, as described in Materials and Methods. Blank spaces represent isolates that were cadmium susceptible and not tested.
[d] NK, not known.
[e] ND, not determined (isolates were not ECI, ECIa, or ECII).

DNA-based characterizations. DNA extractions and PCR-based detection of the cadmium resistance determinants (*cadA1*, *cadA2*, and *cadA3*) were done as described previously (25). Epidemic clonal groups for serotype 4b isolates were determined using DNA-DNA hybridizations as described previously (3). Specifically, isolates were identified as ECI based on serotype 4b status, hybridization with the ECI-specific probe 85M, and resistance of genomic DNA to digestion by Sau3AI, as described previously (32) while ECII status was determined by hybridization with probe 1, specific to LMOh7858_1168 (3). ECIa isolates were identified based on hybridization with probe LMSG, constructed

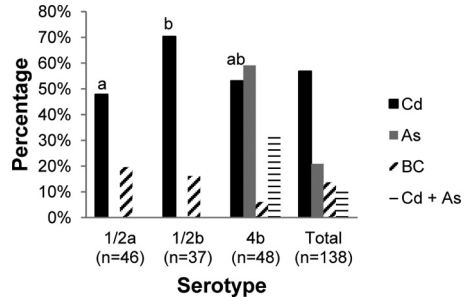

FIG 1 Prevalence of resistance to cadmium (Cd), arsenic (As), and benzalkonium chloride (BC) in *L. monocytogenes* isolates of different serotypes from food and environmental sources. Those that were resistant to both cadmium and arsenic are indicated by horizontal lines. Different letters above the bars indicate statistically significant differences between serotypes ($P < 0.05$). The total includes seven strains of serotype 1/2c (or 3c) listed in Table 1.

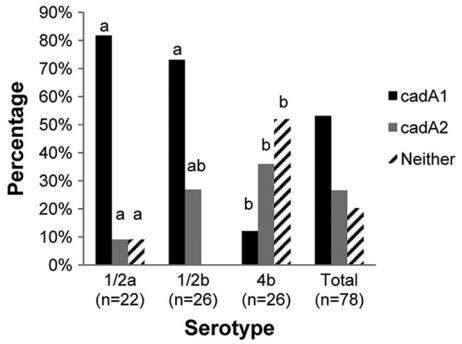

FIG 2 Prevalence of the cadmium resistance determinants *cadA1* and *cadA2* in *L. monocytogenes* isolates of different serotypes from food and environmental sources. Isolates lacking any of the three known determinants (*cadA1*, *cadA2*, or *cadA3*) are indicated with black and white diagonals. Different letters above the bars indicate statistically significant differences between serotypes ($P < 0.05$). The determinants were detected by PCR, as described in Materials and Methods. The total includes four isolates of serotype 1/2c (or 3c).

using primers LMSG_01573F (5′-TACAATTGGTCGGACACGTG-3′) and LMSG_01573R (5′-AGAATCCGCTCATAAACAGC-3′) and by PCR for *mcrB* using primers H7858_0337F (5′-ATATCCATGCCCATCACCA C-3′) and H7858_0337R (5′-CGGGAGGAATCTCGTTATAC-3′). Hybridization with probe LMSG and absence of an amplicon with the *mcrB* primers were indicative of ECIa status (S. Lee and S. Kathariou, unpublished data). ECIa status was confirmed for randomly selected isolates by multilocus genotyping as described below.

PFGE and MLGT. Pulsed-field gel electrophoresis (PFGE) was conducted with AscI (New England BioLabs, Ipswich, MA) and ApaI (Roche, Indianapolis, IN) as described previously (11). BioNumerics (Applied Maths, Austin, TX) was employed for analysis and clustering of PFGE profiles. Multilocus genotyping (MLGT) for selected serotype 4b isolates was conducted as described previously (6).

Statistical analysis. Fisher's exact test was used to make pairwise comparisons among strains for their resistance to arsenic, cadmium, or BC. Summary statistics and *P* values for these comparisons were obtained using the FREQ procedure of the statistical software package SAS (version 9.1.3; SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Serotype-associated trends in prevalence of resistance to BC, cadmium, and arsenic. BC resistance ranged from approximately 6% in serotype 4b to 20% among isolates of serogroup 1/2 (Fig. 1). With the exception of two strains (LW-A22 and LW-A112, of serotypes 1/2b and 4b, respectively), all BC-resistant isolates were also resistant to cadmium (Tables 1 to 3). This strong association between BC resistance and resistance to cadmium was also noted in a study of isolates from the environment of turkey processing plants, where, as mentioned earlier, all BC-resistant isolates were also resistant to cadmium (24).

Resistance to cadmium was common among isolates of all serotypes, ranging from 48% (serotype 1/2a) to approximately 70% (serotype 1/2b) (Fig. 1). In a previous study of food and clinical isolates, 77 to 80% of those of serotypes 1/2a and 1/2b were resistant to cadmium, in contrast to 34% of serotype 4b isolates (23). In the present study, the prevalence of resistance to cadmium among serotype 4b isolates was higher (54%), and it was also higher than observed earlier in our survey of serotype 4b isolates from the environment of turkey processing plants (19%) (24).

In contrast to the findings with cadmium resistance, arsenic resistance was markedly more prevalent among isolates of serotype 4b (60%) than among those of serogroup 1/2 ($P < 0.0001$). In fact, all but one of the arsenic-resistant isolates were of serotype 4b (with the single remaining isolate being of serotype 1/2c); resistance to arsenic was not encountered among any of the isolates of serotype 1/2a or 1/2b. Of the 28 serotype 4b isolates that were arsenic resistant, 15 (54%) were also resistant to cadmium (Fig. 1). Significantly higher prevalence of resistance to arsenic among serotype 4b than among serogroup 1/2 isolates was also noted among isolates from the turkey processing plant environment (24) as well as in an earlier study of food-derived isolates (31% of serotype 4b versus only 1.6% among those of serogroup 1/2) (23).

Serotype-associated differences in prevalence of different cadmium resistance determinants. PCR-based analysis of the cadmium-resistant isolates in the present study ($n = 78$) revealed that overall *cadA1* was more prevalent than *cadA2* (53 versus 27%), while *cadA3* was not detected in any of the isolates. A noticeable fraction (15/78 [19%]) of the cadmium-resistant isolates were negative for all three determinants (Fig. 2). No isolates harbored both *cadA1* and *cadA2*. Our earlier study of environmental isolates also failed to identify cadmium-resistant isolates harboring *cadA3*. However, in that study a significant fraction (30%) of the cadmium-resistant isolates harbored both *cadA1* and *cadA2*, while only 5% of the cadmium-resistant isolates were devoid of any of the three determinants (25). The findings may reflect differences in strain composition in the two studies. They may also reflect outcomes of different selective pressures operating in turkey processing plants in comparison to the food processing plants and foods that were the sources of the isolates in the present study.

Noticeable differences were observed in the prevalence of *cadA1* versus *cadA2* among isolates of different serotypes. The prevalence of *cadA1* was higher among isolates of serotypes 1/2a (82%) and 1/2b (73%) than those of serotype 4b (12%) ($P < 0.0001$). In contrast, *cadA2* prevalence was markedly more common among isolates of serotypes 1/2b (27%) and 4b (38%) than those of serotype 1/2a (9%) ($P < 0.0001$) (Fig. 2). The distribution of *cadA1* differed from that observed in the earlier study of turkey processing plant environmental isolates, where *cadA1* was noticeably more common among those of serotype 1/2a than among those of serotype 1/2b (25). However, in both studies *cadA2* was found to be more common among isolates of serotypes 1/2b and 4b than among those of serotype 1/2a. Such findings suggest a general predilection of this determinant for serotypes 1/2b and 4b,

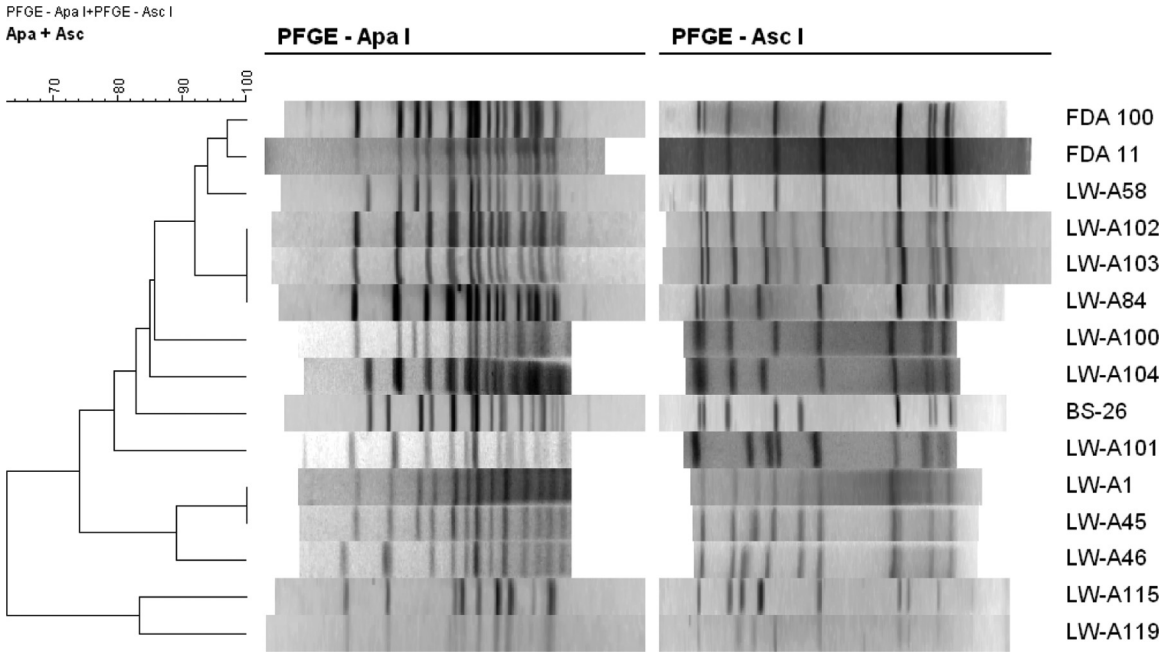

FIG 3 PFGE dendrogram of cadmium-resistant isolates negative for known resistance determinants. Isolates were of serotype 4b, except for LW-A115 and LWA119 (serotype 1/2a). PFGE and cluster analysis were done as described in Materials and Methods.

which belong to the same major genomic division of *L. monocytogenes*, designated lineage I (13, 30). The underlying mechanisms remain uncharacterized but may involve differences in the ecology and microbial community associations of *L. monocytogenes* of different serotypes; it is possible, for instance, that lineage I strains are more likely to inhabit environments populated with bacteria (including other *Listeria* spp.) that may serve as donors of *cadA2* (e.g., via conjugative plasmids).

Cadmium-resistant isolates with putative novel resistance determinants are primarily of serotype 4b and are also resistant to arsenic. As mentioned, an estimated 19% of the cadmium-resistant isolates lacked any of the three known cadmium resistance determinants. Surprisingly, these were found to be noticeably more prevalent among isolates of serotype 4b than among those of other serotypes ($P < 0.0001$): of the 15 isolates in this category, 13 were of serotype 4b, while the remaining two were of serotype 1/2a (Fig. 2 and Tables 1 to 3). All 13 of these serotype 4b isolates were resistant to both cadmium and arsenic. PFGE analysis of these isolates revealed that they represented several different strain types (Fig. 3). The prevalence of such isolates was noticeably higher than that observed in the earlier study of turkey processing plant environmental isolates, where 5% of the cadmium-resistant isolates lacked any of the three known cadmium resistance determinants. However, it is noteworthy that those previously identified isolates without the known determinants were also of serotype 4b and resistant to arsenic (25).

Relative prevalence of cadmium resistance determinants and arsenic resistance varies among different epidemic clones of serotype 4b *L. monocytogenes*. Numerous outbreaks of listeriosis have involved strains of three major serotype 4b clonal groups, designated epidemic clones (ECs) such as ECI, ECII, and ECIa (also referred to as ECIV) (3, 4, 13). Analysis of the serotype 4b isolates in the present study revealed that ECI, ECII, and ECIa were all represented among the isolates, accounting for ca. 92% of the total serotype 4b population. MLGT analysis of randomly selected ECI and ECIa isolates confirmed that they belonged to these clonal groups. All tested ECI isolates had haplotype 1.13_4b_Sw87_EC1, while haplotypes 1.4_4b_EC1a and 1.2_4b_UK88_EC1a were encountered among the ECIa isolates that were analyzed by MLGT (Table 3). Haplotype 1.13_4b_Sw87_EC1 has been found to be ECI specific, while 1.4_4b_EC1a and 1.2_4b_UK88_EC1a are specific for ECIa (6). Each of the four serotype 4b strains outside ECI, ECII, or ECIa (LW-A125, LW-A13, BS-26, and LW-A109) had unique genotypes: three strains had MLGT haplotypes 1.59_4b, 1.46_4b, and 1.16_4b, and strain LW-A109 had the PCR-based serogrouping profile IVb-v1, as previously described (22) (Table 3).

Several of the isolates belonging to ECI, ECII, and ECIa were resistant to cadmium. It was noteworthy that cadmium-resistant isolates lacking any of the three known resistance determinants were especially common in one clonal group, ECIa: none of the nine cadmium-resistant isolates of ECIa harbored *cadA1*, *cadA2*, or *cadA3*. As mentioned above, such isolates were also resistant to arsenic (Fig. 4).

It was earlier noted that arsenic resistance was primarily encountered among serotype 4b isolates (Fig. 1). However, within serotype 4b the prevalence of resistance was disproportionally high (20/22 [91%]) among ECIa isolates, followed by ECI (7/18 [39%]) (Fig. 4). The findings suggest that ECIa isolates, followed by ECI isolates, are the primary contributors of arsenic resistance in *L. monocytogenes* from foods and food processing plants. Further studies are needed to determine whether arsenic resistance prevalence follows similar trends in serotype 4b strains from other sources.

One can only speculate as to the reasons for the observed tendency of arsenic resistance to be associated with these two epi-

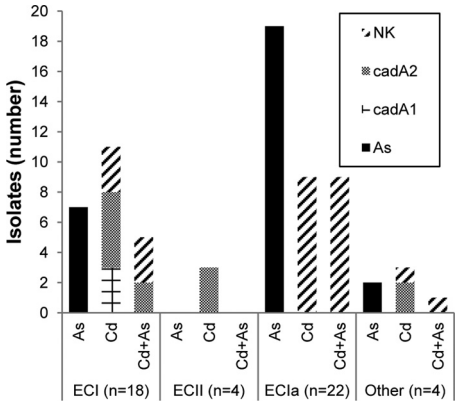

FIG 4 Heavy metal resistance and cadmium resistance determinants in different clonal groups of serotype 4b strains. Columns are as indicated in the inset. Epidemic clones (ECs) ECI, ECII, and ECIa, resistance, and *cadA* types were determined as described in Materials and Methods. "Other," serotype 4b isolates not belonging to ECI, ECII, or ECIa; NK, not known (cadmium-resistant isolates that did not harbor *cadA1*, *cadA2*, or *cadA3*).

demic-associated clonal groups. It is possible, for instance, that ancestral ECIa and ECI strains evolved in natural environments with high concentrations of arsenic, as hypothesized for other bacteria (15, 29). Further studies are needed to determine whether ECIa and ECI isolates may indeed be overrepresented in habitats with high levels of arsenic of natural or anthropogenic origin. The finding that most cadmium-resistant serotype 4b isolates that lacked the known cadmium resistance cassettes were also resistant to arsenic suggests that they may harbor heavy metal detoxification systems that mediate efflux of both cadmium and arsenic (and possibly other heavy metals). Genetic analysis (e.g., mutagenesis) will be needed to characterize the molecular mechanisms mediating cadmium and arsenic resistance in these strains.

The association between arsenic resistance and serotype 4b is especially intriguing, as strains of this serotype, including ECI and ECIa, contribute not only to numerous outbreaks but also to a significant fraction of sporadic human listeriosis (4, 13). The predilection of serotype 4b strains to exhibit resistance to arsenic appears to be an intrinsic feature of this serotype and is not limited to isolates from foods or the environment. Characterization of *L. monocytogenes* from human cases of disease in Belgium and Portugal revealed that approximately 28% of the isolates were resistant to arsenic and that in all cases these isolates were of serotype 4b (1, 31).

It is intriguing that serotype 1/2b strains rarely, if ever, exhibit resistance to arsenic in the present study and in others (1, 23, 24, 31), even though they are members of the same major genomic division (lineage I) as serotype 4b (13, 30). Further studies are needed to elucidate the evolution of arsenic and cadmium resistance in *L. monocytogenes*, especially in serotype 4b strains, and to assess their possible impact on other adaptations, including virulence.

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