Conservation and Distribution of the Benzalkonium Chloride Resistance Cassette *bcrABC* in *Listeria monocytogenes*

Vikrant Dutta,^{a,*} Driss Elhanafi,^{a,b} Sophia Kathariou^a

Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina, USA^a; Biomanufacturing Training and Education Center, North Carolina State University, Raleigh, North Carolina, USA^b

Analysis of a panel of 116 *Listeria monocytogenes* strains of diverse serotypes and sources (clinical, environment of food processing plants, and food) revealed that all but one of the 71 benzalkonium chloride-resistant (BC^r) isolates harbored *bcrABC*, previously identified on a large plasmid (pLM80) of the 1998–1999 hot dog outbreak strain H7858. In contrast, *bcrABC* was not detected among BC-susceptible (BC^s) isolates. The *bcrABC* sequences were highly conserved among strains of different serotypes, but variability was noted in sequences flanking *bcrABC*. The majority of the BC^r isolates had either the pLM80-type of organization of the *bcrABC* region or appeared to harbor *bcrABC* on the chromosome, adjacent to novel sequences. Transcription of *bcrABC* was induced by BC (10 µg/ml) in strains of different serotypes and diverse *bcrABC* region organization. These findings reveal widespread dissemination of *bcrABC* across BC^r *L. monocytogenes* strains regardless of serotype and source, while also suggesting possible mechanisms of *bcrABC* dissemination across *L. monocytogenes* genomes.

Listeria monocytogenes is a food-borne pathogen that can cause severe illness and death in susceptible individuals (pregnant women and their fetuses, the elderly, and patients with various types of immunosuppression) (1, 2). An array of adaptations, including biofilm formation, cold tolerance, and resistance to disinfectants and to phage, can contribute to the ability of this pathogen to persist in food processing environments and thus contaminate ready-to-eat products (3–8). However, our understanding of the mechanisms and factors affecting such adaptations remains limited.

Quaternary ammonium compounds such as benzalkonium chloride (BC) are extensively used in food processing and health care environments (9, 10). BC resistance has been detected in *L. monocytogenes* strains of different serotypes and from diverse sources (11–14). Characterization of *L. monocytogenes* from foods and food processing plants revealed that most BC-resistant (BC^r) isolates were also resistant to the heavy metal cadmium, although the reverse was not always the case (14). Two distinct cadmium resistance determinants (*cadA1* and *cadA2*) were identified among these BC^r isolates, alone or together (14, 15). Of these, *cadA1* is harbored on Tn5422, associated with plasmids of various sizes (16–18), whereas *cadA2* has been identified on large plasmids, such as pLM80 of *L. monocytogenes* H7858, implicated in the 1998–1999 hot dog-associated outbreak of listeriosis, and pLI100 of *L. innocua* CLIP 11262 (17, 19, 20).

In pLM80 of *L. monocytogenes* H7858, *cadA2* appears to be a component of a composite transposon flanked by IS1216 elements (IS1216^{left} and IS1216^{center} in Fig. 1A). In the vicinity of *cadA2* a three-gene cassette (*bcrABC*) was found to be associated with resistance to quaternary ammonium disinfectants such as BC. This cassette also appears to be a component of two putative transposable units, one flanked by IS1216^{center} and IS1216^{right} and the other flanked by IS1216^{left} and IS1216^{right} (Fig. 1A) (21).

Cloning of *bcrABC* in a pLM80-cured derivative of *L. monocytogenes* H7550, a strain obtained from the 1998–1999 outbreak, confirmed that *bcrABC* was associated with BC resistance in this strain (21). A conserved *bcrABC* cassette was detected on a similar plasmid of a different strain (J0161), implicated in an outbreak

involving turkey deli meats in 2000 (17, 21, 22). However, limited information is available on distribution or conservation of *bcrABC* in BC^r *L. monocytogenes* of diverse serotypes and sources. Our objectives here were to determine whether such strains harbored *bcrABC* and to characterize the genomic location and transcriptional expression of *bcrABC* upon exposure to BC among diverse BC^r strains of *L. monocytogenes*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *L. monocytogenes* strains used in the present study were from our laboratory's *Listeria* collection at North Carolina State University. The 71 BC^r strains are listed in Table 1. Bacteria were grown in tryptic soy broth with 0.7% yeast extract (TSBYE; Becton Dickinson [BD], Sparks, MD), brain heart infusion broth (BHI; BD), on BHI agar (BHI with 1.2% Bacto agar [BD]) or on blood agar plates containing 5% sheep blood (Remel, Lenexa, KS), as described previously (21). Propagation of bacteria was performed at 37°C for 24 to 36 h or as indicated. Long-term storage occurred at –80°C in BHI with 20% glycerol (Fisher Scientific, Fairlawn, NJ).

Determination of BC resistance and MICs. BC resistance was assessed as described previously (21, 23). Briefly, a single colony from a blood agar plate (Remel) was suspended in 100 µl of TSBYE; 5 µl of this cell suspension was spotted in duplicate on Mueller-Hinton agar (Mueller-Hinton broth with 1.2% Bacto agar (BD) containing 10 µg of BC (Acros, Fair Lawn, NJ)/ml and 2% defibrinated sheep blood (BBL, Sparks, MD). The plates were incubated at 37°C for 48 h. Strains H7550 and H7550-Cd^s (H7550 cured of pLM80) were used as BC^r and BC-susceptible (BC^s) controls, respectively (21). Strains showing confluent growth of the spots upon exposure to BC (10 µg/ml) were considered resistant (23). For MIC determinations, a single colony from a blood agar plate (Remel) was resuspended in 100 µl of Mueller-Hinton broth (BD), 5 µl of the

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Address correspondence to Sophia Kathariou, sophia_kathariou@ncsu.edu.

* Present address: Vikrant Dutta, EnviroLogix, Inc., Portland, Maine, USA.

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TABLE 1 BC^r *L. monocytogenes* strains used in this study

Strain ^a	Isolation (mo-yr or yr-yr)	Serotype	Category ^b	<i>bcrABC</i> ^c	<i>cadA</i> type ^d	Source ^e (reference)
J0161*	2000	1/2a	I	+	A2	O (17)*
176A	04-2004	1/2a, 3a	I	+	A1, A2	E (23)
192A	06-2004	1/2a, 3a	I	+	A1, A2	E (23)
201A*†	06-2004	1/2a, 3a	I	+	A1, A2	E (23)
231b-1	08-2004	1/2a, 3a	I	+	A1, A2	E (23)
14LA*	06-1905	1/2a, 3a	I	+	A1, A2	C
210A	06-2004	1/2a, 3a	I	+	A2	E (23)
F6825	01-1989	1/2a, 3a	I	+	A2	F
F6972	01-1989	1/2a, 3a	I	+	A2	F
L1712a	06-2005	1/2a, 3a	I	+	A2	E (23)
171D*	04-2004	1/2b, 3b	I	+	A1, A2	E (23)
201B	06-2004	1/2b, 3b	I	+	A1, A2	E (23)
128b-2	02-2004	1/2b, 3b	I	+	A1, A2	E (23)
223a-1	08-2004	1/2b, 3b	I	+	A1, A2	E (23)
326b-5	12-2004	1/2b, 3b	I	+	A1, A2	E (23)
34-2a	09-2003	1/2b, 3b	I	+	A1, A2	E (23)
360b-1	04-2005	1/2b, 3b	I	+	A1, A2	E (23)
414a-1	10-2005	1/2b, 3b	I	+	A1, A2	E (23)
91a-1	12-2003	1/2b, 3b	I	+	A1, A2	E (23)
26LA*	06-1905	1/2b, 3b	I	+	A1, A2	C
210B	06-2004	1/2b, 3b	I	+	A2	E (23)
4 MI-16*†	12-2004	1/2b, 3b	I	+	A2	E (23)
2 MI 40	07-2004	1/2c, 3c	I	+	A2	E (23)
J2446†	01-2003	4b	I	+	A1, A2	C
34-6a†	09-2003	4b	I	+	A2	E (23)
H7550	1998-1999	4b	I	+	A2	O (21)†
H7738	1998-1999	4b	I	+	A2	O†
H7762	1998-1999	4b	I	+	A2	O†
H7858	1998-1999	4b	I	+	A2	O† (20)
283a-3†	10-2004	1/2c, 3c	II	+	A1	E (23)
516b-1*†	03-2006	1/2c, 3c	II	+	A1	E (23)
LW-A131	07-2006	1/2a, 3a	III	+	A1	F (14)
LW-A111	02-2006	1/2b, 3b	IV	+	A1	F (14)
266A	10-2004	1/2a, 3a	V	+	A1	E (23)
266a-1	10-2004	1/2a, 3a	V	+	A1	E (23)
J3916	01-2006	4b	V	+	No A1/A2	C
J5202	09-2008	4b	V	+	Cd ^s	C
265E	10-2004	1/2a, 3a	VI	+	A1	E (23)
101b-4	12-2003	1/2a, 3a	VI	+	A1	E (23)
139a-5	02-2004	1/2a, 3a	VI	+	A1	E (23)
176b-1	04-2004	1/2a, 3a	VI	+	A1	E (23)
201a-4	06-2004	1/2a, 3a	VI	+	A1	E (23)
206a-5	06-2004	1/2a, 3a	VI	+	A1	E (23)
210b-1*†	06-2004	1/2a, 3a	VI	+	A1	E (23)
223b-4	08-2004	1/2a, 3a	VI	+	A1	E (23)
239a-2	08-2004	1/2a, 3a	VI	+	A1	E (23)
26-1a	09-2003	1/2a, 3a	VI	+	A1	E (23)
279a-2	10-2004	1/2a, 3a	VI	+	A1	E (23)
339b-5	12-2004	1/2a, 3a	VI	+	A1	E (23)
367b-1	04-2005	1/2a, 3a	VI	+	A1	E (23)
369a-1	04-2005	1/2a, 3a	VI	+	A1	E (23)
L1624a†	05-2005	1/2a, 3a	VI	+	A1	E (23)
494b-1†	03-2006	1/2a, 3a	VI	+	A1	E (23)
FSL F2-515	08-2000	1/2a1	VI	+	A1	F (26)
258a-1	10-2004	1/2b, 3b	VII	+	A2	E (23)
#18	09-2003	1/2a, 3a	ND	+	A1, A2	E (23)
#192C	06-2004	1/2b, 3b	ND	+	A2	E (23)
#206A	06-2004	1/2a, 3a	ND	+	A1, A2	E (23)
143a-2	02-2004	1/2a, 3a	ND	+	A1, A2	E (23)
158b-2	04-2004	1/2b, 3b	ND	+	A2	E (23)

(Continued on following page)

TABLE 1 (Continued)

Strain ^a	Isolation (mo-yr or yr-yr)	Serotype	Category ^b	<i>bcrABC</i> ^c	<i>cadA</i> type ^d	Source ^e (reference)
171-b1	04-2004	1/2a, 3a	ND	+	A1, A2	E (23)
179b-1	04-2004	1/2a, 3a	ND	+	A1, A2	E (23)
192b-1	06-2004	1/2b, 3b	ND	+	A2	E (23)
201a-1	06-2004	1/2b, 3b	ND	+	A1, A2	E (23)
231a-1	08-2004	1/2b, 3b	ND	+	A1, A2	E (23)
236a-1	08-2004	1/2a, 3a	ND	+	A1, A2	E (23)
266a-2	10-2004	1/2a, 3a	ND	+	A2	E (23)
369a-2	04-2005	1/2a, 3a	ND	+	A2	E (23)
369a-4	04-2005	1/2b, 3b	ND	+	A2	E (23)
LW-A22	06-2001	1/2b, 3b	ND	+	A2	F (14)
J5074	05-2008	4b	ND	–	Cd ^s	C

^a *, strains used to assess *bcrABC* transcription; †, strains used for sequencing *bcrABC*.

^b Categories I to VII are described in the text. ND, category not determined.

^c Based on PCR as described in Materials and Methods.

^d A1 and A2 indicate *cadA1* and *cadA2*, respectively. “A1, A2” indicates the presence of both *cadA1* and *cadA2*. “No A1, A2” indicates that the strain was negative for both *cadA1* and *cadA2*. Cd^s, cadmium susceptible.

^e C, human clinical origin; E, environment of food processing plants; F, food; O, outbreak associated. *, U.S. multistate turkey deli meats outbreak in 2000 (22); †, U.S. multistate hot dog outbreak in 1998–1999 (20, 21)

–10 and –35 sequences were identical to those of H7858 (20, 21). Similar conservation was noted for two strains (J0161 and F2-515, both of serotype 1/2a), except that the putative Shine-Dalgarno sequence for strain F2-515 (<http://www.broadinstitute.org/>) was different (AGGAAAGG) from the rest of the sequenced strains (AGGAGG) (17, 21).

Deduced BcrA sequences were identical in all sequenced strains. No amino acid substitutions were detected among the deduced BcrB polypeptides, but strain 210B lacked tryptophan at residue 79 in BcrB. In the case of BcrC, a glycine-to-serine substitution at residue 13 was noted in four strains (516b-1, 494b-1, L1624-a, and F2-515), while one strain (J2446) had a valine-to-alanine substitution at residue 78 (data not shown).

TABLE 2 Oligonucleotides used in this study

Primer ^a	Sequence (5′–3′) ^b
BcF2	GACTGGATCCGATTCTGGAACATCCCTATC
BcR	GTATGAATTCGTATAATCCGGATGCTGCC
<i>cadA2</i> _F	GCACCAAAGTTTACTGTTGC
c1F*	CAGTCATATTCAGTTTCTCCTTCC
c1R*	GACAATAGCGGTAATTAATCTTTCAC
<i>fsl</i> _F	CCAATGCTTCATGCGTTAC
<i>fsl</i> _R	CGCTTAACCTTCTCGTAACT
0076R	CTCTGTAACCTTCTAAAGCG
0076F	CGCCTTTAGAAAGTTACAGAG
<i>bcrA</i> _R	GATTACCTCTCTTTTGTATTTC
<i>LtrC</i> _F	CAGAGTGAACGTGATCAGCATC
p1*	CATTAGAAGCAGTCGCAAAGCA
p2*	GTTTTCGTGTGTCAGCAGATCTTTGA
<i>tnpR</i>	CTAATTGGTTAAACACGAGCC
0067R	CTGAAATTACGGCAGCGATCTATGAC
0067F	GTCAAATCATTGCCATCGTGCGTA
0065R	CTTCACTCGTTCTACCGTGTGG

^a *, primers used for reverse transcription. For *cadA1*, c1R was used for reverse transcription, and c1F and c1R were used for PCR. For *cadA2*, c2 was used for reverse transcription, and c1 and c2 were used for PCR (21). For *bcrABC*, p2 was used for reverse transcription, and p1 and p2 were used for PCR (21). For housekeeping gene *spoVG*, s2 was used for reverse transcription, and s1 and s2 were used for PCR (21).

^b Underlined sequences correspond to restriction enzyme sites: GGATCC, BamHI; GAATTC, EcoRI. Primers p1, p2, 0067F, 0067R, BcF2, and BcR have been reported before (21).

Diversity among sequences flanking *bcrABC* suggests at least seven categories of *bcrABC*-harboring regions. All but two BC^r isolates in the panel were also resistant to cadmium; the two exceptions were the clinical strains J5074, which was also the only BC^r strain lacking *bcrABC*, and J5202, which harbored *bcrABC* (Table 1). Most BC^r isolates (65/71, 91%) harbored one or both of the previously reported (15) cadmium resistance determinants *cadA1* and *cadA2* (Table 1). To determine whether the organization of the region harboring *bcrABC* in different strains was similar to that in pLM80 (21), we used PCR with pLM80-derived primers (Table 2; Fig. 1A and B) to screen a subset of 55 BC^r isolates of different serotypes and sources (Table 1). PCR results suggested the presence of seven categories of *bcrABC*-harboring regions (Fig. 1B). Two of these categories (I and VI) accounted for the majority of the isolates.

Category I isolates harbor a pLM80-like *bcrABC* region and *cadA2* (alone or together with *cadA1*). Category I was the largest ($n = 29/55$, 52% of the screened BC^r isolates) and consisted of isolates of diverse serotypes (1/2a, 1/2b, 1/2c, and 4b) and sources (both clinical and environmental). These isolates harbored *bcrABC* flanked by the same IS1216-associated sequences present on pLM80 (Table 1; Fig. 1B and 2B). All category I isolates harbored *cadA2*, either alone or together with *cadA1*. Category I isolates harboring *cadA1* alone (in the absence of *cadA2*) were not identified (Table 1). The PCR data suggested that *cadA2* was in the same location as in pLM80, in the vicinity of *bcrABC* (Fig. 1).

Further analysis is needed to determine the location of *cadA1* in category I isolates harboring both *cadA1* and *cadA2*. As mentioned earlier, *cadA1* has been shown to be harbored on plasmid-borne Tn5422 (16–18), and it may be located on the same plasmid that harbors *bcrABC* and *cadA2*; alternatively, *cadA1* and *cadA2* may be associated with different plasmids harbored simultaneously by such isolates.

Evidence for a chromosomal *bcrABC* cassette in category VI isolates. Category VI ($n = 17/55$, 30% of the screened BC^r isolates) consisted exclusively of isolates of serotype 1/2a (or 3a). In these isolates, the PCR data provided evidence only for *bcrABC* and the putative transposon resolvase downstream of *bcrABC* in pLM80, while none of the other pLM80 open reading frames

FIG 2 PCR-based analysis of *L. monocytogenes* *bcrABC* regions. (A) Detection of *bcrABC* in BC^r but not in BC^s strains. Lanes: 1 and 3, BC^r strains 4MI-16 and H7550, respectively; 2 and 4, BC^s strains 24-1b (serotype 1/2a or 3a, processing plant environment) and H7550-Cd^s (21), respectively. PCR was performed using the primers p1 and p2. (B) Category I strains with pLM80-like *bcrABC* region. PCR was performed using the primers *cadA2*_F and 0076R (lanes 1 and 2), 0076F and *bcrA*_R (lanes 3 and 4), p1 and 0067R (lanes 5 and 6), and 0067F and 0065R (lanes 7 and 8). Lanes: 1, 3, 5, and 7, DNA from strain H7550; 2, 4, 6, and 8, DNA from strain 4MI-16. (C) Region harboring *bcrABC* in category VI strains. PCR was performed using the primers *LtrC*_F and p2 (lanes 1 and 2), p1 and p2 (lanes 3 and 4), and p1 and 0067R (lanes 5 and 6). Lanes: 1, 3, and 5, strain H7550; 2, 4, and 6, strain 210b-1. (D) Sequences upstream to *bcrABC* and the downstream transposon resolvase are novel and conserved in category VI isolates but not in the category VII strain 258a-1. Lanes 1 to 10, PCR with the primers *fsl*_F and p2 (a) and the primers p1 and *fsl*_R (b). The template was genomic DNA from strains 223b-4, 279a-2, 210a-4, 258a-1, 210b-1, 239a-2, H7550-Cd^s (negative control), 265E, 339b-5, and 367b-1, respectively. With the exception of H7550-Cd^s (negative control) and 258a-1 (category VII), all other isolates are category VI. (E) Region harboring *bcrABC* in category II strains. PCR was performed using the primers *cadA2*_F and 0076R (lanes 1 and 2) and 0076F and *bcrA*_R (lanes 3 and 4). Lanes: 1 and 3, strain H7550; 2 and 4, strain 516b-1. (F) Region harboring *bcrABC* in category III and IV strains. PCR was performed using the primers *LtrC*_F and p2 (lanes 1 to 4) and 0067F and 0065R (lanes 5 to 8). Lanes: 1 and 5, strain LWA111 (category IV); 2 and 6, strain LWA131 (category III); 3 and 7, strain H7550; 4 and 8, H7550-Cd^s (negative control). (G) Region harboring *bcrABC* in category V. PCR was performed using the primers *LtrC*_F and p2 (lanes 1 and 2), p1 and 0067R (lanes 3 and 4), and 0067F and 0065R (lanes 5 and 6). Lanes: 1, 3, and 5, strain H7550; lanes 2, 4, and 6, strain J5202. (H) Evidence for a truncated transposon resolvase immediately downstream of *bcrABC* in strain 258a-1 (category VII). Lanes 1 to 4 represent PCR with the p1 and *tnpR* primers using genomic DNA from strains F2-515, 258a-1, H7550, and H7550-Cd^s (negative control), respectively. M, 100- to 2,686-bp DNA molecular marker XIV (Roche). Arrows indicate the expected band size of the PCR amplicons.

(ORFs) in the vicinity of *bcrABC* were detected (Table 1; Fig. 1B and 2C).

Analysis of the partially sequenced genome of one of these isolates (*L. monocytogenes* F2-515) revealed that this strain harbored a plasmid with *cadA1*; however, *bcrABC* was not present on this plasmid (<http://www.broadinstitute.org>; 17) and instead appears to be on the chromosome, localized on a 3.1-kb supercontig (<http://www.broadinstitute.org>). Immediately upstream of *bcrABC* was part (238 nt) of an ORF encoding a hypothetical protein without homologs in other *Listeria* genomes in the database, while orf0069 was truncated and followed by novel sequences (537 nt on the contig), also without known homologs in the database (data not shown). PCR with primers derived from these novel F2-515 sequences (upstream of *bcrABC* and downstream of orf0069), in combination with the primers p1 and p2, revealed that the F2-515 gene organization was conserved in other category VI isolates (Fig. 2D). Further genome sequence information for F2-515 and other category VI strains will be needed to identify the location of the *bcrABC* region on the chromosomes of such strains.

The other four categories (II to V) consisted of a small number

of isolates (1 to 4 each) and harbored *bcrABC* flanked by at least some of the *IS1216*-associated ORFs flanking *bcrABC* in pLM80 (Table 1, Fig. 1B, and Fig. 2E to H). *L. monocytogenes* isolates of these categories were of various serotypes. The PCR amplicon with primers *LtrC*_F and p2 was considerably larger for strain LWA111 (category IV) than for the category III strain LWA131 (Fig. 2D). These findings were confirmed in independent trials, but the underlying reasons remain unclear. With the exception of *L. monocytogenes* J3916 and J5202, which lacked known cadmium resistance determinants (J5202 was cadmium susceptible), these isolates harbored *cadA1* (Table 1). Strains J3916 and J5202, along with two others of serotype 1/2a (or 3a) and positive for *cadA1*, comprised a unique category (V) that harbored three of the ORFs downstream of *bcrABC* in pLM80 but not any of those upstream (Table 1, Fig. 1B, and Fig. 2G).

As mentioned earlier, all category I isolates harbored *cadA2*, alone or together with *cadA1*. We failed to detect *cadA2* in isolates of other categories, with the sole exception of the category VII strain 258a-1 (serotype 1/2b or 3b). This strain was *cadA2* positive but lacked any of the other known sequences upstream of *bcrABC* (data not shown); downstream of *bcrABC*, it appeared to harbor a

TABLE 3 BC MICs for representative strains from different categories

Category	Strain	BC MIC ($\mu\text{g/ml}$)
I	201A	40
	4MI-16	40
	210B	40
	26LA	40
II	516b-1	40
	283a-3	40
III	LWA131	40
IV	LWA111	40
V	J3916	40
	J5202	40
VI	F2-515	30
	494b-1	30
	210b-1	30
VII	258a-1	30

shorter variant of orf0070 (transposon resolvase), unlike category VI strains with truncated orf0069 (Fig. 1B and 2H). Strain 258a-1 lacked other downstream sequences detected in pLM80 (data not shown).

BC MICs in isolates of different categories. The MIC for BC was 40 $\mu\text{g/ml}$ for strains in categories I to V, as reported before for the pLM80-harboring strain H7550 (21). However, the MICs were slightly (but consistently) lower (30 $\mu\text{g/ml}$) for strains in categories VI and VII (Table 3). Similar MICs (30 $\mu\text{g/ml}$) were

noted with a recombinant derivative of H7550-Cd^s in which *bcrABC*, with its upstream intergenic region, but not any other pLM80 ORFs had been integrated into the chromosome (V. Dutta and S. Kathariou, unpublished findings). The lower MICs of category VI isolates may be associated with the chromosomal nature (and hence a single copy) of *bcrABC* in this category. Alternatively, some of the sequences flanking *bcrABC* in other categories but absent from categories VI and VII may contribute to the observed levels of resistance to BC. MICs for all tested BC^s strains were 10 $\mu\text{g/ml}$ (data not shown), similarly to the previously reported MIC for H7550-Cd^s (21).

The organization of *bcrABC*-harboring regions suggests putative mechanisms for *bcrABC* dissemination in *L. monocytogenes* genomes. The strong association between *cadA2* and the pLM80-type flanking gene arrangement of *bcrABC* in category I isolates suggests the dissemination of a pLM80-like plasmids harboring *cadA2* and *bcrABC* among *L. monocytogenes* of diverse serotypes. Indeed, the category I serotype 1/2a strain J0161 harbors a large plasmid highly similar to pLM80 (17). Since the majority of the isolates belonged to either category I or VI, it can be hypothesized that *bcrABC* dissemination in *L. monocytogenes* has taken place either via plasmids (pLM80 and related replicons) that also harbor *cadA2* (category I) or via chromosomal integration events (category VI).

Tentative evidence for possible transposable units harboring *bcrABC* could also be obtained for isolates of certain other categories. For instance, category II isolates harbored a part of the pLM80-like IS1216 transposon (IS1216^{center} to IS1216^{right}; Fig. 1A) and may be able to mobilize *bcrABC*, along with its flanking genes. Category V isolates harbored *bcrABC* and the downstream genes up to orf0067, but sequences upstream of *bcrA* were

FIG 3 Impact of exposure to BC and cadmium on transcriptional levels of *bcrABC* and *cadA* (*cadA1*, *cadA2*, or both). (A) RT-PCR of *bcrABC* and *cadA1* for category II strain 516b-1 (harbors *bcrABC* and *cadA1*). The data for *bcrABC* and for *cadA1* expression are shown in the left-hand and right-hand panels, respectively. Lanes: 1, bacteria exposed to no BC or cadmium; 2 and 3, bacteria exposed to BC (10 $\mu\text{g/ml}$) and cadmium (10 $\mu\text{g/ml}$), respectively (30 min, 25°C). Lanes 4 and 5 represent H7550 genomic DNA and total RNA as positive and negative RT-PCR controls, respectively. (B) *bcrABC* and *cadA2* RT-PCR for category I strain 4MI-16 (harbors *bcrABC* and *cadA2*). The data for *bcrABC* and for *cadA2* are shown in the left-hand and right-hand panels, respectively. Lanes: 1, bacteria exposed to no BC or cadmium; 2 and 3, bacteria exposed to cadmium (10 $\mu\text{g/ml}$) and BC (10 $\mu\text{g/ml}$), respectively (30 min, 25°C). Lanes 4 and 5 are as in panel A. (C) *bcrABC*, *cadA1*, and *cadA2* RT-PCR for category I strain 201A (harbors *bcrABC*, *cadA1*, and *cadA2*). The data for *bcrABC*, *cadA1*, and *cadA2* are shown in the left-hand, middle, and right-hand panels, respectively. Lanes are as described in panel B. M, 100- to 2,686-bp DNA molecular marker XIV (Roche). Arrows point to the expected PCR product for *bcrABC* (1,130 bp), *cadA1* (2,526 bp), and *cadA2* (592 bp). RT-PCR was performed as described in Materials and Methods.

different than those in pLM80. Further data are needed to characterize the *bcrABC*-harboring regions in these isolates and also to determine whether *bcrABC* is located on *cadA1*-harboring plasmids. These categories were represented by only a few (1–4) isolates each; it is possible that the environmental fitness of these organisms may be relatively low, for reasons that remain to be identified.

Transcription of *bcrABC* was induced by BC in BC^r strains, regardless of serotype or category. Transcription of *bcrABC* was analyzed using RT-PCR for selected strains representing categories I, II, and VI (Table 1). With all tested strains, transcription increased upon exposure to BC (10 µg/ml) and was not affected by the type of cadmium resistance genes (*cadA1*, *cadA2*, or both) harbored by the strain or by category type. Transcription of *bcrABC* was also not impacted by exposure to cadmium (Fig. 3 and data not shown). On the other hand, transcription of *cadA1*, *cadA2*, or both *cadA1* and *cadA2* increased in the presence of cadmium (10 µg/ml CdCl₂) but not after exposure to BC (Fig. 3 and data not shown). No significant changes were observed in the levels of transcripts of the reference housekeeping gene *spoVG* when cells were exposed to either cadmium or BC (data not shown). These data were similar to those observed before for strain H7550, harboring *bcrABC* on pLM80 (21).

In conclusion, these findings highlight the dissemination of *bcrABC* in *L. monocytogenes* strains of different serotypes and provide clear evidence that the vast majority of BC^r strains harbor *bcrABC*, regardless of serotype or source. Our findings suggest mobility of *bcrABC* on putative transposons harbored by pLM80-like plasmids that also harbor the cadmium efflux determinant *cadA2*, as observed in *L. monocytogenes* H7858 and H7550 (1998–1999 hot dog outbreak) and in *L. monocytogenes* J0161 (2000 turkey deli meats outbreak) (17, 20). Sequencing of other plasmids from category I isolates will be needed to adequately assess their similarity to pLM80 and related plasmids. Additional sequencing will be also needed to elucidate the genomic locations of *bcrABC* in strains of other categories (including category VI isolates that appear to harbor *bcrABC* on the chromosome). Furthermore, it will be of interest to characterize the *bcrABC*-harboring regions of nonpathogenic *Listeria* spp., which may serve as reservoirs for *bcrABC* and other resistance determinants for *L. monocytogenes* (25).

Regardless of their location and flanking sequence content, the pronounced conservation of the *bcrABC* cassettes suggests their relatively recent acquisition from sources that remain to be identified. Further studies are needed to characterize the potential impact of *bcrABC* on environmental persistence and possibly virulence-related adaptations of *L. monocytogenes* strains representing the different categories of *bcrABC*-harboring regions described here.

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