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Characterization of *bla*$_{SHV}$ Genes on Plasmids from *Escherichia coli* and *Salmonella enterica* Isolates from Canadian Food Animals (2006-2007)

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***bla*$_{SHV}$ genes from *Escherichia coli* and *Salmonella enterica* isolates from chicken ($n = 19$) and pork ($n = 1$) were identified as *bla*$_{SHV-2}$ ($n = 5$) or *bla*$_{SHV-2a}$ ($n = 15$). Eighteen were on plasmids of the incI1 ($n = 15$), incP ($n = 2$), and incFIB ($n = 1$) incompatibility groups. These plasmids were all transferable by conjugation between *E. coli* and *S. enterica*.**

Although SHV enzymes remain among the major extended-spectrum β-lactamases (ESBLs) in bacteria from humans (1), data on their prevalence in bacteria from food animals are sparse (2), particularly in North America. Broad-spectrum cephalosporin resistance seen in bacteria from farm animals in North America, and Canada in particular, has been dominated by cephamycinase resistance determinants (3, 4). However, in addition to numerous *bla*$_{CMY-2}$-positive isolates, we recently detected *Escherichia coli* and *Salmonella enterica* isolates carrying *bla*$_{SHV}$ genes in Canadian farm animals. Therefore, the objective of this study was to investigate *bla*$_{SHV}$ and associated plasmids from these *E. coli* and *S. enterica* isolates, obtained by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) in 2006 to 2007 (5, 6), to provide a baseline for comparison with isolates from later years and of human origin.

PCR screening for *bla*$_{SHV}$ (7) was performed on 675 *E. coli* isolates (448, 187, and 40 isolates of chicken, porcine, and bovine origins, respectively) and 205 *Salmonella enterica* isolates (142, 63 isolates of chicken and porcine origins, respectively) resistant to ampicillin recovered by CIPARS in 2006 and 2007 from retail meat and abattoir cecal samples across Canada. Eighteen *E. coli* isolates (2.7%) of chicken ($n = 17$) and porcine ($n = 1$) origins and two *S. enterica* isolates (0.9%) of chicken origin were positive. These 20 isolates were screened for the presence of *bla*$_{TEM}$ and *bla*$_{CMY-2}$ (7). One *Salmonella* isolate (SA18) was positive for *bla*$_{TEM}$. The isolates were serotyped at the Laboratory for Food-Borne Zoonoses (LFZ; Guelph, Ontario, Canada) and tested in duplicate on different days by broth microdilution for susceptibility to a panel of 27 antimicrobial agents, including 16 β-lactams (8). Each isolate belonged to a different serotype. The two *S. enterica* isolates had the highest MICs for most β-lactams (Table 1).

Plasmid preparations from each original isolate (Qiagen plasmid midikit; Qiagen, Hilden, Germany) were electroporated into ElectroMAX *E. coli* DH10B cells (Invitrogen, Carlsbad, CA). Transformants were recovered with the plasmid preparations from 18 isolates on Mueller-Hinton agar containing 50 μg/ml ampicillin or 25 μg/ml tetracycline. However, despite repeated attempts, transformants were not recovered with plasmid preparations of two *E. coli* isolates. Southern blot hybridizations (9) of total DNA (DNeasy blood and tissue kit; Qiagen) and of plasmid preparations with a digoxigenin-labeled *bla*$_{SHV}$ probe (PCR DIG probe synthesis kit; Roche, Mannheim, Baden-Württemberg, Germany) confirmed the *bla*$_{SHV}$ from these isolates was chromosomal. DNA sequencing (described below) showed that these two isolates carried *bla*$_{SHV-2a}$, and they were not investigated further. The *bla*$_{SHV}$ genes from the remaining 18 isolates were carried on plasmids of sizes ranging from approximately 95 kbp to 200 kbp.

DNA sequencing (Laboratory Services, University of Guelph) with a combination of PCR and primer walking and comparison with DNA sequences in NCBI's GenBank (10) and with SHV amino acid sequences (http://www.lahey.org/studies/webt.asp#SHV) was used to identify *bla*$_{SHV}$ variants. Five and 13 plasmids carried *bla*$_{SHV-2}$ and *bla*$_{SHV-2a}$, respectively. All *bla*$_{SHV-2}$ and *bla*$_{SHV-2a}$ genes were identical to GenBank accession no. AF148851 and X53817, respectively (11), and matched the SHV-2 and SHV-2a amino acid sequences in the Lahey Clinic database. The median MICs of the original isolates for β-lactams were generally identical for SHV-2a and SHV-2. However, they were 1 dilution higher for SHV-2a than for SHV-2 with most β-lactams when transformants were compared (Table 1). Thus, the slight differences in β-lactam resistance phenotypes provided by the substitution between SHV-2 and SHV-2a and in their promoter regions may be detectable when expressed under a standardized genetic background, but not readily in natural bacterial populations.

As the two *S. enterica* isolates had median MICs at least 2 dilution steps higher than the *E. coli* isolates for most β-lactams (Table 1), *bla*$_{SHV}$ promoter regions of the two *S. enterica* isolates (SA17 and SA18) and of two *E. coli* isolates (EC7 and EC15) were characterized by PCR (12). The promoter regions for *bla*$_{SHV-2}$ for *E. coli* EC7 and *Salmonella* SA18 were identical in sequence, and so were *bla*$_{SHV-2a}$ promoter sequences for *E. coli* EC15 and *Salmonella* SA17, thus suggesting the observed difference in MICs between bacterial host species was not due to promoter variants affecting SHV expression levels.

Although some minor variation resulting in seven antimicrobial susceptibility profiles was observed, transformants were similar overall, with most *bla*$_{SHV}$ plasmids associated with resistance

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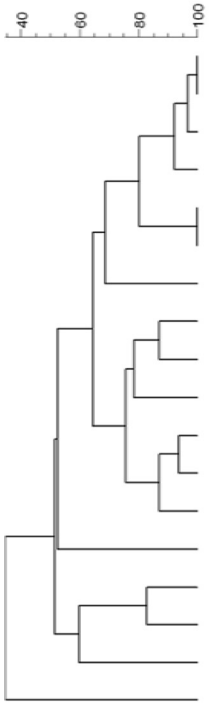
TABLE 1 β-Lactam MICs in the original *E. coli* and *Salmonella* isolates and in *E. coli* DH10B transformants and *Salmonella* Typhimurium LB500 transconjugants

β-Lactam[a]	MIC (μg/ml) for[b]:							
	Original isolates		Transformants		Original isolates		*E. coli* transformants	*Salmonella* Typhimurium transconjugants
	bla_{SHV-2}	bla_{SHV-2a}	bla_{SHV-2}	bla_{SHV-2a}	*E. coli*	*Salmonella*		
CZO	20 (≤8–32)	16 (≤8–32)	16 (≤8–32)	32 (16–32)	16 (≤8–32)	32 (32–32)	32 (≤8–32)	32 (≤8–32)
FEP	≤1 (≤1–≤1)	≤1 (≤1–2)	≤1 (≤1–2)	≤1 (≤1–≤1)	≤1 (≤1–≤1)	2 (2–8)	≤1 (≤1–2)	≤1 (≤1–2)
CTX	0.75 (0.25–4)	1 (0.25–8)	0.75 (0.25–1)	2 (1–2)	1 (0.25–4)	8 (4–16)	1 (0.25–2)	4 (0.5–4)
FOX	≤4 (≤4–16)	≤4 (≤4–8)	8 (≤4–8)	8 (≤4–16)	≤4 (≤4–16)	≤4 (≤4–≤4)	8 (≤4–16)	≤4 (≤4–≤4)
CPD	4 (1–16)	4 (1–>32)	6 (4–8)	12 (4–32)	4 (1–16)	32 (32–64)	8 (4–32)	16 (4–32)
CAZ	0.5 (0.25–1)	0.5 (0.25–2)	1 (0.25–2)	2 (1–2)	0.5 (0.25–2)	4 (2–8)	2 (0.5–2)	2 (0.5–4)
CCV	≤0.12 (≤0.12–≤0.12)	≤0.12 (≤0.12–0.25)	0.25 (0.25–0.25)	0.25 (0.25–0. 5)	≤0.12 (≤0.12–0.25)	0.25 (≤0.12–0.5)	0.25 (≤0.12–0.5)	0.25 (≤0.12–0.25)
CRO	≤1 (≤1–4)	≤1 (≤1–8)	≤1 (≤1–2)	2 (≤1–4)	≤1 (≤1–4)	8 (4–16)	1.5 (≤1–4)	4 (≤1–8)

[a] CZO, cefazolin; FEP, cefepime; CTX, cefotaxime; FOX, cefoxitin; CPD, cefpodoxime; CAZ, ceftazidime; CCV, ceftazidime-clavulanic acid; CRO, ceftriaxone.
[b] Numbers in parentheses represent the MIC ranges (μg/ml) observed in each respective group. Data for ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cephalothin, cefotaxime-clavulanic acid, and carbapenems (imipenem and meropenem) are not shown because the MICs were monomorphic and outside the testing range.

to sulfonamides, tetracycline, streptomycin, and occasionally trimethoprim or gentamicin (Fig. 1). PCR screening (7) of transformants confirmed the presence of associated resistance genes (Fig. 1) for sulfonamides ($n = 17$), tetracycline ($n = 15$), and streptomycin ($n = 17$). PCR screening for *intI1* and *intI2* genes (13) indicated that 17 of the 18 bla_{SHV} plasmids carried class 1 integrons. Sequencing of PCR products (14) confirmed integron variable regions of 1,200 bp ($n = 10$) and 900 bp ($n = 7$), consisting of *dfrA1* for trimethoprim resistance plus *aadA1* for streptomycin resistance or *aadA1* alone (Fig. 1). The presence of these additional resistance determinants suggests a potential for coselection and persistence of bla_{SHV} plasmids in the absence of cephalosporin use. The plasmids belonged to three different replicon types (2), but the vast majority were of the incI1 type (Fig. 1). EcoRI restriction analysis of transformant plasmids was conducted following standard protocols (3) and analyzed using BioNumerics v5.1 software (Applied Maths, Austin, TX). Only partial correlations between plasmid clusters, replicon types, antimicrobial



ID[a]	Source	Year[b]	Serotype	Replicon	SHV	Reduced antimicrobial susceptibility[c]	Antimicrobial resistance genes[d]
EC3	Abattoir chicken cecum	2006	O73:H10	I1	2a	**AMP-CZO-CEP-CPD-STR-SSS-SXT-TET**	*tet*(A), *sul1*, *aadA*, *dfrA1*
EC16	Retail chicken meat	2007	O53:H4	I1	2a	**AMP-CZO-CEP-CPD**-NAL-**STR-SSS-SXT-TET**	*tet*(A), *sul1*, *aadA*, *dfrA1*
EC15	Retail chicken meat	2007	O153:NM	I1	2a	**AMP-CZO-CEP-CPD**-NAL-**STR-SSS-SXT-TET**	*tet*(A), *sul1*, *aadA*, *dfrA1*
SA17	Abattoir chicken cecum	2006	Kiambu	I1	2a	**AMP-CZO-CEP-CPD-CTX**-CRO-**STR-SSS-SXT**	*aadA*, *sul1*, *dfrA1*
EC10	Retail chicken meat	2007	O41:H6	I1	2a	**AMP-CZO-CEP-CPD-STR-SSS-SXT-TET**	*tet*(A), *sul1*, *aadA*, *dfrA1*
EC14	Retail chicken meat	2007	O?:H6	I1	2a	**AMP-CZO-CEP-CPD**-TIO-**STR-SSS-SXT-TET**	*tet*(A), *sul1*, *aadA*, *dfrA1*
EC11	Abattoir chicken cecum	2006	O103:H7	I1	2a	**AMP-CZO-CEP-CPD-STR-SSS-SXT-TET**	*tet*(A/B), *sul1*, *strA/B*, *aadA*, *dfrA1*
EC7	Retail pork	2006	O21:H16	I1	2	**AMP-CZO-CEP-CPD**-CRO-**GEN-STR-SSS-TET**	*tet*(A), *sul1*, *aadA*
SA18	Retail chicken meat	2007	Heidelberg	I1	2	**AMP-CZO-CEP-CTX-CPD-TIO-CRO-TZP-STR-SSS-GEN**	*sul1*, *aadA*
EC2	Abattoir chicken cecum	2006	O135:NM	I1	2a	**AMP-CEP-CPD-TIO-STR-SSS-TET**	*tet*(A), *sul1*, *aadA*
EC4	Abattoir chicken cecum	2007	O178:H7	I1	2a	**AMP-CZO-CEP-CPD**-TIO-**STR-SSS-TET-GEN**	*tet*(A), *sul1*, *aadA*
EC8	Retail chicken meat	2007	O160:H28	I1	2a	**AMP-CZO-CEP-CPD-STR-SSS-TET-GEN**	*tet*(A), *sul1*, *aadA*
EC5	Abattoir chicken cecum	2007	O25:NM	FIB	2	**AMP-CZO-CEP-CPD**-NAL-**STR-SSS-TET-GEN**	*tet*(A), *sul1*, *aadA*
EC6	Retail chicken meat	2006	O88:NM	I1	2	**AMP-CEP-CPD-CRO**-STR-SSS-SXT	-
EC12	Retail chicken meat	2006	O?:H10	P	2a	**AMP-CZO-CEP-CPD**-TIO-**STR-SSS-SXT-TET**	*tet*(A/B), *sul1*, *strA/B*, *aadA*, *dfrA1*
EC9	Retail chicken meat	2007	O?:H10	P	2a	**AMP-CZO-CEP-CPD**-CRO-**STR-SSS-SXT-TET**	*tet*(A/B), *strA/B*, *sul1*, *aadA*, *dfrA1*
EC13	Abattoir chicken cecum	2006	O172:H16	I1	2a	**AMP-CZO-CEP-CPD-STR-SSS-SXT-TET**	*tet*(A), *sul1*, *aadA*, *dfrA1*
EC1	Abattoir chicken cecum	2006	O40:H48	I1	2	**AMP-CEP-STR-SSS-TET-GEN**	*tet*(A), *sul1*, *aadA*

FIG 1 EcoRI restriction analysis of bla_{SHV} plasmids and characteristics of bla_{SHV} isolates. The scale on the top left of the figure indicates the percentage of similarity for the EcoRI restriction profiles of the bla_{SHV} plasmids. Footnotes: a, isolate identification number (all isolates are *E. coli*, except SA17 and SA18, which are *Salmonella enterica*); b, year isolate was collected; c, antimicrobial for which the original isolates' MICs were in the intermediate or resistant range; d, antimicrobial resistance genes detected in transformants. Genes that were shown to be part of a class 1 integron variable region are underlined. Antimicrobial abbreviations: AMP, ampicillin; CEP, cephalothin; CPD, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; CZO, cefazolin; GEN, gentamicin; NAL, nalidixic acid; SSS, sulfonamides; STR, streptomycin; SXT, sulfonamide-trimethoprim; TET, tetracycline; TIO, ceftiofur; TZP, piperacillin-tazobactam. Reduced susceptibilities that were transferred to the respective transformants are in boldface and underlined.

susceptibility, integron size, antimicrobial resistance genes, and bla_{SHV} variants were visible (Fig. 1), suggesting numerous recombinations in the history of these plasmids. However, the *S. enterica* isolates and some *E. coli* isolates shared highly related replicon type I1 plasmids, and two pairs of *E. coli* isolates (EC3 and EC16 and EC10 and EC14) from spatially and temporally distinct sources carried indistinguishable I1 plasmids (Fig. 1). This suggests the ability of bla_{SHV} plasmids to move across bacterial populations and from commensal *E. coli* to *Salmonella*. To confirm this, the 2 *S. enterica* and 13 *E. coli* isolates were conjugated with a nalidixic acid-resistant derivative of *S. enterica* serovar Typhimurium strain LB5000 (15) by overnight mating in Luria-Bertani (LB) broth using a 1:1 ratio of donor and recipient cells. Transconjugants were selected using LB agar containing 50 μg/ml ampicillin and 50 μg/ml nalidixic acid. Three *E. coli* isolates (EC5, EC15, and EC16) were resistant to nalidixic acid and could not be tested. The bla_{SHV} plasmids of all 15 isolates tested were transferable by conjugation. Efficiencies of 7.36×10^{-2} and 1.41×10^{-6} per donor, respectively, were recorded for the transfer of the bla_{SHV} plasmid between two randomly selected *E. coli* isolates (EC2, replicon type I1, and EC9, replicon type P) and *S. enterica* serovar Typhimurium LB5000. Overall, these results demonstrate widespread horizontal transfer of bla_{SHV} plasmids, in particular of replicon type incI1, rather than clonal spread of a few SHV-2- and SHV-2a-producing strains. Furthermore, these plasmids appear to move easily between *E. coli* and *S. enterica*.

Previous reports have suggested that lower cephalosporin MIC values should be used for *E. coli* than for *S. enterica* to trigger investigations of the presence of ESBLs (16). Although not extensive enough for statistical evaluation, the results from the present study strongly support these findings in the case of bla_{SHV}. The two *S. enterica* isolates had median MICs at least 2 dilution steps higher than those of the *E. coli* isolates for cefotaxime, ceftriaxone, ceftazidime, and cefpodoxime and 1 dilution step higher for most other β-lactams (Table 1). Similar trends were visible when comparing the bla_{SHV} plasmids in the *S.* Typhimurium LB5000 host with the *E. coli* DH10B host or with the original *E. coli* isolates, whereas transfer from the original *S. enterica* isolate into *S.* Typhimurium LB5000 resulted in minor changes only (Table 1).

In conclusion, although their prevalence was low (3.2% and 0.4% of ampicillin-resistant isolates from chicken and porcine origins, respectively), the detection of plasmid-borne bla_{SHV-2} and bla_{SHV-2a} in bacteria from Canadian food animals and retail meat with a high potential for horizontal transfer between *E. coli* and *S. enterica* is a public health concern. It is certainly not a coincidence that the first bla_{SHV} characterized in a Canadian non-Typhi *Salmonella* isolate of human origin was also a bla_{SHV-2a} (17). Despite their high mobility, the vast majority of bla_{SHV} plasmids were found in bacteria originating from chicken. Further testing of 12 *E. coli* isolates from 2010 (9 chicken and 2 bovine isolates and 1 porcine isolate) and of two *Salmonella* isolates from 2008 (*S. enterica* serovar Kiambu from chicken) and 2010 (*S. enterica* serovar Derby from swine) from the CIPARS collection confirmed that bla_{SHV-2a} ($n = 11$) and bla_{SHV-2} ($n = 2$) are still persisting in bacteria from food animals in Canada (P. Boerlin, G. Chalmers, and L. Martin, unpublished data), in particular from chickens. Concerns have been expressed previously regarding use of broad-spectrum cephalosporins and the presence of cephamycinases in *E. coli* and *Salmonella* isolates from chickens (4). The data presented here suggest that these concerns may also extend to the selection of ESBLs, such as bla_{SHV}.

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