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Characterization of bla_{CMY-2} Plasmids in *Salmonella* and *Escherichia coli* Isolates from Food Animals in Canada

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One hundred thirty-four bla_{CMY-2} plasmids from *Salmonella* and *Escherichia coli* strains from animals and food in Canada were characterized. Five plasmid groups were identified based on replicon type and restriction profiles. Three groups contained *E. coli* plasmids only. IncA/C plasmids included most multiresistant plasmids and all those of bovine origin.

In North America, *Salmonella enterica* serovar Newport (8) and *Salmonella enterica* serovar Heidelberg (1, 7) carrying the plasmid-borne bla_{CMY-2} gene have been sources of concern in animal production and public health. However, these plasmids have also been found in isolates of other *Salmonella enterica* serovars (8) and *Escherichia coli* (2, 3). Previous studies in Canada have characterized bla_{CMY-2} plasmids in bacteria from human, cattle, and environmental sources (12–14, 16). The objective of this study was to characterize bla_{CMY-2} plasmids in *S. enterica* and *E. coli* isolates from a broader spectrum of food and food-producing animals in Canada.

Bacterial isolates were obtained by passive and active surveillance from poultry, cattle, swine, and related meat products. *S. enterica* isolates, collected between 1999 and 2007 by the World Organization for Animal Health (OIE) Reference Laboratory for Salmonellosis at the Laboratory for Food-borne Zoonoses and by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (10), served as a basis for this study. *E. coli* isolates obtained by CIPARS between 2003 and 2007 completed this collection (10).

Antimicrobial susceptibility testing was performed on OIE Reference Laboratory isolates (1999 to 2004) by agar dilution (18, 19) and on CIPARS isolates (2003 to 2007) by broth microdilution (6, 10). Isolates with a cefoxitin MIC of ≥16 mg/liter were eligible for inclusion. Sixty-nine cefoxitin-resistant *Salmonella* isolates were selected to include the broadest possible diversity of serovars and of sources. Forty-nine cefoxitin-resistant *E. coli* isolates were randomly selected. Eight pairs of cefoxitin-resistant *S. enterica* and *E. coli* isolates from the same chicken ceca were included.

bla_{CMY} was detected by PCR (17, 21), and plasmid DNA (Qiagen plasmid kit; Qiagen, Hilden, Germany) was electroporated into *E. coli* ElectroMAX DH10B (Invitrogen, Carlsbad, CA). Transformants were selected on Mueller-Hinton agar (BD, Franklin Lakes, NJ) containing 8 mg/liter ceftiofur (Sigma-Aldrich, St. Louis, MO).

Transformants underwent PCR and antimicrobial susceptibility testing (described above), as well as replicon typing (5) and plasmid restriction analysis using BglII (New England BioLabs, Ipswich, MA). Restriction fragments of ≥3 kb were identified, and profiles were clustered using Dice similarity coefficients and the unweighted pair group method using arithmetic means (BioNumerics version 5.1; Applied Maths, Belgium).

Sixty-four restriction profiles were identified and grouped into five clusters based on replicon type (clusters A to E in Fig. 1). Sequencing of the bla_{CMY} PCR product of one transformant of each profile confirmed that all were bla_{CMY-2}.

Plasmids from clusters A and B (repA/C and repI1, respectively) were the most frequent and diverse. These clusters correspond to common groups of bla_{CMY-2} plasmids previously described in North America (4, 12, 13, 16, 20) and contain plasmids from *S. enterica* and *E. coli*, with four restriction profiles found in both species (Fig. 1). Plasmids from the three remaining clusters (repK, repFIB, and unidentified replicon type) were found in *E. coli* only. Other Canadian studies have identified *E. coli* isolates from water that contain plasmids from all five clusters (12, 13) and isolates from hospitalized patients that contain plasmids from clusters A, B, C, and E (2). Thus, bla_{CMY-2} plasmids from these five clusters have disseminated in *E. coli* from a variety of sources. However, those from clusters C, D, and E may not have spread extensively in *S. enterica*. No clear association between plasmid types or clusters and time of isolation was visible.

bla_{CMY-2} plasmids from seven pairs of *S. enterica* and *E. coli* isolates originating from the same samples belonged to different replicon types. One pair contained identical repI1 plasmids, and the restriction profiles of this pair of plasmids were indistinguishable and identical to those of 28 other *S. enterica* and *E. coli* plasmids (Fig. 1), suggesting the coincident occurrence of two isolates independently acquiring the same plasmid rather than plasmid transfer *in vivo*.

Plasmids belonging to all five clusters were identified in bacteria from poultry (primarily chickens) and poultry meat. Plasmids encoding resistance to β-lactams only were overwhelmingly found in poultry and poultry products, thus suggesting a possible association with the use of this class of antimicrobial agents (including ceftiofur) in poultry (7). This contrasts with repA/C plasmids, which were found in multiple isolates from all sources investigated. This probably reflects the potential of these multiresistance plasmids to be coselected by the use of antimicrobial agents other than β-lactams. Plasmids in bacteria from cattle and beef were all repA/C (Fig. 1). Together, these data suggest different dynamics and perhaps different selective and coselective pressures

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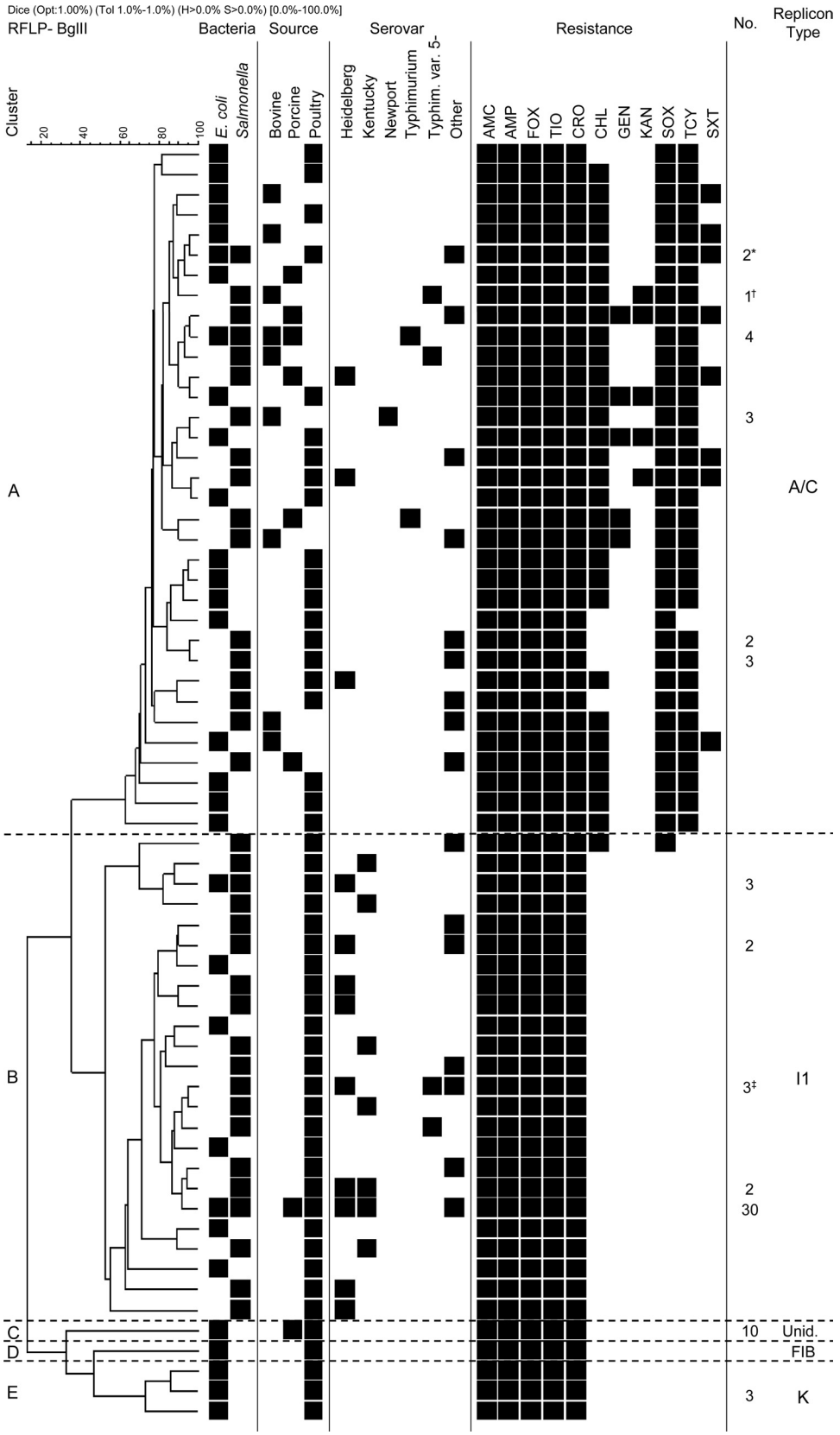

FIG 1 Restriction fragment length polymorphism (RFLP) analysis of *bla*CMY-2 plasmids, associated characteristics, and source data. *Salmonella enterica* serovars other than those represented included Agona, Bredeney, Derby, Enteritidis, Infantis, Mbandaka, Reading, and Thompson and monophasic variants I:4,(5),12:r:−, I:4,12:i:−, I:6,8:eh:−, I:RoughO:r:1,2, and I:RoughO:fgs:−. Resistance to streptomycin was not assessed, as the recipient strain was resistant to streptomycin. AMC, amoxicillin-clavulanic acid; AMP, ampicillin; FOX, cefoxitin; TIO, ceftiofur; CRO, ceftriaxone; CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; SOX, sulfisoxazole; TCY, tetracycline; SXT, trimethoprim-sulfamethoxazole. Numbers of isolates are indicated where more than one plasmid was identified with the same restriction profile. *, one of these plasmids conferred resistance to SOX but not to TCY and SXT; †, the replicon types of this plasmid were A/C and FIIs; ‡, the replicon type of one of three plasmids in this cluster was unidentified (Unid.).

for bla_{CMY-2} in the major animal commodities. Resistance to extended-spectrum cephalosporins (ESCs) is more frequent in poultry than in cattle and swine in Canada (11), and it appears that bla_{CMY-2} plasmids are also more diverse in poultry than in other animals.

Plasmids from repA/C and repI1 were found in 13 and 10 *Salmonella* serovars, respectively, and both were found in *S.* Heidelberg, *S. enterica* serovar Infantis, and *S. enterica* serovar Typhimurium variant 5− (Fig. 1). *S. enterica* serovar Newport is less frequent in Canadian farm animals than in the United States (11, 20), but as expected, bla_{CMY-2} plasmids from this serovar were all repA/C multiresistance plasmids. The majority of bla_{CMY-2} plasmids from *S.* Heidelberg were repI1 plasmids, as was found previously (1). However, repA/C plasmids were also found in this serovar, confirming the repeated acquisition of bla_{CMY-2} plasmids by *S.* Heidelberg from poultry (7). In contrast, chicken-associated *S. enterica* serovar Kentucky contained only repI1 plasmids, possibly reflecting the more recent appearance of ESC resistance in this serovar (9, 11).

As observed by others (4, 13, 14), resistance to agents other than β-lactams was observed in repA/C plasmids only, with one exception (Fig. 1). The most frequent additional resistances were to tetracycline, sulfonamides, and chloramphenicol. One plasmid from a porcine *Salmonella* strain encoded additional resistance to trimethoprim, kanamycin, and gentamicin. As in other studies (4, 15), chloramphenicol resistance was encoded by the *floR* gene (data not shown), thus confirming the potential for selection of bla_{CMY-2} multiresistance plasmids by the use of florfenicol in the animal industry (15).

In conclusion, a large diversity of bla_{CMY-2} plasmids of five replicon types were identified in *S. enterica* and *E. coli* isolates from food animals and derived food products in Canada. These plasmids are similar to those found in bacteria from the environment and from human hospitals. This confirms that, despite a few exceptions, bla_{CMY-2} plasmids are promiscuous and have diffused across bacterial populations from a wide spectrum of ecological and epidemiological compartments. This should be a source of concern, since a broad and complex reservoir of ESC resistance determinants associated with mobile genetic elements has arisen and may be difficult to control.

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