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Genetic Characterization of Antimicrobial Resistance in Canadian Isolates of *Salmonella* Serovar Typhimurium DT104

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PCR was used to identify antibiotic resistance determinants in 31 Canadian *Salmonella* serovar Typhimurium DT104 isolates. Genes encoding resistance to ampicillin (*pse1* or *blaP1*), chloramphenicol (*pasppflo*-like), streptomycin-spectinomycin (*aadA2*), sulfonamide (*sulI*), and tetracycline [*tet*(G)] were mapped to a 13-kb region of DNA of one isolate. Two copies of *sulI* were identified and mapped to the 3′ end of either *pse1* or *aadA2* integrons. The two integrons were separated by the *pasppflo*-like gene and the *tet*(G) gene. The kanamycin resistance determinant (*aphA-1*) was present on a 2.0-MDa plasmid (five isolates) or on the chromosome (three isolates).

Recently in Canada there has been an increase in occurrence of *Salmonella* serovar Typhimurium DT104 isolates (17, 18). Many of these isolates are resistant to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Su), and tetracycline (T), like those found in the United Kingdom (23) and the United States (7, 9), and some are also kanamycin resistant (KAN^r). This study describes the identification of the resistant genes involved in 31 Canadian isolates of *Salmonella* serovar Typhimurium DT104 as well as the location and gene order in a selected isolate (96-5227) with the ACSSuT resistance phenotype.

The antimicrobial susceptibilities (15, 20) of the 31 *Salmonella* serovar Typhimurium DT104 are shown in Table 1, and the PCR primers designed to identify their resistance genes are shown in Table 2. PCR conditions were optimized based on the melting temperature (T_m) of the primers, and long PCR was performed in accordance with the supplier's protocol (Roche Diagnostics, Laval, Quebec, Canada). All primers were synthesized with a Beckman Oligo 1000M DNA synthesizer, and DNA sequencing was carried out with an ABI Prism 377 DNA sequencer (Applied Biosystems Division of Perkin-Elmer, Foster City, Calif.). DNA sequences were aligned with sequences in the National Center for Biotechnology Information database by using Blast version 2.0.4 (1).

To obtain a detailed map and elucidate the order of the ACSSuT resistance phenotype genes, Southern blots of isolate 96-5227 DNA digested with *Bam*HI, *Hin*dIII, *Eco*RI, *Bgl*I, *Xba*I, and corresponding double digests were probed sequentially with amplicons of the identified genes.

Class 1 integrons which have conserved regions (5′CS and 3′CS) often contain antimicrobial resistance gene cassettes (8, 19). PCR amplification using primers 3′-CS and 5′-CS on genomic DNA from all of the DT104 ACSSuT-resistant isolates resulted in 1.0- and 1.2-kb amplicons. DNA sequence analyses (1) of the 1.0-kb amplicon from one selected isolate (96-5227) showed a sequence identical to that of a region on InCg, an integron on *Corynebacterium glutamicum* plasmid pCG4 (emb|Y14748), containing *aadA2*, which encodes streptomycin-spectinomycin resistance (3, 16), while the 1.2-kb amplicon contained sequence with 100% identity to *pse1* (*blaP1*) (*carb-2*, emb|Z18955, coordinates 35 to 1150), which encodes ampicillin resistance (25). In addition, PCR amplification using intragenic primers (Table 2) identified the presence of *pse1* in the 1.2-kb amplicons from 18 (8 ACSSuT^r, 4 ACSSuT-KAN^r, and 6 ACSuT^r) isolates and of *aadA2* in the 1.0-kb amplicon of 12 (8 ACSSuT^r and 4 ACSSuT-KAN^r) isolates (Table 1). Other studies also found the *pse1* and *aadA2* genes within 1.0- and 1.2-kb amplicons in DT104 isolates with ACSSuT resistance (5, 20, 21).

Class 1 integrons may contain the *qacEΔ1-sulI* (sulfonamide resistance)-*orf5* region (8, 19). All 18 isolates that had 5′CS and 3′CS sequence produced a PCR amplicon with *qacEΔ1* and *orf5* primers (Table 2). Partial DNA sequence analysis and PCR using intragenic *sulI* primers confirmed the presence of the sulfonamide resistance gene in these isolates. Furthermore, these isolates also contained the integrase (*intI1*) gene sequence as determined by PCR.

PCR using primers specific for tetracycline resistance genes (Table 2) showed that *tet*(A) was identified in one isolate with an SSuT resistance profile while *tet*(B) was found in three isolates with T-KAN^r profiles. In addition, *tet*(G) was identified in 18 DT104 isolates with ACSSuT resistance, ACSSuT-KAN^r, and ACSuT resistance profiles. DNA sequence analysis of the *tet*(G) PCR product from isolate 96-5227 (gb|AF11924) showed 100% identity with published sequences from *Salmonella* serovar Typhimurium DT104 (gb|AF071555) (5) and *Pseudomonas* plasmid pPSTG2 (gb|AF133140) and 93% identity (CG instead of GC at coordinates 1351 and 1352 of gb|S52437) with that of *Vibrio anguillarum* (24).

The chloramphenicol resistance determinant in 96-5227 was identified as the previously described *pasppflo*-like (*flor*) gene (2, 5) by using PCR primers (Fig. 1) specific for this gene (5) (gb|AF071555).

All of the identified resistance genes as well as the integrase gene were mapped to an 11.8-kb *Xba*I fragment, and their order is shown in Fig. 1. Although Ridley and Threlfall (20) have suggested that two integron-associated genes in DT104 isolates map to a single 10-kb *Xba*I chromosomal fragment, our results showed that the *pse1* gene, which has an internal *Xba*I site, actually extends to an adjacent 4.2-kb *Xba*I fragment (Fig. 1). This difference is probably due to the elution of the 4.2-kb *Xba*I fragment from their pulsed-field gel electrophore-

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TABLE 1. Source, antibiograms, antibiotic resistance genes, and plasmid profiles of Canadian *Salmonella* serovar Typhimurium DT104 isolates

Source	Total no. of isolates	Antibiogram[a]	Genotype identified	Plasmid size profile (MDa)
Human	6	ACSSuT^r	*pse1 pasppflo*-like[c] *aadA2 sulI tet*(G)	60
	3	ACSSuT-KAN^r	*pse1 aadA2 sulI tet*(G) *aphA-1*	60, 2.0
	1	ACSuT^r	*pse1 sulI tet*(G)	60
	1	SSuT^r	*tet*(A)	60
	3	T-KAN^r	*tet*(B) *aphA-1*	60
	6	Susceptible[b]		60
	1	Susceptible[b]		60, 2.2, 1.4
Nonhuman	2	ACSSuT^r	*pse1 aadA2 sulI tet*(G)	60
	1	ACSSuT-KAN^r	*pse1 aadA2 sulI tet*(G) *aphA-1*	60, 2.0
	5	ACSuT^r	*pse1 sulI tet*(G)	60
	2	Susceptible[b]		60
Unknown	14[d]	ASSu-TMP^r	*NA*[f]	60, 50, 4.6, 3.7
	16[e]	ASSu-KAN^r	*sulI aphA-1*	60, 3.4, 2.3, 2.0, 1.4

[a] TMP^r, trimethoprim resistant.
[b] Susceptible to all antibiotics tested.
[c] Putative chloramphenicol resistance determinant (2, 5), identified in strain 96-5227 by the PCR method.
[d] A phage-type-atypical (AT) isolate containing TMP resistance on a self-transmissible 4.6-MDa plasmid was used as a control for the mating experiment.
[e] Phage type PT120.
[f] NA, unable to be determined in this study.

sis gels (20). The relative positions and orientation of genes on the restriction map were confirmed by using various combinations of PCR primers (Fig. 1). The structure of the integron containing *aadA2* is similar to the map published by Briggs and Fratamico (5). However, we did not detect the partial integrase gene (*groEL-intI1b*) (5) in the 5′ region of the *pse1* gene by hybridization with the *intI1* probe.

It had been reported that the ACSSuT resistance genes in DT104 were located on the chromosome and were not self-transmissible (22, 23). All ACSSuT-resistant isolates in this study harbored only the 60-MDa virulence plasmid which has not been reported to confer antimicrobial resistance (23). Furthermore, this plasmid was also found in susceptible isolates (Table 1). Therefore, it is likely that the ACSSuT resistance determinants are on the chromosome of isolate 96-5227.

Kanamycin resistance of eight isolates, including four with ACSSuT-KAN^r, was encoded by the *aphA-1* gene. The amplicons were verified to contain *aphA-1* by *Alu*I and *Dde*I digestion (gb|U63147). The *aphA-1* probe hybridized to a 2-MDa plasmid present in five of the eight isolates (Table 1). This suggests that the other three isolates, containing only the 60-MDa plasmid, have *aphA-1* located on the chromosome. Mating experiments showed that the 2-MDa plasmid in a selected *Salmonella* serovar Typhimurium isolate 97-5025 was not self-transmissible.

In summary, the order, orientation, and location of the ACSSuT resistance genes in the Canadian isolate (96-5227) were similar to those found by others (5, 20, 21). Although the origin of DNA containing the ACSSuT resistance genes remains unknown, our observations together with other published data (5, 20, 21) suggested the possible clonal dissemination of these isolates in the population. The rapid increase of ACSSuT-resistant DT104 isolates in Canada and other countries may suggest that there are other genes in the DNA region which account for the rapid dissemination of this isolate. In an attempt to better understand this unique region of the genome, we have constructed DNA libraries in order to identify other genes and determine the size of the inserted DNA in isolate 96-5227. To this end, sequence analysis and hybridization ex-

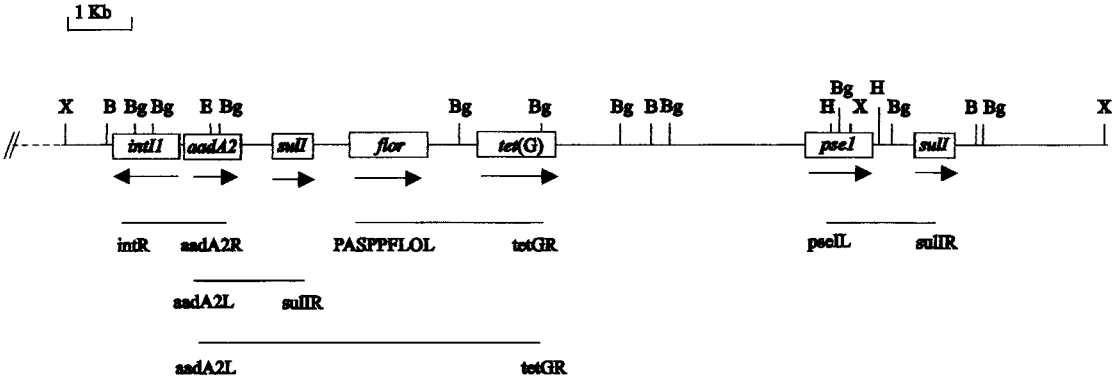

FIG. 1. Restriction map of *Salmonella* serovar Typhimurium DT104 multidrug resistance region from isolate 96-5227. The orientations of the genes are indicated by arrows. The respective positions of the antibiotic resistance genes are shown in boxes. Lines below the map indicate the sizes of products obtained by using the primer sets listed. Restriction enzyme abbreviations: X, *Xba*I; B, *Bam*HI; Bg, *Bgl*I; H, *Hin*dII.

TABLE 2. PCR primers used in the identification of integrons and resistance genes

Gene	PCR primer sequences 5′-3′	Expected size (bp)	GenBank accession no.	Reference strain (plasmid)	Source (reference)
Integron	5′-CS, GGC ATC CAA GCA GCA AG; 3′-CS, AAG CAG ACT TGA CCT GA	?[a]	M73819	NA[b]	P. Roy (11)
pse1	L, AAT GGC AAT CAG CGC TTC CC; R, GGG GCT TGA TGC TCA CTC CA	586	Z18955	*Salmonella* serovar Newport E1572	G. Jacoby (10)
aadA2	L, TGT TGG TTA CTG TGG CCG TA; R, GCT GCG AGT TCC ATA GCT TC	381	X68227	*Escherichia coli*(pSa)	D. Taylor (3)
sulI	*qacEΔ1*, TAG TTG GCG AAG TAA TCG CA; *orf5*, AGC TTG TGC AGA TAT GCG G; *sulI*, TGA AGG TTC GAC AGC AC	1,581	U49101	*Proteus mirabilis* 88071820	P. Roy (11)
aphA-1	L, TTA TGC CTC TTC CGA CCA TC; R, GAG AAA ACT CAC CGA GGC AG	489	U63147	*Escherichia coli* JE2571(pHH1457)	D. Taylor (4)
intI1	L, GCC TTG CTG TTC TTC TAC GG; R, GAT GCC TGC TTG TTC TAC GG	558	X12870	NA	P. Roy (11)
pasppflo-like	L, CAC GTT GAG CCT CTA TAT GG; R, ATG CAG AAG TAG AAC GCG AC	868	AFO71555	NA	GenBank (5)
tet(A)	L, GCT ACA TCC TGC TTG CCT TC; R, CAT AGA TCG CCG TGA AGA GG	210	X61367	*Escherichia coli* D20-15(pSL18)	S. Levy (14)
tet(B)	L, TTG GTT AGG GGC AAG TTT TG; R, GTA ATG GGC CAA TAA CAC CG	659	J01830	*Escherichia coli* D20-16(pRT11)	S. Levy (13)
tet(C)	L, CTT GAG AGC CTT CAA CCC AG; R, ATG GTC GTC ATC TAC CTG CC	418	J01749	*Escherichia coli* D20-6(pBR322)	S. Levy (13)
tet(D)	L, AAA CCA TTA CGG CAT TCT GC; R, GAC CGG ATA CAC CAT CCA TC	787	L06798	*Escherichia coli* D22-2(pSL106)	S. Levy (13)
tet(E)	L, AAA CCA CAT CCT CCA TAC GC; R, AAA TAG GCC ACA ACC GTC AG	278	L06940	*Escherichia coli* HB101(pSL1504)	S. Levy (12)
tet(G)	L, GCT CGG TGG TAT CTC TGC TC; R, AGC AAC AGA ATC GGG AAC AC	468	S52437	*Escherichia coli*(pJA8122)	T. Aoki (24)
tet(G)	L, CAG CTT TCG GAT TCT TAC GG; R, GAT TGG TGA GGC TCG TTA GC	844	S52437	*Escherichia coli*(pJA8122)	T. Aoki (24)
tet(S)	L, CAT AGA CAA GCC GTT GAC C; R, ATG TTT TTG GAA CGC CAG AG	667	X92946	*Escherichia coli*(pAT451)	M. Roberts (6)

[a] ?, variable size depending on the inserted gene(s).
[b] NA, not available.

periments showed that the insertion site of the unique region is between 24 and 30 kb upstream of the *intI1* gene.

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