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Chromosomal Transferable Multidrug Resistance Genes of *Salmonella enterica* Serovar Infantis

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In Japan, *Salmonella enterica* serovar Infantis, together with *S. enterica* serovar Typhimurium, has been one of the major causes of *Salmonella* infections during the past decades, though in 1989 *S. enterica* serovar Enteritidis suddenly emerged and its epidemic continues (1-3). While rare among *S.* Enteritidis (3), multidrug resistance (MDR) was frequent among *S.* Infantis and *S.* Typhimurium (1,2). MDR genes were transferable along with or without a class 1 integron among them (1,2) and also among enterohemorrhagic *Escherichia coli* (2). The present communication shows the chromosomal localization of MDR genes in *S.* Infantis.

Among 32 *S.* Infantis isolates (Inf01 - Inf32) from 1992 - 2003 in Hyogo Prefecture, 23 isolates were implicated in three food poisoning outbreaks and 15 sporadic infections and were of human sources (see the legend to Fig. 1). The remaining nine isolates were from other than humans, one from causative food in an outbreak (Inf25), six from chickens/eggs (Inf03, Inf04, Inf05, Inf07, Inf22, and Inf31), and two from the environment (Inf06 and Inf30). They had no mutual epidemiological relations except between Inf08 and Inf10 from an epidemic in 1996, between Inf15 and Inf17 in an epidemic in 1999, and between Inf23 and Inf25 in an epidemic in 2001.

All the isolates were tested for drug sensitivities by the disk method (Becton Dickinson Microbiology Systems, Cockeysville, Md., USA) (1,2) and by the dilution method. For the dilution method, lactose (0.5%) - MacConkey agar (Lac-MAC) plates were used for testing sensitivities to kanamycin (Km, 25 μg/ml), streptomycin (Sm, 12.5, 25, and 50 μg/ml), and tetracycline (Tc, 25 μg/ml), and Mueller-Hinton (MH) plates for testing sensitivities to trimethoprim (Tm, 25 μg/ml) and sulfamethoxazole (Su, 125 μg/ml). Twelve isolates had MDR that could be classified into three groups. Eight isolates (Inf07, Inf13, Inf14, Inf18, Inf27, Inf28, Inf29, and Inf32) were resistant to Km, Sm, Tc, Tm, and Su,

Table 1. Conjugal transfer of MDR determinants in two strains of *Salmonella* serovar Infantis

Donor	Selection (transfer of freguency/h)	Number of transconjugants obtained/tested	Antibiogram[1)]				
			Km	Sm	Tc	Tm	Su
Inf18	Km (0.3×10^{-7})	33/33	R	R	R	R	R
	Tc (1.8×10^{-7})	29/30	R	R	R	R	R
		1[2)]/30	R	R	R	S	R
	Tm	33[2)]/34	R	R	R	R	R
		1[2)]/34	S	R	R	R	R
	Su	31/31	R	R	R	R	R
Inf32	Km (0.2×10^{-7})	29/31	R	R	R	R	R
		1[2)]/31	R	S	R	R	R
		1[2)]/31	R	R	R	S	R
	Tc (3.2×10^{-7})	28/30	R	R	R	R	R
		1[2)]/30	R	R	R	S	R
		1[2)]/30	S	R	R	S	R
	Tm	33[2)]/34	R	R	R	R	R
		1/34	S	R	R	R	R
	Su	29/31	R	R	R	R	R
		1[2)]/31	S	R	R	R	R
		1[2)]/31	S	R	R	S	R

[1)] Km: kanamycin, Sm: streptomycin, Tc: tetracycline, Tm: trimethoprim, Su: sulfamethoxazole.
[2)] Each of the transconjugants indicated was examined for the presence of class 1 integron by PCR as previously reported (1,2). All the strains tested were positive.

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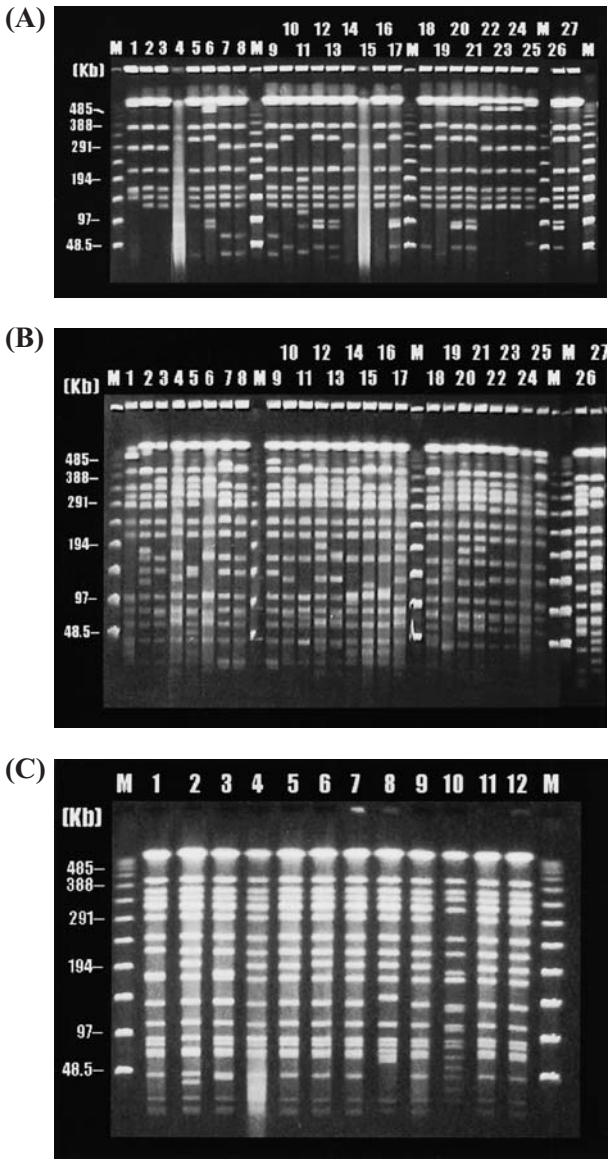

Fig. 1. PFGE patterns of *Bln*I- and *Xba*I-digests of chromosomal DNA of *Salmonella* Infantis isolates. (A), *Bln*I-digests; and (B) and (C), *Xba*I-digests. The lane numbers and the strain names are common in (A) and (B). In (A) and (B), the patterns for 19 antibiotic-sensitive isolates and eight MDR isolates are shown; and in (C), those of 10 MDR isolates and a sensitive strain, Inf30. In explanations below, the names of MDR isolates are indicated in italics, and origins (H: human, C: chicken/egg, and E, Environment) and year of isolation are shown in parentheses. Cases A, B, and C indicate separate food poisoning outbreaks.

(A) (B) Lane 1: Inf01 (H, 1992). Lane 2: Inf02 (H, 1992). Lane 3: Inf03 (C, 1994). Lane 4: *Inf05* (C, 1994). Lane 5: Inf06 (E; drain at a Chinese restaurant, 1994). Lane 6: *Inf07* (C, 1994). Lane 7: Inf08 (H in case A, 1996). Lane 8: Inf09 (H in case A, 1996). Lane 9: Inf10 (H in case A, 1996). Lane 10: Inf11 (H, 1996). Lane 11: Inf12 (H, 1997). Lane 12: *Inf13* (H, 1998). Lane 13: *Inf14* (H, 1998). Lane 14: Inf15 (H in case B, 1999). Lane 15: Inf16 (H in case B, 1999). Lane 16: Inf17 (H in case B, 1999). Lane 17: *Inf18* (H, I999). Lane 18: Inf19 (H, 1999). Lane 19: Inf20 (H, 2000). Lane 20: *Inf21* (H, 2001). Lane 21: *Inf22* (C, 2001). Lane 22: Inf23 (H in case C, 2001). Lane 23: Inf24 (H in case C, 2001). Lane 24: Inf25 (sushi-lunch box in case C, 2001). Lane 25: Inf26 (H, 2001). Lane 26: *Inf27* (H, 2001). Lane 27: Inf04 (C, 1994). M: λ ladder.

(C) Lane 1: *Inf05*. Lane 2: *Inf13*. Lane 3: *Inf14*. Lane 4: *Inf18*. Lane 5: *Inf21*. Lane 6: *Inf22*. Lane 7: *Inf27*. Lane 8: *Inf28* (H, 2001). Lane 9: *Inf29* (H, 1995). Lane 10: Inf30 (E; conveyer on a chicken farm, 1993). Lanes 11 and 12: *Inf31* (C, 2002). M: λ ladder.

two (Inf05 and Inf22) to Km, Sm, Tc, and Su, and two (Inf21 and Inf31) to Sm, Tc, Tm, and Su (2). Among them Inf05, Inf07, Inf22, and Inf31 were from chickens/eggs and the remaining eight were from humans (2).

Figure 1 shows the results of pulsed-field gel electrophoresis (PFGE) (Gene Path Typing System, Program No. 5; Bio-Rad Laboratories, Hercules, Calif., USA) of *Bln*I- or *Xba*I-digests of chromosomal DNA. The patterns of all of the isolates digested with *Bln*I (Fig. 1A) were not sharply differentiated. The pattern was dissimilar among the *Xba*I-digests of the isolates (Fig. 1B), though less variable among those of the MDR isolates (Fig. 1B, lanes 4, 6, 12, 13, 17, 20, 21, and 26; Fig. 1C), which may indicate a common genetic background of these MDR strains.

To locate drug resistance, we mated donor isolates Inf18 and Inf32, which were $Km^rSm^rTC^rTm^rSu^r$, with a recipient *S. enterica* serovar Litchfield $AOLac^+Nal^r$-01 (lac^+Na^r) (4) for 4 h in liquid cultures at 37°C. The transconjugants were selected on Lac-MAC- (for Km^r or Tc^r) or MH-plates (for Tm^r or Su^r). The donor was eliminated by nalidixic acid (Na) (25 μg/ml). Segregation of drug resistance genes during the conjugation was rare, however it was certainly observed (Table 1). Plasmids from nine MDR isolates failed to transfer resistance genes (data not shown). Thus, drug resistance genes were not on a plasmid but on the chromosome.

Southern blot analysis was conducted on Inf18, Inf21, and Inf22 by using *aphA1-LAB* as Km^r-probe, *TetA* as Tc^r-probe, and *qacEΔ1sul1* as Su^r-probe (2). The probes were prepared by polymerase chain reaction, which incorporated digoxigenin-11-dUTP (Boehringer GmbH, Mannheim, Germany) in the reaction mixture, using template Inf18 ($Km^rSm^rTc^rTm^rSu^r$) DNA and appropriate primer pairs (2). Genomic DNA of Inf18, Inf21 ($Sm^rTc^rTm^rSu^r$), and Inf22 ($Km^rSm^rTc^rSu^r$) were digested with *Xba*I, run on PFGE (Gene Path Typing System, Program No. 5; Bio-Rad) and transferred to nylon membranes. Figure 2 shows the presence of Km^r and Su^r genes on the chromosomes of Inf18 and Inf22 (lanes 1-6). As expected, chromosomal DNA of Inf21 hybridized with Tc^r probe

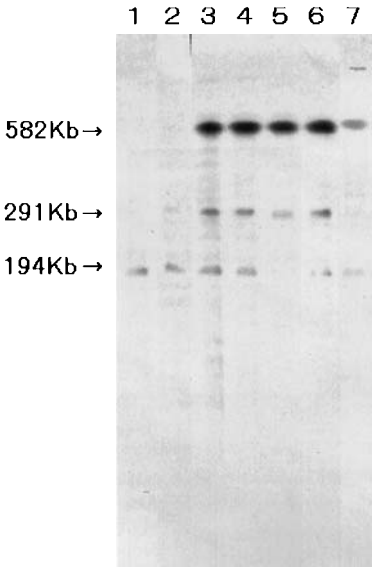

Fig. 2. Southern blot analysis of three MDR *Salmonella* Infantis strains (Inf18, Inf21, and Inf22).
Lane 1: Inf18 (hybridized with Km^r-probe). Lane 2: Inf22 (with Km^r-probe). Lanes 3-4: Inf18 (with Su^r-probe). Lanes 5-6: Inf22 (with Su^r-probe). Lane 7: Inf21 (with Tc^r-probe).

(lane 7) but not with *Km*^{r}-probe (data not shown).

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