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Increasing Incidence and Comparison of Nalidixic Acid-Resistant *Salmonella enterica* subsp. *enterica* Serotype Typhimurium Isolates from Humans and Animals

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We determined the resistance to quinolone of 309 *Salmonella enterica* subsp. *enterica* serotype Typhimurium strains isolated from humans and animals (cattle, pigs, or poultry) in 1995 or 1996. Nalidixic acid resistance increased from 8.5% in 1995 to 18.6% in 1996. The highest resistance levels correlated with a mutation at Ser-83 (or Asp-82). All strains remained ciprofloxacin susceptible. Human and animal isolates were compared by pulsed-field gel electrophoresis, and the banding patterns of the human isolates most closely matched those of the bovine isolates.

Salmonella infection is the most frequent food-borne gastrointestinal disease transmitted from animals to humans (5). *Salmonella enterica* subsp. *enterica* serotype Typhimurium (*S.* Typhimurium) is one of the most common serotypes both in animal and in human isolates (5). Fluoroquinolones are drugs of choice for treatment of human invasive salmonellosis, and some, namely, enrofloxacin, danofloxacin, and marbofloxacin, are also specifically approved for therapeutic veterinary use in France (4). The emergence of quinolone resistance in *S.* Typhimurium is a matter of concern (2), especially in animals whose products are often suspected sources of infection for human gastroenteritis. There have been several reports of human ciprofloxacin treatment failure (11, 12, 17, 20). Genetic analysis indicated that quinolone resistance often resulted from a Ser-83→Phe-83 mutation in the *gyrA* gene coding for the A subunit of the active DNA gyrase (9). This mutation leads to the loss of a *Hin*fI restriction site (GANTC) present in the wild-type *gyrA* sequence (7). On *Hin*fI digestion, the codon 82- or 83-mutated quinolone genes and the quinolone-sensitive genes yield 376- and 277-bp *Hin*fI fragments, respectively.

The purpose of the present study was to investigate a potential link between human and animal *S.* Typhimurium isolates collected in the same rural area. The isolates were compared by pulsed-field gel electrophoresis (PFGE) (18), and quinolone-resistant strains were examined for the presence of a mutation at gyrase A codon 82 or 83 by restriction fragment length polymorphism (RFLP) analysis.

We studied a total of 309 *S.* Typhimurium strains isolated from humans (53 patients) and animals (181 cows, 34 pigs, and 41 chickens) in the department of Ille-et-Vilaine (France) in 1995 to 1996. The animal strains were provided by the Laboratoire Départemental Vétérinaire d'Ille-et-Vilaine. Antimicrobial susceptibility to nalidixic acid, pefloxacin, and ciprofloxacin was assessed by the standardized disk diffusion method, and determination of the MICs was performed according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (1). Statistical analysis of antimicrobial susceptibility was performed by chi-square analysis.

Preparation of whole cellular DNA for PFGE followed the protocol of Allardet-Servent et al. (3). Slices of DNA containing agarose plugs were incubated for 4 h in the presence of 20 U of *Xba*I (13). DNA fragments were separated by PFGE in a 1% agarose gel (Appligene) that was prepared and run in an 0.5× Tris-borate-EDTA buffer on a contour hexagonal electric field (Pharmaco-LKB; Gene Navigator). The pulse times were 5 to 15 s for 12 h and 15 to 40 s for 24 h. Electrophoresis was run at 150 V and at +8°C for 42 h. PFGE patterns were compared by calculation of the Dice correlation coefficient with the Gel Compar software (Applied Maths, BVAD, Kortrijk, Belgium) and were clustered into a dendrogram by the unweighted pair group method with the arithmetic average clustering technique.

*Hin*fI RFLP analysis of the gyrase A gene (*gyrA*) in bacterial DNA was described by Fisher et al. (7). The following modification was made. The filter was hybridized to a digoxigenin-labelled 347-bp fragment of the *gyrA* gene which was prepared by PCR.

Of the 309 *S.* Typhimurium strains screened for resistance to quinolones, 41 isolates (13.3%) were resistant to nalidixic acid. They were found among cattle, pig, poultry, and human iso-

TABLE 1. Susceptibilities to nalidixic acid of 309 *S.* Typhimurium isolates and number (percent) of strains with decreased susceptibility according to their origin

Yr	Phenotype[a]	No. (%) of strains from:				Total
		Humans	Cattle	Poultry	Pigs	
1995	S	21	107	10	12	150
	I or R	1 (4.5)	9 (8.6)	3 (23)	1 (7.7)	14 (8.5)
1996	S	26	53	22	17	118
	I or R	5 (16.7)	12 (18.5)	6 (21.4)	4 (20)	27 (18.6)

[a] S, susceptible to nalidixic acid; I, intermediate; R, resistant.

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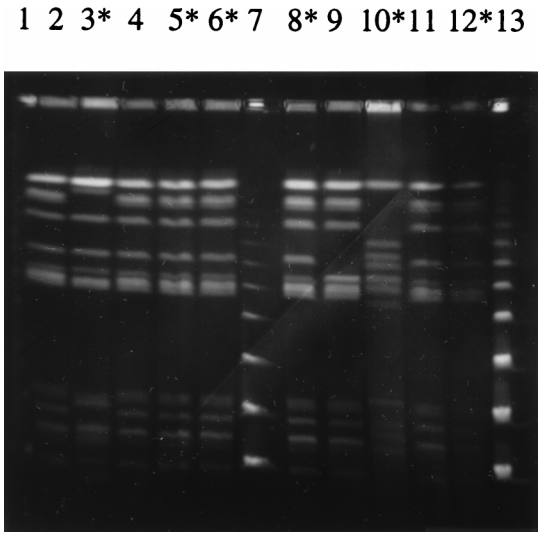

FIG. 1. PFGE patterns of human *S.* Typhimurium isolates. Lanes: 2, SH96-21; 3, SH96-23; 4, SH96-12; 5, SH96-11; 6, SH96-17; 8, SH96-14; 9, SH96-9; 10, strain Haddar; 11, SH96-7; 12, SH95-18; 1, 7, and 13, molecular size markers which were successively larger concatamers of bacteriophage lambda DNA (Fluka-Biochemika). Lanes with asterisks indicate nalidixic acid-resistant strains.

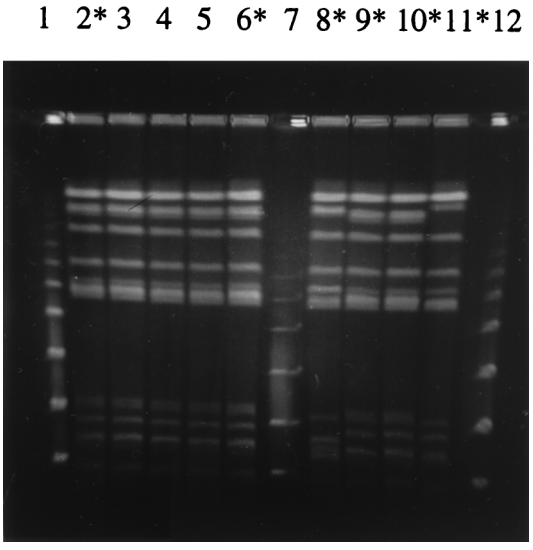

FIG. 2. PFGE patterns of bovine *S.* Typhimurium isolates. Lanes: 2, SB95-67; 3, SB96-24; 4, SB96-25; 5, SB96-17; 6, SB96-6; 8, SB96-21; 9, SB95-24; 10, SB95-33; 11, SB96-15; 1, 7, and 12, molecular size markers which were successively larger concatamers of bacteriophage lambda DNA (Fluka-Biochemika). Lanes with asterisks indicate nalidixic acid-resistant strains.

lates. Twenty-four strains (7.8%) had a decreased pefloxacin susceptibility, but all were susceptible to ciprofloxacin. The distribution of these isolates according to their origin is shown in Table 1. Comparison of the total number of nalidixic acid-resistant strains between the two collection years revealed a significant increase in the percentage, from 8.5% in 1995 to 18.6% in 1996 ($P < 0.01$).

The 41 nalidixic acid-resistant and -susceptible strains of human and animal origin were tested by PFGE (Fig. 1 and 2). Comparison of the banding patterns of 30 *S.* Typhimurium strains (7 from humans, 8 from cattle, 8 from pigs, and 7 from poultry) showed a close relationship among human and bovine isolates (cluster I) with a Dice correlation coefficient of 90% (Fig. 3). The comparison of pig and poultry isolates showed a Dice coefficient of 87.5% (cluster II). These two clusters exhibited different patterns (Dice coefficient = 81%). The comparison of human and animal strains revealed clonal similarity between human and bovine strains and clonal diversity between human and poultry or pig strains. Resistant or susceptible strains cannot be distinguished by PFGE.

*Hin*fI RFLP analysis of 36 nalidixic acid-resistant *S.* Typhimurium strains revealed two different patterns (Fig. 4): 19 strains had a 376-bp *Hin*fI *gyrA* fragment whereas the other 17 had a 277-bp fragment like the LT2 reference strain. The 19 strains with a mutation at codon 82 or 83 were highly resistant to nalidixic acid with at least a 32-fold increase in MICs (MIC > 256 μg/ml). Many of these (17 of 19) had a decreased pefloxacin susceptibility (MIC = 2 μg/ml). MICs of ciprofloxacin were also increased at 0.25 μg/ml. Strains without a mutation at codon 82 or 83 were less resistant. Many of them (10 of 17) had only a twofold increase in the MIC of nalidixic acid (MIC = 16 μg/ml) and remained susceptible to fluoroquinolones (Table 2). The reason for their resistance maps elsewhere. Other mutations have been described in the *gyrA* gene of *Escherichia coli* (15, 16, 23) and *Salmonella* (9). The mutation at codon 83 of subunit A of DNA gyrase greatly reduces the binding of quinolone to the gyrase-DNA complex (22) and can explain the link between this mutation and the MIC (14, 23).

Quinolone-resistant strains have been reported in Asia and Europe. In the United States, multidrug-resistant *S.* Typhimurium definitive type 104 (DT 104) has become a widespread pathogen (8), but the prevalence of quinolone resistance in *Salmonella* remains low, although increasing from 0.1% in 1989 to 1991 to 0.5% in 1994 to 1995 (10). In Vietnam, nalidixic-acid-resistant *Salmonella* serotype Typhi, first described in 1993, is now associated with a treatment failure rate of 50% with short-course treatments which have proved remarkably effective for treatment of multidrug-resistant typhoid fever (21). In the United Kingdom, *S.* Typhimurium DT 104 was the second most prevalent strain of *Salmonella* (after *Salmonella* serotype Enteritidis PT4) isolated from humans in 1996. The incidence of fluoroquinolone resistance of this strain increased from 0% in 1994 to 7% in 1995 and 14% in 1996 (19). Emerging quinolone resistance in animal *S.* Typhimurium is worrying, since these resistant strains are responsible for human pathologies (6) and can lead to treatment failure. Continued monitoring of *Salmonella* for resistance to fluoroquinolone antimicrobial drugs is essential.

TABLE 2. Quinolone susceptibilities of 36 nalidixic acid-resistant *Salmonella* Typhimurium strains according to the presence of a mutation at Ser-83 (or possibly Asp-82)

Quinolone	Mutation	No. of strains	MIC (μg/ml)		
			Range	50%	90%
Nalidixic acid	At Ser-83 (or Asp-82)	19	256–512	512	512
	No mutation at codon 82 or 83	17	16–256	16	256
Pefloxacin	At Ser-83 (or Asp-82)	19	1–2	2	2
	No mutation at codon 82 or 83	17	0.112–1	0.5	1
Ciprofloxacin	At Ser-83 (or Asp-82)	19	0.25–1	0.25	0.25
	No mutation at codon 82 or 83	17	0.03–0.25	0.06	0.12

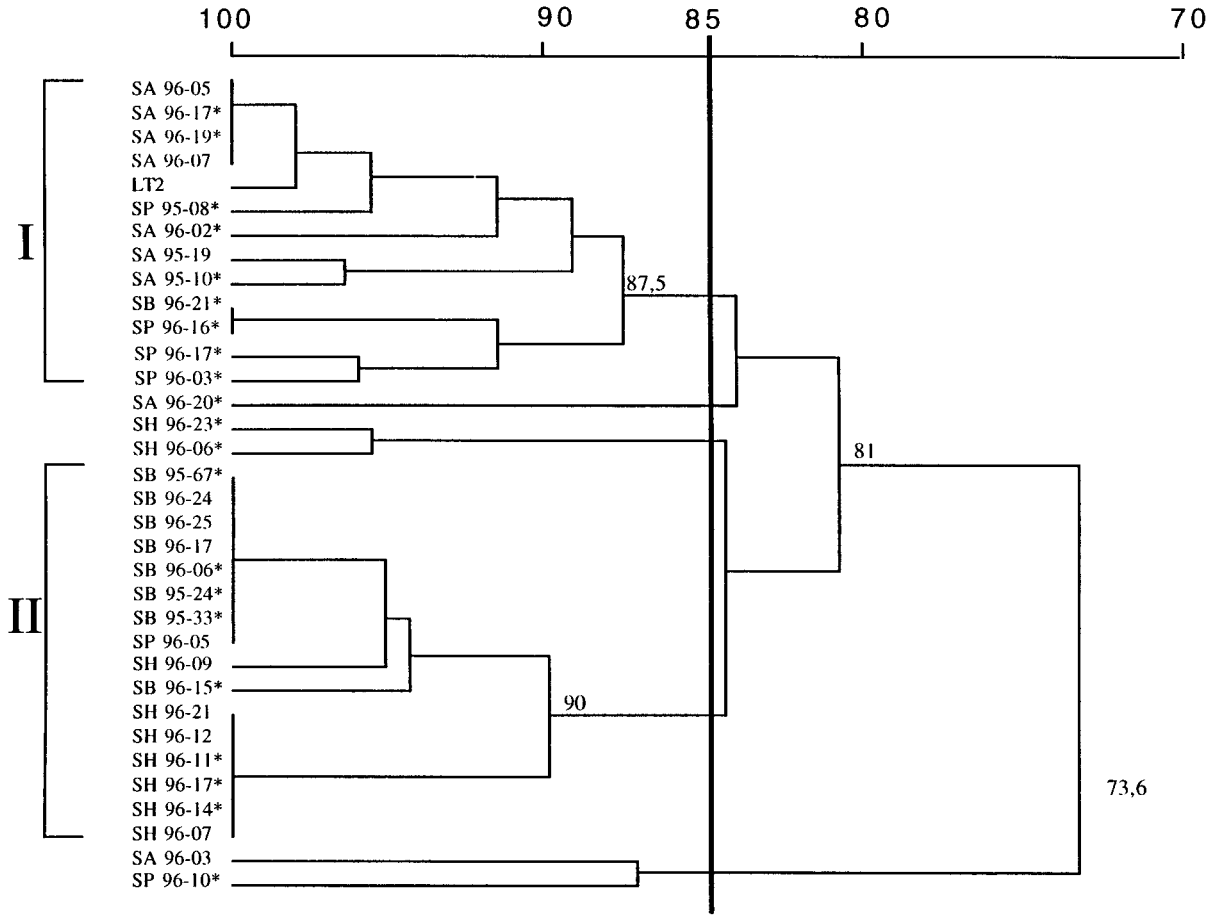

FIG. 3. Dice similarity coefficients (percent). The dendrogram was obtained from cluster analysis of *Xba*I macrorestriction patterns of 30 *S.* Typhimurium isolates from humans (SH), cattle (SB), poultry (SA), and pigs (SP) isolated between 1995 and 1996. Asterisks indicate nalidixic acid-strains. SH96-6 is strain Haddar, SA96-3 is strain Bovis morbificans, and SP96-10 is nontypeable.

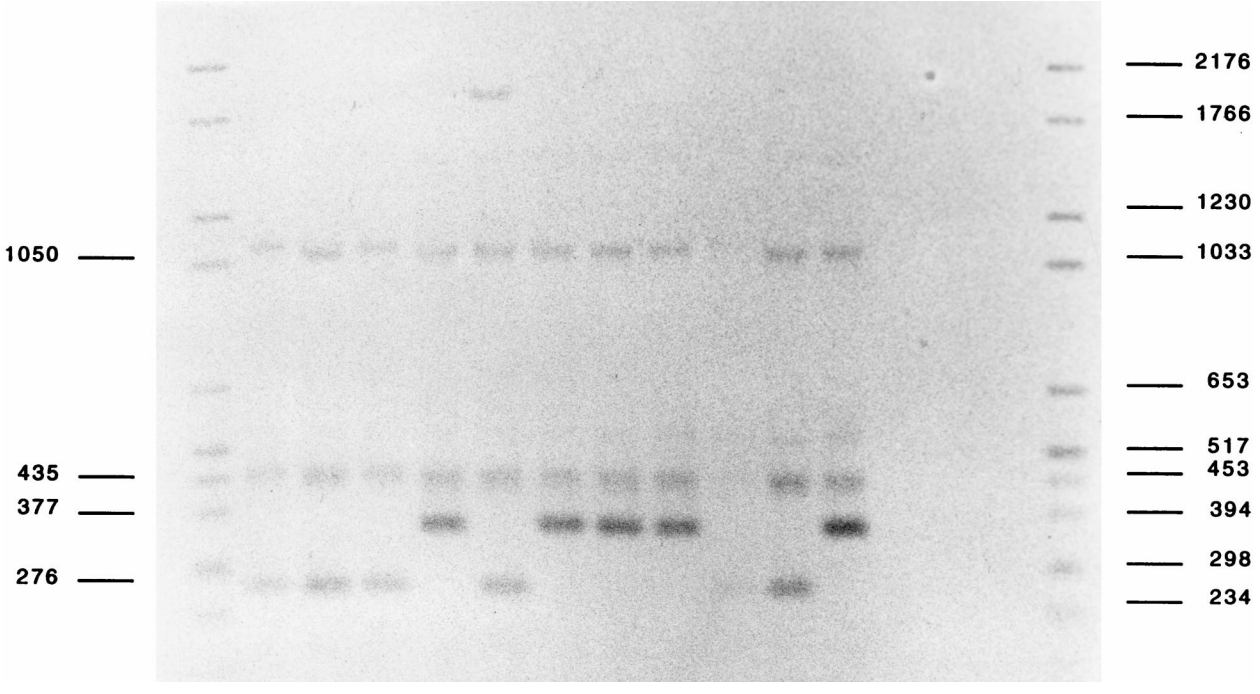

FIG. 4. *Hin*fI RFLP. Genomic DNA was digested with *Hin*fI and probed on Southern blots with a digoxigenin-labelled 347-bp fragment of the *gyrA* gene. Quinolone-sensitive strains produced a 277-bp *Hin*fI fragment, whereas nalidixic acid-resistant strains yielded a 376-bp fragment, indicating the loss of the *Hin*fI site at position 244. Lanes: 2, SB96-65; 3, SA96-9; 4, SB96-52; 5, SH96-14; 6, LT2; 7, SB95-25; 8, SA95-10; 9, SB95-6; 10, SB96-11; 11, SB96-21; 12, SP95-111; 1 and 13, Marker VI (Boehringer) molecular size markers (in base pairs) labelled with digoxigenin. Lanes 5, 7, 8, 9, and 12 contain nalidixic acid-resistant strains.

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