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Comparison of Plasmid Profiles, Phage Types, and Antimicrobial Resistance Patterns of *Salmonella enteritidis* Isolates in the United States

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To evaluate the laboratory techniques for subtyping isolates of *Salmonella* enteritidis, we compared the plasmid profiles (PP), phage types (PT), and antimicrobial susceptibility patterns (AS) of two nationally representative samples of sporadic human *S. enteritidis* isolates from 1979 (n = 28) and 1984 (n = 37), 43 isolates from 20 outbreaks of *S. enteritidis* infections between 1983 and 1987, and 46 animal isolates selected from the U.S. Department of Agriculture Veterinary Services Laboratory in 1986 and 1987. Sporadic and outbreak isolates from humans showed similar rates of resistance to at least one of a panel of antimicrobial drugs (23 and 14%, respectively), PT (91 and 98%, respectively), and PP (97 and 100%, respectively). Sixteen different PP were identified in sporadic, outbreak, and animal isolates; two PP accounted for 76% of sporadic and outbreak isolates. Sporadic human isolates were of PT 8 (42%), of PT 13a (37%), nontypeable (9%), of PT 14b (8%), of PT 9a (3%), and of PT 13 (2%). Outbreak human isolates had similar distributions of PT. PT 8 was associated with poultry: 58% (7 of 12) of the poultry isolates but only 24% (8 of 34) of the isolates from other animals were of PT 8 ($P < 0.04$). Although antimicrobial susceptibility patterns do not appear as useful as an epidemiologic marker, PP and PT effectively subtyped *S. enteritidis*.

Salmonella enteritidis infections have increased in the United States and internationally over the past 10 years (3, 16). Between 1979 and 1989, *S. enteritidis* infections increased sixfold in the northeastern and mid-Atlantic regions of the United States (3). In both community and nosocomial outbreaks, bacterial epidemic strains have often been defined by serotype, biotype, and antimicrobial resistance patterns. However, these phenotypic determinations have not always differentiated *S. enteritidis* isolates. To evaluate epidemiologically useful methods of subtyping *S. enteritidis*, we compared sporadic, outbreak, and animal isolates by use of plasmid profiles (PP), phage types (PT), and antimicrobial susceptibility patterns.

MATERIALS AND METHODS

S. enteritidis isolates were selected from three sources: sporadic isolates from two national *Salmonella* surveillance studies conducted in 1979 and 1984 (10, 15), outbreaks of infections between 1983 and 1987 from which isolates were submitted to the Centers for Disease Control for confirmation, and a random selection of animal isolates submitted between 1986 and 1987 to the U.S. Department of Agriculture Veterinary Services Laboratory.

Isolates were tested by the disk diffusion method (1) for susceptibility to the following antimicrobial agents: sulfamethoxazole, trimethoprim-sulfamethoxazole, nalidixic acid, gentamicin, streptomycin, kanamycin, tetracycline, chloramphenicol, carbenicillin, cephalothin, and ampicillin. An isolate was defined as antimicrobially resistant if it was resistant to at least one of the tested antimicrobial agents and multiply resistant if it was resistant to more than one antimicrobial agent.

PT were determined by use of the system described by Ward et al. with 10 typing phages obtained from the International Reference Laboratory for Enteric Phage Typing, London, United Kingdom (21). PT were identified with a series of numbers and letters which corresponded to phage lysis patterns previously reported (21). Additional PT not previously reported were also identified.

Plasmid DNA was extracted from *S. enteritidis* cells by the technique described by Birnboim and Doly (2). Extracted plasmid DNA was electrophoresed for 2.5 h at 35 mA on a 0.7% vertical agarose gel in TBE buffer (89 mM Tris base, 2.5 mM disodium EDTA, 89 mM boric acid) as described by Meyers et al. (14). After the gels were stained with ethidium bromide (1.5 mg/liter for 20 min), they were photographed under UV illumination. The approximate molecular masses of plasmids in megadaltons were determined by comparison with plasmids of known molecular masses (11, 14). Plasmid DNA from selected PP strains was further purified with a cesium chloride gradient before the determination of plasmid mass (12).

The chi-square test, Fisher's exact test, and Student's t test were used for statistical analysis.

RESULTS

Sixty-five sporadic case isolates were available for analysis from the national *Salmonella* surveillance surveys in 1979 (n = 28) and 1984 (n = 37), as were 43 isolates from 20 outbreaks of *S. enteritidis* infections between 1983 and 1987 and 46 animal isolates from the U.S. Department of Agriculture Veterinary Services Laboratory.

Sporadic, outbreak, and animal isolates had similar rates

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TABLE 1. Antimicrobial resistance of U.S. *S. enteritidis* isolates studied by source of isolate

Source (*n*)	No. (%) of isolates resistant to[a]:										
	Amp	Sulf	Carb	Nitx	Strp	Tetr	Kana	Gent	Chlo	Ceph	Tmpx
Sporatic (65)	7 (11)	3 (5)	0 (0)	5 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Outbreak (43)	0 (0)	6 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Animal (46)	11 (24)	0 (0)	11 (24)	0 (0)	4 (9)	5 (11)	4 (9)	1 (2)	0 (0)	0 (0)	0 (0)

[a] Amp, ampicillin; Sulf, sulfamethoxazole; Carb, carbenicillin; Nitx, nitrofurantoin; Strp, streptomycin; Tetr, tetracycline; Kana, kanamycin; Gent, gentamicin; Chlo, Chloramphenicol; Ceph, cephalothin; Tmpx, trimethoprim-sulfamethoxazole.

of antimicrobial resistance: 23, 14, and 24%, respectively (Table 1). Eleven (92%) of 12 resistant animal isolates were multiply resistant; none of the human isolates were.

Sixteen PP were identified among the three groups of isolates (Fig. 1). Sporadic and outbreak isolates had similar distributions of PP (Table 2). PP 1 and 3 accounted for 76% of sporadic and outbreak isolates. Among the 12 PP identified for the animal isolates, PP 1 and 3 accounted for 54%.

Sixteen PT were identified among the three groups of isolates. Animal isolates had 14 PT, and human isolates had 5. PT 13a and 8 accounted for 83% of human isolates (Table 3) and 42% of animal isolates. When the data were stratified by the origins of the isolates from animals, PT 8 was found associated with poultry; 7 (58%) of 12 poultry isolates but only 8 (24%) of 34 isolates from other animals were PT 8 ($P < 0.04$; Fisher's exact test, two-tailed).

Multiple PP could be identified within one PT, and multiple PT could be identified within one PP, with a total of 36 subtypes being identified among 154 isolates (Table 4). The most common PP among *S. enteritidis* isolates from humans, PP 3, could be further subtyped into seven PT; similarly, the most common PT, PT 8, could be subtyped into five PP. However, a dominant PP subtype was noted for each of the three most common PT: 52 of 58 PT 8 isolates were PP 3, and 38 of 50 PT 13a isolates were PP 1.

Marker diversity increased among sporadic human isolates collected between 1979 and 1984; 5 PP subtypes and 3 PT were identified in 1979, and 11 and 5, respectively, were identified in 1984. Although diversity increased, PT 8 remained the predominant PT, accounting for 43% of all isolates in both years.

For eight outbreaks, more than one isolate was available for analysis. In two of these eight outbreaks, multiple PP or PT were identified in patient isolates. Antimicrobial resistance was identified in isolates from two outbreaks for which more than one *S. enteritidis* isolate was submitted; the antimicrobial resistance patterns were different for isolates from the same outbreak in one large multistate outbreak associated with pasta.

DISCUSSION

As *S. enteritidis* infections continue to increase in the United States and internationally, serotype alone becomes less effective as an epidemiologic marker (20). PP and PT have been used successfully in a number of epidemiologic investigations of other *Salmonella* serotypes and appear to be useful means of subtyping *S. enteritidis* (13, 18).

Antimicrobial susceptibility patterns were not as useful for typing *S. enteritidis* as were PP and PT, as most outbreak and sporadic isolates were nonresistant. This result is similar to the experience with *S. typhimurium*, for which antimicrobial resistance patterns were of little use for typing (8). In this North American series, PP and PT complemented each other in subtyping *S. enteritidis*, in contrast to the situation in the United Kingdom, where PP were not as discriminatory as PT for the primary subdivision of *S. enteritidis* (19). These differences may be explained by the differences in the

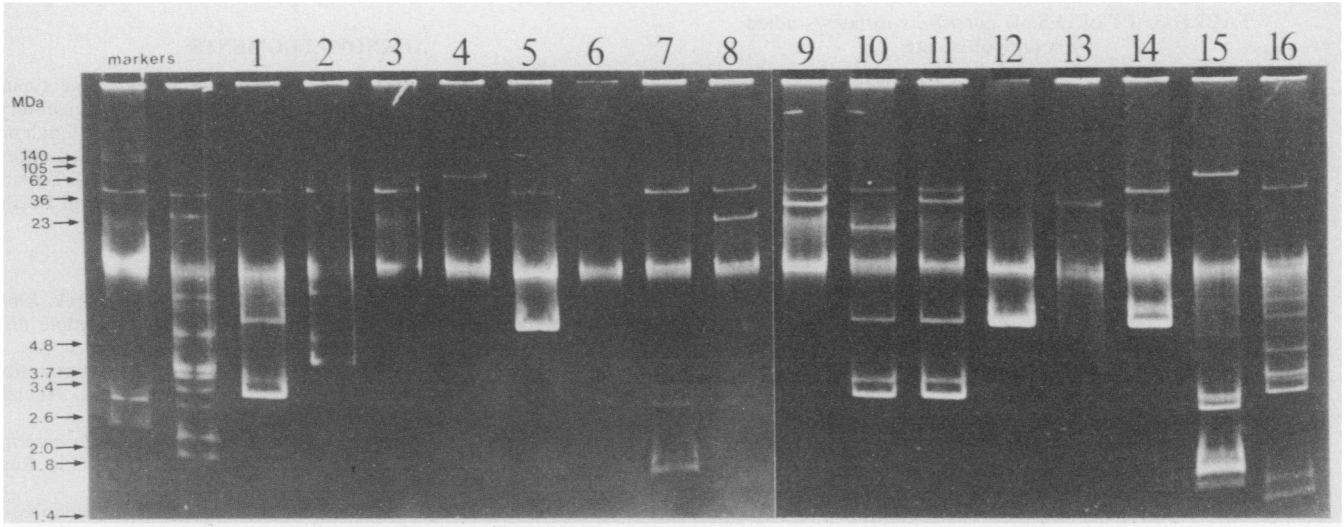

FIG. 1. PP identified in U.S. *S. enteritidis* isolates. Each PP pattern is indicated by the number used in the text. The two leftmost lanes are dye markers. Sizes are given on the left in megadaltons.

TABLE 2. PP of U.S. *S. enteritidis* isolates studied by source of isolate

PP	Molecular mass (MDa)	No. (%) of isolates		
		Sporadic	Outbreak	Animal
1	36, 3.1	15 (23)	22 (51)	1 (2)
2	36, 3.7	2 (3)	3 (7)	1 (2)
3	36	28 (43)	16 (37)	24 (52)
4	62	2 (3)	1 (2)	5 (11)
5	36, 5.5	6 (9)	0 (0)	7 (15)
6	No plasmid DNA	2 (3)	0 (0)	0 (0)
7	36, 1.4	0 (0)	0 (0)	1 (2)
8	36, 14	0 (0)	0 (0)	1 (2)
9	36, 28	1 (2)	0 (0)	1 (2)
10	36, 10, 3.1	1 (2)	0 (0)	0 (0)
11	36, 28, 3.1	2 (3)	0 (0)	2 (4)
12	5.5	3 (5)	0 (0)	0 (0)
13	20	0 (0)	0 (0)	1 (2)
14	62, 36, 5.5	0 (0)	0 (0)	1 (2)
15	62, 2.6, 1.4	0 (0)	0 (0)	1 (2)
16	36, 3.1, 1.4	3 (5)	1 (2)	0 (0)

TABLE 4. PP related to PT of 154 human and animal U.S. *S. enteritidis* isolates

PP	No. of isolates with the following PT:																
	1	2	2a	3v	4	4v	8	9	9a	9b	13	13a	14b	24	28	34	NT[a]
1	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0
3	0	1	1	0	1	0	52	0	0	1	3	0	0	1	0	0	8
4	1	0	0	0	0	0	1	0	2	3	0	0	0	0	0	0	1
5	0	0	0	0	0	0	3	0	0	0	0	4	1	0	2	0	3
6	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
9	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
11	0	0	1	0	1	0	0	0	0	0	2	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1
13	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
15	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	1	4	0	0	0	0

[a] NT, nontypeable.

selection of *S. enteritidis* isolates. Threlfall et al. (19) selected type strains of 27 PT and examined their PP. We selected sporadic, outbreak, and animal *S. enteritidis* isolates on the basis of epidemiologic data and without prior knowledge of PT, and we then examined PT and PP. Both studies demonstrated that certain PT and PP predominated. Although there are differences in the interpretation of the usefulness of PP between these two studies, both reported that common PT such as PT 4 in the United Kingdom and PT 13a in the United States, were divisible by PP (19).

PT 8 and 13a accounted for 83% of the *S. enteritidis* subtypes, similar to the distribution of 79% reported in Canada for *S. enteritidis* isolates collected between 1976 and 1989 (9). This distribution is in contrast to the predominance of PT 4 and 8 reported for 85% of *S. enteritidis* isolates in the United Kingdom between 1981 and 1986 (21). The reasons for the geographic distribution of molecular markers remain unclear. Further study is needed to determine whether the predominance of certain PP and PT may be related to an increased virulence in humans or in the associated animal reservoirs from which humans become infected through food-borne transmission (4, 7). Geographic differences in PT and PP may also reflect differences in food production, antibiotic use, or other environmental factors.

The statistical association between PT 8 and poultry is based on a small number of isolates. It is interesting, however, that PT 8 was also a common PT in human sporadic and outbreak *S. enteritidis* isolates; the association with poultry supports the epidemiologic connection between *S. enteritidis* infections and eggs (5, 6, 17). Further systematic comparisons of human and animal isolates are needed to address this issue. The similarity in these laboratory markers between human sporadic and outbreak isolates should also be interpreted with caution. Although the collection time periods overlapped between the groups, they were not identical. Nevertheless, it is plausible that the similar distribution of markers between sporadic and outbreak isolates indicates that they share a common reservoir. The dominant vehicle for *S. enteritidis* outbreaks in North America may also be an important source for sporadic *S. enteritidis* infections.

TABLE 3. PT of U.S. *S. enteritidis* isolates studied by source of isolate

PT	No. (%) of isolates		
	Sporadic	Outbreak	Animal
8	27 (42)	16 (37)	15 (33)
13a	24 (37)	22 (51)	4 (9)
14b	5 (8)	4 (9)	0 (0)
Nontypeable	6 (9)	1 (2)	7 (15)
9a	2 (3)	0 (0)	0 (0)
9b	0 (0)	0 (0)	4 (9)
9	0 (0)	0 (0)	1 (2)
13	1 (2)	0 (0)	2 (4)
1	0 (0)	0 (0)	1 (2)
2	0 (0)	0 (0)	2 (4)
2a	0 (0)	0 (0)	2 (4)
3v	0 (0)	0 (0)	1 (2)
4	0 (0)	0 (0)	1 (2)
4v	0 (0)	0 (0)	2 (4)
24	0 (0)	0 (0)	2 (4)
28	0 (0)	0 (0)	2 (4)
34	0 (0)	0 (0)	1 (2)

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