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Development and Application of a New Scheme of Phages for Typing and Differentiating *Salmonella* Strains from Different Sources

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A set of 25 phages for typing and differentiating *Salmonella* strains from different origins (food, water, and disease outbreaks) is described. All the strains were typeable by use of the phages, whereas by the serological method more than 5% of the strains could not be classified. By using the phage typing scheme, 75 phage types were established, and all the results were reproducible 1 and 6 months later. Some phages were serotype and serogroup specific, which may be useful in additional tests for the identification of strains of some *Salmonella* serotypes. In addition, the strains responsible for an outbreak possessed the same phage type, which implies the potential epidemiological use of these phages.

Salmonellosis is one of the main communicable foodborne diseases that affect humans today (28). *Salmonella* infections occur naturally not only in humans but also in many other warm-blooded animals and in cold-blooded vertebrates (5). Because of the ubiquitous nature of these microorganisms in several habitats, the use of a powerful epidemiological tool is necessary to discriminate among the strains involved in an outbreak. Epidemiological investigations have traditionally relied on biochemical and serological methods for the primary identification of strains. In many cases, the species identification and antimicrobial susceptibility pattern are sufficient to confirm the epidemiological relationship between different isolates; however, there is an increasing need for more detailed discrimination or typing of *Salmonella* strains.

The ability of bacteriophages to distinguish varieties among isolates with apparently identical serotypes has led to the development of schemes that use phages for typing (phage typing schemes) as an appropriate epidemiological procedure (7, 18, 23). Many phage typing systems have been proposed and widely applied to the identification of the following *Salmonella* serotypes: *S. adelaide*, *S. anatum*, *S. bareilly*, *S. blockley*, *S. bovis-morbificans*, *S. braenderup*, *S. dublin*, *S. enteritidis*, *S. gallinarum-pullorum*, *S. good*, *S. hadar*, *S. heidelberg*, *S. minnesota*, *S. newport*, *S. oranienburg*, *S. panama*, *S. paratyphi* type A, *S. potsdam*, *S. schottmuelleri*, *S. senftenberg*, *S. thompson*, *S. typhi*, *S. typhimurium*, *S. virchow*, and *S. weltevreden*. However, the wide variety of phages involved in each phage typing set and its complex methology make the system based on individual sets inappropriate for use in a routine microbiology laboratory (22).

In recent years, several efforts have been made to obtain a reduced set of phages for typing all salmonellae (15, 16, 22); this would reduce the costs and time needed to determine strain types.

One of the most important properties of the phages is their host specificity. Phages may be extremely specific for a single species (29) or even for a certain subdivision of that species (13). On the basis of this fact, Le Minor and Chalon (21) used the *S. typhimurium*-specific bacteriophage ES18 as an additional test for the identification of *Salmonella* strains that are not serologically related. More recently, Gershman and Markowsky (16) described a set composed of 27 bacteriophages for the differentiation of several *Salmonella* serogroups.

This study was conducted to develop a new system of phages for typing and differentiating *Salmonella* serovars involved in disease outbreaks in comparison with those isolated from water and food and to evaluate the potential application of the system as a tool in epidemiological and surveillance studies.

MATERIALS AND METHODS

Bacterial cultures. The 857 isolates of *Salmonella* spp. used in this study were obtained from naturally polluted waters (n = 292), contaminated food (n = 115), and humans involved in disease outbreaks (n = 450) that occurred in different areas of Spain (Madrid, San Sebastian, and Malaga). All isolates with biochemical profiles that indicated that they belong to the genus *Salmonella* (20) were confirmed serologically by using type O and H antisera supplied by Difco Laboratories (Detroit, Mich.), and then all isolates were sent to the Spanish National Salmonella Reference Center (Majadahonda, Madrid) so that their identities could be confirmed.

Phage isolation and propagation. The source of bacteriophages was raw sewage from both an effluent which is discharged directly into Huelin Beach (Malaga, Spain) and a wastewater pipe on the Guadalhorce River.

Phages were isolated by enriching individual, untreated sewage samples (10 ml) with 10 ml of one of the serovars that was to be used as a specific bacterial host (about 10^8 organisms per ml) and that had been incubated for 3 h in tryptic soy broth (Difco). The culture was shaken vigorously to allow as much contact between phage and host as possible. After incubation for 24 h at 36 ± 1°C, 5 ml of the incubated broth was centrifuged at 8,000 × g for 20 min, and

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TABLE 1. *Salmonella* strains used for propagation of phages

Strain no.	Strain used for propagation	Reference no.	Sero-group	Source of isolate
1	*S. typhimurium*	716	B	Seawater
2	*S. typhimurium*	22	B	Disease outbreak
3	*S. braenderup*	3	C_1	Food
4	*S. infantis*	725	C_1	Seawater
5	*S. infantis*	8	C_1	Food
6	*S. muenchen*	1386	C_2	Seawater
7	*S. montevideo*	88	C_1	Food
8	*S. ohio*	1079	C_1	Seawater
9	*S. ohio*	33	C_1	Food
10	*S. oranienburg*	7000	C_1	Seawater
11	*S. paratyphi* type C	1117	C_1	Seawater
12	*S. potsdam*	1143	C_1	Seawater
13	*S. potsdam*	13	C_1	Food
14	*S. thompson*	698	C_1	Seawater
15	*S. virchow*	36	C_1	Food
16	*S. blockley*	706	C_2	Seawater
17	*S. blockley*	91	C_2	Food
18	*S. bovis-morbificans*	1099	C_2	Seawater
19	*S. bovis-morbificans*	140	C_2	Food
20	*S. gold-coast*	1	C_2	Seawater
21	*S. enteritidis*	714	D_1	Disease outbreak
22	*S. enteritidis*	7	D_1	Food
23	*S. london*	1401	E_1	Seawater
24	*S. london*	4	E_1	Food
25	*S. weltevreden*	661	E_1	Freshwater
26	*S. senftenberg*	1115	E_4	Seawater
27	*S. senftenberg*	102	E_4	Food
28	*S. taksony*	696	E_4	Seawater

the supernatant was assayed for the presence of specific phages by the drop technique (2) by using the same serovar as the serovar of the host strain.

When phage activity was demonstrated, the isolation and quantification of the phages in the supernatant were carried out by the double-agar-layer technique described by Bell (4). Purification and propagation of the phages were conducted by serial passage of a well-isolated single plaque three or five times by the method described by Eisenstark (9).

The host strains used for the isolation of phages as well as the serotypes and sources of the host strains are listed in Table 1.

Assay of the phage lysates and storage. The procedure for testing the phage lysates involved the determination of the routine test dilution (RTD) and the lytic pattern of the phages on all the host strains used in this study. RTD was calculated by 10-fold serial dilution of the lysate in 0.1% peptone water and assay of the effect of each dilution of phage on the strain used for phage propagation. The highest dilution of phage that produced complete or confluent lysis on its host strain was considered the RTD.

The lytic pattern of each phage on the strains used for phage propagation was determined by following the methodology of Krzywy et al. (19). The lytic actions of the phages on the host strains were detected by the presence of lysis zones on bacterial lawns after 12 to 14 h of incubation.

Both the RTD and the phage lysates were sealed and stored in triplicate at 4°C (24) and were tested at least once a month. In the case of bacteriophages with proven resistance to chloroform, another storage system was used for this study. This system consisted of incubation of the phage-bacteria mixture for 24 h at 36 ± 1°C. Then, 2-ml portions of the culture were transferred to a sterile tube with a 1/10 dilution (vol/vol) of chloroform. The mixture was shaken vigorously for a few minutes and was stored at 4°C.

TABLE 2. Mnemonic for reporting bacteriophage types[a]

Triplet (no. representation)	Doublet (letter representation)
+++ (1)	++ (A)
++− (2)	+− (B)
+−+ (3)	−+ (C)
−++ (4)	−− (D)
+−− (5)	
−+− (6)	
−−+ (7)	
−−− (0)	

[a] For example, for results of tests with typing phages 9, 10, 11, 14, 22, 23, and 25, the mnemonic classification is 0072-600-AC, which corresponds to the following reactions:

−−−/−−−/−−+/++−/−+−/−−−/−−−/++/−+
0 0 7 2 6 0 0 A C

Characterization tests. Heat inactivation studies on the phages were performed by exposing 1-ml portions of the phage lysate to 60, 70, and 80°C for 30 min in a shaking water bath and by comparing the number of plaques formed after treatment with the number of plaques formed in an untreated phage suspension.

For electron microscopy studies, phages were prepared by the techniques specified by Dawes (8) and Borrego (6). Specimens were deposited on Formvar-coated grids and stained with 2% potassium phosphotungstate (pH 7.2; Sigma Chemical Co., St. Louis, Mo.) or 1% uranyl acetate (pH 7.0; Sigma). The grids were examined at 60 kV with a Phillips EM-201 electron microscope.

Typing technique. The *Salmonella* cultures to be typed were grown in 5 ml of tryptic soy broth until turbidity was barely perceptible (4 to 6 h). A small quantity of each culture was then plated individually onto nutrient agar (Difco) and was allowed to dry at room temperature. After 5 min, one drop of each phage lysate was spotted onto the *Salmonella*-inoculated plate by using a 1-ml syringe with a 27-gauge needle. The plates were then incubated at 36 ± 1°C overnight, and the results were read on the following day. Phage patterns were determined by viewing them through the bottom of the plate, sometimes with the aid of a magnifying lens. Susceptibility to phages was demonstrated by areas of clearing that varied from isolated plaques to confluent lysis. Phage activity was recorded on the basis of the reactions described by Anderson (3).

A modification of the mnemonic pattern described by Farmer (10) for reporting bacteriophage types was used to simplify the results (Table 2). Briefly, this system consists of eight triplets and two doublets of reactions on the basis of lysis or no lysis of the strains to the phages used for typing.

RESULTS

Isolation and characterization of the bacteriophages used for typing. A total of 461 phages were isolated, primarily from sewage. Because of their sources, all the phages were considered to be wild-type phages (18). The phages were characterized by the following criteria: sensitivity to chloroform, size and morphology of the plaque lytic zone, inactivation by heat, and host range.

The results obtained in the tests described here show that the phages can be placed into the following three categories: serotype-specific phages (monovalent), serogroup-specific

TABLE 3. Characteristics of the phage typing scheme

Scheme no.	Phage no.	Strain used for propagation	Lysed plaque characteristics[a]				Chloroform sensitivity[b]	PFU/ml (36°C)	RTD
			Diam (mm)	Morphology	Halo	Margins			
1	527	*S. infantis* 725	0.5	C	N	M	R	6.7×10^9	10^{-4}
2	48	*S. infantis* 725	1.5	T	N	M	R	6.4×10^{10}	10^{-4}
3	505	*S. potsdam* 1143	0.5	O	N	D	S	9.0×10^8	10^{-3}
4	363	*S. potsdam* 13	0.7	O	N	M	R	2.9×10^7	10^{-2}
5	367	*S. potsdam* 13	0.5	C	N	M	S	4.8×10^7	10^{-2}
6	388	*S. oranienburg* 7000	0.5	C	N	I	R	1.4×10^8	10^{-3}
7	425	*S. ohio* 1079	2.7	C	Y	M	R	9.1×10^8	10^{-3}
8	345	*S. ohio* 33	1.7	C	N	M	R	3.2×10^9	10^{-4}
9	322	*S. infantis* 8	≤0.5	C	N	M	R	3.6×10^7	10^{-2}
10	124	*S. enteritidis* 714	≤0.5	O	N	M	R	1.1×10^8	10^{-3}
11	279	*S. london* 4	0.5	C	N	M	R	6.3×10^8	10^{-3}
12	331	*S. taksony* 696	1.0	O	N	D	R	1.5×10^{10}	10^{-4}
13	417	*S. blockley* 91	≤0.5	C	N	M	R	1.3×10^{10}	10^{-3}
14	521	*S. muenchen* 1386	0.5	C	N	M	R	1.0×10^8	10^{-3}
15	208	*S. gold-coast* 1	1.0	C	N	M	R	1.5×10^9	10^{-3}
16	396	*S. gold-coast* 1	≤0.5	O	N	M	R	1.4×10^9	10^{-3}
17	28	*S. typhimurium* 716	0.7	C	N	M	R	1.4×10^{10}	10^{-4}
18	108	*S. typhimurium* 716	0.5	C	N	M	R	1.0×10^9	10^{-3}
19	214	*S. enteritidis* 7	0.5	C	N	M	R	1.0×10^8	10^{-3}
20	259	*S. enteritidis* 7	≤0.5	O	N	M	S	6.5×10^6	10^{-2}
21	268	*S. enteritidis* 7	1.0	C	N	M	R	2.7×10^9	10^{-3}
22	4	*S. senftenberg* 1115	0.5	C	N	M	S	8.4×10^8	10^{-3}
23	449	*S. senftenberg* 1115	≤0.5	C	N	M	S	2.4×10^8	10^{-3}
24	216	*S. london* 4	1.0	C	N	M	R	4.1×10^8	10^{-3}
25	315	*S. london* 1401	≤0.5	C	N	M	R	2.7×10^8	10^{-3}

[a] Morphology, C, clear; T, turbid; O, opaque. Halo, Y, yes; N, no. Margins, I, no margin (regular); D, difuse margin; M, patent margin.
[b] R, resistant; S, sensitive.

phages, and phages that lyse different strains of *Salmonella* with distinct serological properties (polyvalent). In total, 210 lytic patterns were obtained from the 461 phages tested, and 150 were serogroup specific (only 21.3% of which presented lytic activity exclusively on the strain used for propagation); the rest of the phages were polyvalent to different degrees (9.1% of the phages were polyvalent on all the strains used for propagation).

Development of the phage typing system. On the basis of the bacteriophage specificity at the serotype or serogroup level and the degree of polyvalence, 46 bacteriophages were selected. Of these, 28 were serogroup specific, 11 were active against strains belonging to two serogroups, and the rest (7 phages) presented different profiles of polyvalence that included a wide lytic range. These phages were tested in relation to the host range on 50 strains belonging to the same serotypes as those of the *Salmonella* serovars used for propagation of the phages. The results allowed us to choose 25 bacteriophages (Table 3 lists the characteristics of these phages) which constitute the scheme of phages for typing developed in the present study.

The phages used for typing belonged to seven of the morphological groups of Ackermann (1) (Table 4); eight belonged to serogroup A_2, seven belonged to serogroup B_1, four belonged to serogroup A_1, three belonged to serogroup B_2, two belonged to serogroup C_1, and one belonged to serogroup E. The A_1 and A_2 serogroups were characterized by their contractile tails with isometric (serogroup A_1) or elongated (serogroup A_2) heads, and they generally presented base plates and fibers. Phages 259, 28, 527, 363, 345, 108, 367, 396, 388, 208, 417, and 4 are included in groups A_1 and A_2. Serogroup B_1 comprised two varieties of phages with isometric heads and long, noncontractile tails. The first

TABLE 4. Morphology of the bacteriophages that made up the typing scheme

Scheme no.	Phage	Morphology				Ackermann group
		Head	Tail[a]	Base plate	Fibers	
1	527	Isometric	C	+	−	A_1
2	48	Isometric	NC	−	−	B_1
3	505	Isometric	NC	−	−	B_1
4	363	Elongated	C	+	+	A_2
5	367	Elongated	C	+	+	A_2
6	388	Elongated	C	+	+	A_2
7	425	Isometric	NC	+	−	B_2
8	345	Elongated	C	+	+	A_2
9	322	Isometric		−	−	E
10	124	Isometric	NC	−	−	B_1
11	279	Isometric	NC	+	−	B_2
12	331	Isometric	S	−	−	C_1
13	417	Elongated	C	+	+	A_2
14	521	Isometric	NC	−	−	B_1
15	208	Elongated	C	+	+	A_2
16	396	Isometric	C	+	−	A_1
17	28	Elongated	C	+	+	A_2
18	108	Elongated	C	+	+	A_2
19	214	Isometric	NC	−	−	B_1
20	259	Isometric	C	−	−	A_1
21	268	Isometric	S	−	−	C_1
22	4	Isometric	C	+	+	A_1
23	449	Isometric	NC	−	−	B_1
24	216	Isometric	NC	−	−	B_1
25	315	Isometric	NC	−	−	B_1

[a] C, contractile; NC, noncontractile; S, short.

TABLE 5. Phage types obtained from the *Salmonella* isolates tested

Phage type	Code	% Occurrence in:		
		Disease outbreak (n = 450)	Food (n = 115)	Water (n = 292)
1	2175-000-DD	—[a]	—	1.37
2	2170-000-DD	—	—	4.11
3	2065-066-DC	—	6.08	—
4	3445-000-DD	—	—	1.37
5	4145-000-DD	—	—	1.37
6	5015-066-DC	—	4.35	—
7	5045-600-DD	1.55	2.61	—
8	5045-000-DD	1.55	4.35	—
9	5010-055-DC	1.55	4.35	—
10	5643-640-AC	1.55	5.22	—
11	5040-000-DD	1.55	—	—
12	5005-044-BC	1.55	—	—
13	5005-046-BC	1.55	—	—
14	5070-071-DC	0.44	—	—
15	6110-000-DD	—	—	1.37
16	6140-000-DD	1.55	—	—
17	6522-000-DD	—	4.35	—
18	6700-000-DD	1.55	—	1.37
19	7425-000-DD	1.55	—	—
20	7715-000-DD	2.66	—	—
21	7745-000-DD	—	—	0.68
22	7765-000-DD	—	—	4.11
23	7052-000-DD	—	—	1.37
24	7005-044-AC	1.55	—	—
25	0410-000-DD	4.00	—	—
26	0674-606-DD	—	3.48	—
27	0676-607-DD	21.55	—	—
28	0032-046-BC	9.78	—	—
29	0032-046-DC	1.55	4.35	—
30	0035-046-BC	1.55	—	—
31	0041-600-DD	—	7.83	—
32	0045-606-DD	—	0.87	—
33	0045-046-DC	1.55	—	—
34	0046-041-AC	1.55	—	1.37
35	0056-600-DD	—	—	1.37
36	0065-041-AC	—	6.96	—
37	0072-350-DD	—	—	2.74
38	0072-600-AC	—	—	2.05
39	0072-600-CD	—	—	2.74
40	0072-041-BB	—	—	1.37
41	0072-041-CA	—	—	1.37
42	0072-000-CD	—	—	1.37
43	0074-600-DD	—	1.74	—
44	0075-041-AC	4.66	—	5.48
45	0075-044-AC	—	—	2.74
46	0075-046-BC	1.55	—	1.37
47	0075-071-AC	—	—	1.37
48	0076-700-DD	—	—	1.37
49	0076-000-AD	—	—	2.40
50	0070-003-DD	0.44	—	—
51	0002-041-AC	—	4.35	—
52	0002-046-DC	—	—	1.37
53	0002-000-AA	—	—	1.37
54	0002-000-AC	—	—	4.45
55	0005-300-DD	—	—	2.74
56	0005-641-BC	—	5.22	—
57	0005-041-AA	1.55	3.48	6.85
58	0005-041-AC	19.78	13.04	10.96
59	0005-041-BC	1.11	—	—
60	0005-044-AC	1.55	—	—
61	0005-045-DC	—	—	1.37
62	0005-046-DC	3.11	4.35	1.37
63	0005-061-AC	1.55	—	—
64	0005-066-DC	—	4.35	—
65	0005-074-BA	—	4.35	—
66	0006-350-DD	—	—	1.37
67	0006-650-DD	—	—	2.40
68	0006-006-AC	1.33	2.61	—
69	0006-000-AA	—	—	2.74
70	0006-000-AC	—	—	5.82
71	0000-061-AC	1.55	—	—
72	0000-066-AC	—	—	1.37
73	0000-070-AC	—	—	1.37
74	0000-000-AC	—	—	4.11
75	0000-000-AD	—	—	1.37

[a] —, no strains with the phage type.

variety included phages 124, 214, 315, 505, and 521, which had rigid tails and no distinctive features. The second variety was represented by phages 48 and 216, which had very flexible tails and tiny, terminal fibers. Phages 268 and 331 had isometric heads and short tails and, thus, belonged to serogroup C_1. Phage 322 was an isometric phage without a tail and resembled coliphage MS-2.

Phages used for typing. To evaluate the useful discrimination of the proposed phage typing scheme, 857 strains of different serogroups of *Salmonella* were tested (*S. typhimurium*, 195 strains; *S. braenderup*, 28 strains; *S. menden*, 5 strains; *S. montevideo*, 4 strains; *S. ohio*, 64 strains; *S. oranienburg*, 9 strains; *S. paratyphi* type C, 1 strain; *S. potsdam*, 8 strains; *S. richmond*, 5 strains; *S. thompson*, 45 strains; *S. virchow*, 13 strains; *S. blockley*, 27 strains; *S. bovismorbificans*, 6 strains; *S. muenchen*, 14 strains; *S. gold-coast*, 1 strain; *S. enteritidis*, 269 strains; *S. london*, 70 strains; *S. weltevreden*, 18 strains; *S. senftenberg*, 10 strains; *S. taksony*, 1 strain; self-agglutinable, 44 strains; phase II, 8 strains; and monophasic, 2 strains). All strains were typeable and were divided into 75 phage types (Table 5). The strains belonging to serogroup C_1 presented a lytic pattern different from those of strains of the other serogroups tested. Serogroup C_1 strains showed sensitivity to only 12 phages of the typing system (phages 1 to 12 of the scheme); similarly, serogroup C_2 strains were always resistant to phages 1 to 8, 12, and 18 and phages 20 to 25. Bacteriophage 24 of the system differentiated some self-agglutinable strains and also differentiated between *S. typhimurium* and *S. enteritidis* strains.

The phage types obtained in this study were reproducible when they were typed after 1 and 6 months by using a proportional number of the strains tested that corresponded to the square root of the number of strains of each serovar. The most common phage types were 58 (0005-041-AC) and 27 (0676-607-DD) (15.87 and 11.32% of all isolates, respectively). However, only phage types 29, 57, and 58 from the strains isolated from the three sources were recorded.

The phage typing scheme proposed here was used as an epidemiological tool to establish the relationship among the strains isolated from the same outbreak (Table 6). By use of this typing scheme, we were able to separate different strains involved in a number of disease outbreaks. These strains were indistinguishable and not typeable by the serological characterization method. The food poisoning outbreaks occurred in a 3-year period (1989 to 1991). All specimens were isolated from food suspected of being contaminated and

TABLE 6. Epidemiologically linked isolates in this study

Serotype	Outbreak no.	No. of isolates typed	No. of phage types detected	Predominant phage type[a]
S. enteritidis	1	78	2	27 (98.7)
S. virchow	2	6	1	27 (100)
S. enteritidis	3	114	6	58 (91.2)
S. typhimurium	4	30	4	62 (90.0)
S. infantis	5	7	1	11 (100)
S. virchow	6	3	1	33 (100)
S. typhimurium	7	26	3	28 (84.6)
S. typhimurium	8	36	3	28 (94.4)
S. enteritidis	9	4	1	59 (100)
S. typhi	10	4	2	14 and 50 (50.0)
S. enteritidis	11	80	11	25 (75.0)
S. enteritidis	12	62	6	24 (83.9)

[a] Values in parentheses are frequency of detection (expressed in percent) of the predominant phage type.

from the stools of individuals who were affected. Some of the people examined required the care of a physician, although only those people affected by outbreaks 3 and 11 required hospitalization. All people examined in this study showed typical symptoms of salmonellosis, including fever, diarrhea, nausea and/or vomiting, abdominal pain, and general fatigue. Incubation periods ranged from 10 to 48 h, with an average of 20 h. The epidemiological and bacteriological findings indicated that the following food vehicles were presumably involved: water (outbreak 10), minced meat (outbreaks 4 and 8), sausage omelet (outbreak 7), salads with mayonnaise (outbreaks 1 and 12), contaminated sausage with red peppers (outbreak 2), precooked food (outbreak 9), and hamburger steaks (outbreaks 5 and 6). The sources of poisoning implicated in outbreaks 3 and 11 are unknown, because the samples were collected from the stools of hospitalized patients. On the basis of the results obtained in the study of the sources of infection of some outbreaks, it can be deduced that the presence of *Salmonella* spp. in food was caused by inadequate storage of the samples or contamination in the handling process.

DISCUSSION

Phage typing is a means of differentiating bacteria on the basis of the susceptibility of the bacterial host to specific bacteriophages (23). Its application depends on the most important property of the phages, that is, their host specificity. A typing scheme must facilitate the differentiation of the maximum number of bacteria into types or groups that are easily recognizable, with epidemiological significance attained by using the minimum number of variables (in this case, bacteriophages). Epidemiological investigations of any bacterial infection must differentiate among strains of the same species of organisms. Serotypes are routinely characterized to identify the vehicle and source of infection. More than 2,000 *Salmonella* serotypes have been recognized (20); some of them, however, are quite common and are not the most appropriate tool for use in epidemiological studies.

In the present report, *Salmonella* strains were grouped into 13 serotypes, although 5.13% of the strains tested could not be classified serologically (self-agglutinable strains). By using the phage typing scheme developed in this study, all the strains were included in 75 phage types.

Among strains of the same serotype, the frequency of detection of each phage type was low, according to the results obtained by others investigators (7, 14, 16, 25). The strains of different serotypes presented distinct susceptibilities to the phages used for typing, and only two strains of different serogroups possessed the same phage type. The strains that are associated with an outbreak of disease share the same phage type, which may have epidemiological significance if one considers the various sources.

The phage typing scheme developed in this study may be used as an epidemiological tool to group serologically related *Salmonella* isolates. Similar results have been reported by Sood and Basu (29), who explained the phage sensitivity on the basis of the antigenic structure of the bacteria, the receptor sites on the bacterial surface, and other factors in the cell. Other investigators have used certain bacteriophages to carry out the differential diagnosis of bacterial strains, such as for *Salmonella* spp. (11, 21), *Hafnia* spp. (17), *Aeromonas salmonicida* (27), *Pasteurella multocida* (12), and group D streptococci (26).

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