

# Single Phage-Typing Set for Differentiating *Salmonellae*

M. GERSHMAN

Departments of Microbiology and Animal and Veterinary Sciences, University of Maine, Orono, Maine 04473

Received for publication 9 September 1976

A phage-typing system is described for characterizing commonly isolated salmonellae. Fifty-eight serovars representative of groups A, B, C<sub>1</sub>, C<sub>2</sub>, D, E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, and E<sub>4</sub> were delineated by using a single set of 50 phages isolated from sewage. All of the 735 cultures used in this effort were typable and were distinguished and differentiated on the basis of the 347 phage patterns observed. All results were reproducible. Characteristic phage patterns were produced by a variety of *Salmonella* serovars isolated from a campus incident and a number of hospital and family outbreaks to indicate an existing epidemiological relationship.

*Salmonellae* were first described in the last half of the 19th century and have since been well recognized for their pathogenic properties and ubiquitous distribution. From 2,000,000 to 4,000,000 people are affected annually in the United States alone (11). Fortunately, salmonellosis can be prevented if the presence of the organism is established and control measures are implemented accordingly. Characterization of serovars is routinely used for identifying the vehicle and source of an infection. Some serovars, however, are quite common and cannot be adequately characterized for epidemiological application by serology alone. Under these circumstances, phage typing is an invaluable adjunct.

When phages were first discovered, they were regarded initially as a potential well spring of clinical miracles. Unfortunately success in this area was minimal (12) and, for the most part, disappointing. Phage therapy, at this time, is not generally regarded as promising, but the contributions of phage typing to epidemiology have been well documented. Aside from relating an isolate to an outbreak, phage typing has also been used for surveillance, assessing strain distribution, and ascertaining the effectiveness of therapeutic measures.

Sonnenschein, in the 1920s, isolated specific phages for *Salmonella paratyphi B* and *S. typhi*, and recommended that they be used to rapidly identify these pathogens (13, 14). In 1938 Craigie and Yen introduced a phage-typing system for differentiating strains of *S. typhi*. Its success led to the development of a number of schemes for other *Salmonella* serovars. Some are now in routine use in a number of public health laboratories throughout the world (2).

In keeping with our own immediate interests, we have developed a number of phage sets for some of the *Salmonellae* encountered in a diagnostic laboratory (5-7, 9, 10).

In the course of our research it was observed that our phages could be used to differentiate similar and unrelated serovars (8). As a consequence, the phages that we specifically isolated for *S. heidelberg* (group B), *S. thompson* (group C<sub>1</sub>), *S. newport* (group C<sub>2</sub>), *S. enteritidis* (group D), *S. anatum* (group E<sub>1</sub>), *S. binza* (group E<sub>2</sub>), and *S. senftenberg* (group E<sub>4</sub>) were all incorporated into a single set and used to characterize numerous *Salmonella* serovars indiscriminately.

## MATERIALS AND METHODS

**Media.** Nutrient agar and nutrient broth were used exclusively for testing phage filtrates and phage typing. Before use, agar plates were dried in an incubator for 2 h with lids partially opened. Nutrient agar, nutrient broth, and nutrient broth with 0.5% NaCl and 0.7% agar were used for phage propagation.

**Bacterial cultures.** The host cultures used for phage recovery were isolated from cases of human gastroenteritis and diseased animals of avian and bovine origin. They were obtained from the National Animal Disease Laboratory, Ames, Iowa, the Pasteur Institute, Paris, France, and the University of Maine at Orono.

**Phage isolation and propagation.** Phages were isolated by enriching individual, untreated sewage samples (100 ml) with 6 ml of a 1.5-h-incubated broth culture of one of the serovars under investigation. After an 18-h incubation, the broths were passed through a 0.45- $\mu$ m membrane filter (Millipore Corp.), and the product was assayed for phage by applying the filtrate to the culture used in the enrichment process.

The culture, used to detect the presence of phage,

was prepared by inoculating 6 ml of broth that was subsequently incubated for 1.5 h or until growth was barely evident. At this stage, the microbial population was about  $9 \times 10^7$  organisms per ml. Two milliliters of this broth was then applied evenly over the surface of an agar plate, allowed to dry for 15 min, and spotted with a drop (0.04 ml) of the above filtrate, using a Pasteur pipette. After the drop had been thoroughly absorbed (approximately 15 to 20 min), the plate was inverted, incubated overnight, and examined the following morning. If isolated plaques appeared, they were purified three times by serial, single-plaque passage. In cases where phage activity was too extensive to permit single-plaque isolations, the assaying procedure was repeated by using a series of diluted filtrates. Phages were then propagated on the basis of a method described by Swanstrom and Adams (15). In essence, this procedure involves the lysis of a culture by a homologous phage suspended in a soft, thin agar matrix resting on a thicker base of nutrient agar. Sixty milliliters of melted agar was poured into a 15-cm petri plate and allowed to harden on a leveled support. Nutrient broth with 0.5% NaCl and 0.7% agar was prepared in 15-ml quantities, cooled to 45°C, and inoculated with a mixture consisting of the growth of an overnight agar slope suspended in 1 ml of broth and 2 ml of phage to be propagated. The density of the broth culture was adjusted to equal the concentration used for phage isolations. This combination was gently agitated and poured over the surface of the base layer, allowed to harden, and incubated overnight. The next day, 10 ml of broth was added to the plate and the soft-agar layer was removed with the aid of a sterile tongue depressor, transferred to tubes, shaken vigorously to break up the agar-phage complex, and centrifuged at  $60 \times g$  for 20 min. The supernatant was then decanted, filtered through a 0.45- $\mu$ m membrane filter, and assayed for phage content.

**Testing of phage filtrates.** The testing procedure involved a preliminary titration to determine the routine test dilution (RTD) and a lytic pattern to ascertain the novelty and usefulness of a phage isolate.

Phages were tested for their ability to lyse and to differentiate strains of *Salmonella* at an RTD of not less than  $10^{-3}$ . A phage was discarded if it did not meet this basic requirement. The RTD as defined by Anderson is the highest dilution of phage that produces complete or confluent lysis on its propagating strain (1). Its use minimizes the occurrence of confusing cross-reactions. The RTDs of the typing phages used in this study are listed in Table 1. They were established by titrating phage serially in 10-fold dilutions.

The lytic pattern was ascertained by testing a phage against its own propagating strain and a set of standard test cultures. In addition to the reasons stated, the lytic pattern was also carried out to detect mutations and other aberrant reactions. The lytic patterns of the typing phages are noted in Table 2.

If, on the basis of tests performed on a collection of assorted cultures, a phage was found to be stable

TABLE 1. RTD of typing phages

Phage	RTD
4, 5, 8, 9, 11, 17, 18, 21, 22, 26, 27, 30-32, 34, 36-38, 40, 46, 49, 50	$10^{-3}$
1-3, 6, 10, 13, 15, 19, 20, 23-25, 28, 33, 35, 39, 47, 48	$10^{-4}$
7, 12, 14, 16, 29	$10^{-5}$

and potentially suitable for strain differentiation, it was henceforth used routinely as part of an ongoing evaluation process.

When RTD phage stocks were renewed, the lytic spectra of new and preceding batches were compared to insure that intrinsic properties were being maintained. The phage pattern of each new subculture was also checked for similar reasons.

**Storage.** RTDs were stored at 4°C and tested for potency at least once a week. A test dilution was considered satisfactory for typing as long as it produced confluent lysis on its propagating strain. In general, the test dilution of the majority of phages retained their effectiveness for 4 to 6 weeks and, occasionally, longer. In any event, the stability of a test dilution was not predictable, and frequent, periodic checks were required.

**Typing technique.** Isolates to be phage typed were prepared in a manner analogous to that used in processing cultures to detect phages. To standardize results, however, phages were applied by using a 1-ml syringe with a 26-gauge needle. After overnight incubation, phage patterns were determined by viewing the results through the bottom of the plate with the aid of a  $\times 10$  aplanat hand lens. To facilitate this operation, the plate bottom was marked with a number of squares equal to the number of phage preparations involved. Readings were made by using a Quebec colony counter (Fisher Scientific Co.). Phage activity was recorded on the basis of the reactions described in the legend of Table 2.

## RESULTS AND DISCUSSION

The results of phage typing were manifested by various patterns that reflected the degree of susceptibility of a strain to the collection of phages used. Reactions were reported in terms of those phages that produced strong lysis, i.e., reactions of 120 or more plaques. The "phage pattern" of a strain, sometimes referred to as the "type," is reported in a form such as 17/22/31/44, or 1/17/19/23/36/48, or 10. For convenience and clarity, a "type" is given in terms of a pattern of strong lysis; however, the laboratory can, on occasion, elucidate the epidemiological relationship further by considering weak reactions that may also occur.

Using a single set of 50 phages, we were able to type a variety of *Salmonella* serovars of both human and animal origin. The 735 isolates used in this study were randomly selected from a collection of cultures obtained from a number of state, national, and international sources,



TABLE 2 - Continued

Bacteriophage isolated from:

Type strain	S. anatum												S. binza												S. senftenberg											
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50								
<i>Salmonella heidelberg</i>	CL	-	-	<SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	<SCL	-	-	<SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	-	<SCL	<SCL	OL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	OL	CL	<SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
<i>S. thompson</i>	CL	OL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	-	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
<i>S. newport</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
<i>S. enteritidis</i>	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
<i>S. anatum</i>	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
<i>S. binza</i>	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								
	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-								

TABLE 2—Continued

Type strain	Bacteriophage isolated from:																											
	S. enteritidis										S. anatum					S. binza					S. senftenberg							
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
43	-	CL	CL	-	-	-	-	-	-	-	-	-	CL	OL	-	+++	-	-	-	-	-	-	CL	-	-	-	-	50
44	CL	-	-	<SCL	-	-	-	OL	-	-	-	-	-	-	-	-	-	-	-	-	<SCL	SCL	-	CL	-	-	-	<SCL
45	-	-	-	-	-	-	+++	CL	-	-	CL	-	-	-	CL	-	CL	CL	-	-	-	-	-	CL	OL	-	-	-
46	CL	-	-	-	-	-	-	CL	-	-	-	-	-	CL	-	±	-	-	-	-	-	-	-	-	CL	CL	±	-
47	OL	-	-	-	-	-	-	<SCL	-	-	-	-	-	OL	-	-	-	-	-	-	-	CL	-	-	-	<SCL	CL	-

<sup>a</sup> Abbreviations: CL, confluent lysis; OL, opaque lysis (opacity due to secondary growth); SCL, semiconfluent lysis; <SCL, less than semiconfluent lysis; + + +, 120 plaques; + ±, 81 to 120 plaques; + +, 61 to 80 plaques; + ±, 41 to 60 plaques; +, 21 to 40 plaques; ±, 6 to 20 plaques; -, 0 to 5 plaques.

TABLE 3. Reactions of representative test strains at routine test dilutions<sup>a</sup>

Salmonella type strain	Culture no.	Pattern
<b>Group A</b>		
<i>S. paratyphi A</i>	1	1/5/6/8/12/17/18/19/22/23/24/25/28/30/31/33/34/35/37/40/41/45/47/50
	2	5/10/17/18/19/23/25/27/28/30/31/33/34/35/40/45/47/50
	3	10/17/19/25/28/35/45/47/49/50
<i>S. paratyphi A</i> var <i>durazzo</i>	1	5/8/10/17/18/25/28/31/34/35/40/45/47/50
	3	5/8/10/17/22/28/31/34/35/40/41/47
<b>Group B</b>		
<i>S. agona</i>	1	7/37
	2	7/19/20/21/36/38
	3	7/18/19/20/21/25/30/32/33/35/36/38
	4	5/7/10/18/19/20/21/24/25/26/28/30/32/33/34/35/36/37/38/39/40/41/45/46/47
	5	1/5/7/19/24/26/27/28/30/31/32/33/34/36/37/39/40/41/46/47/48
	6	7/10/18/19/20/21/25/30/32/33/35/36/38/45
<i>S. bredeney</i>	7-23 <sup>b</sup>	5/7/10/18/20/21/23/26/28/30/31/32/33/34/35/36/37/38/39/40/41/43/46/47
	1	5/19/21/23/28/31/36/38/47/48
<i>S. californica</i>	1	5/7/8/10/18/23/24/25/30/31/33/34/35/40/41/45/47
	2	23
	3	7/18/23/30/33/34/35/40/41
<i>S. chester</i>	1	21/30/33/41/47/48
	2	18/20/21/23/28/30/33/41/47
	3-5 <sup>c</sup>	21/23/28/29/30/36/41/47/48
<i>S. derby</i>	1	31/48
	2	7/27/28/36
	3	36
	4	7/19/36
	5	5/7/19/20/23/24/26/28/30/31/32/33/34/36/37/39/40/41/46/47/48
<i>S. heidelberg</i>	8	4/5/6/10/11/16/17/18/22/27/28/30/31/32/33/34/41/46/47
	9	5/10/11/16/18/27/35/41/46/47
	10	1/5/11/16/17/21/22/30/33/34/35/41/46/47
	11	1/26
	12	1/6/10/11/18/22/23/26/28/30/31/32/33/34/37/39/40/41/46/47
	13	3/5/8/10/11/17/26/30/31/32/33/34/37/40/46/47
	14	1/2/8/17/18/22/23/37/39/40/41/46/47
	15	3/7/8/27/28/31/36/41/42/46/47
	16	1/3/5/8/27/28/30/31/32/33/34/41/46/47
	17	3/5/6/7/8/23/26/27/28/30/31/32/33/34/37/39/40/41/43/46/47
	18	5/6/18/37/41
	19	1/3/7/8/24/25/26/28/30/31/34/37/40/41/45/47
<i>S. saint paul</i>	1	5/7/10/18/24/25/26/28/30/31/32/33/34/35/36/37/39/40/41/45/46/47/50
	2	5/7/10/18/26/28/30/31/32/33/34/35/36/37/39/40/41/45/46/47/48/49
	3	1/5/6/7/10/18/23/24/25/26/28/30/31/32/33/34/35/36/37/39/40/41/43/45/46/47/48/50
<i>S. schwarzengrund</i>	4	5/7/10/18/24/25/26/28/30/31/32/33/34/35/36/39/40/41/45/46/47/48
	5-8 <sup>c</sup>	5/28/31/34/36/37/38/40/41/47
	1	1/5/7/10/18/26/28/29/30/31/32/33/34/35/36/37/38/40/41/47/48
	2	1/5/10/18/19/23/28/29/30/31/32/33/34/35/36/37/38/40/41/47
	3	5/10/18/19/23/28/29/30/31/33/34/36/37/38/40/41/47/48
<i>S. typhimurium</i>	1	1/5/7/23/24/26/27/28/30/31/32/33/34/36/37/39/40/41/43/46/47
	2	5/7/23/24/26/27/28/30/31/32/33/34/39/40/41/46/47
	3	1/5/7/17/18/24/25/26/31/32/33/34/36/39/40/41/47
	4	5/6/7/24/26/28/30/31/32/33/34/37/39/40/41/46/47
	5	7/27/28/31/36/41/48
	6	5/6/7/17/22/23/24/26/28/30/31/32/33/34/37/39/40/41/43/46/47
	7	1/5/6/7/17/23/24/26/27/28/30/31/32/33/34/36/39/40/41/43/46/47/48
	8	1/7/23/27/30/31/32
	9	1/3/5/7/23/24/26/27/28/30/31/32/33/34/36/37/39/40/41/43/46/47/48
	10	5/7/24/26/27/28/30/31/32/33/34/36/37/38/41/42/46/47/48
	11	1/5/7/27/28/31/36/41/42/46/47
	12	5/6/7/10/17/24/26/28/30/31/32/33/34/35/36/38/40/41/42/44/47/48
	13	1/5/7/24/31/36/39/41/47

TABLE 3—Continued

Salmonella type strain	Culture no.	Pattern	
<i>S. typhimurium</i>	14	1/5/6/7/10/19/24/26/27/28/29/30/31/32/33/34/35/37/39/40/41/46/47	
	15	1/5/6/7/23/24/26/27/30/31/32/33/34/36/39/43/46/47	
	16	1/5/7/19/23/24/26/28/30/31/32/33/34/37/39/40/41/46/47	
	17	1/5/7/23/24/26/27/28/30/31/32/33/34/36/39/40/41/46/47	
	18	5/7/24/26/28/30/31/32/33/34/37/39/40/41/43/46/47	
	19	7/27/28/30/31/33/36/41/42/47/48	
	20	1/29/48	
	21	1/31	
	22–25 <sup>c</sup>	1/29/48/50	
	26–29 <sup>c</sup>	1/7/19/23/36/48	
	30	1/5/10/18/19/23/26/28/30/31/32/33/34/36/39/46/47	
	31	1/5/19/23/26/28/30/31/32/33/36/47	
	32	1/7/23/27/42/48	
	33	1/7/23/31/36/48	
	34–37 <sup>c</sup>	1/48	
	<i>S. typhimurium</i> var. <i>co-penhagen</i>	1	1/7/19/23/29/36/48
	Group C <sub>1</sub>		
<i>S. bareilly</i>	1	1/10/18/25/35/36/45/50	
	2	1/10/25/35/36/45/48/49/50	
	3	1/9/10/18/25/35/36/45/50	
	4–8 <sup>c</sup>	1/9/10/16/17/18/25/35/36/38/45/50	
<i>S. braenderup</i>	1	10/36/38	
	2	2/17/22	
	3	1/9	
<i>S. cholerae-suis</i>	1	1/2/4/5/6/8/10/12/13/14/15/17/18/22/24/25/26/28/30/31/32/33/34/35/38/40/41/45/47/50	
<i>S. cholerae-suis</i> var. <i>kunzendorf</i>	1	1/21/36/38	
<i>S. decatur</i>	1	20/36/38/48/49/50	
	2	17/28/36/38	
<i>S. eimsbuettel</i>	1	1/5/8/10/17/24/26/28/31/34/36/37/39/40/41/42/46/47	
	2	9/10/25/35/36/38/45/50	
	3	10/17/21/25/35/36/38/45/50	
	4	4/5/10/18/22/25/26/28/30/32/33/34/35/36/41/45/47/50	
	5	10/25/35/36/45/50	
	6	21/25	
	7	10/18/25/35/36/45/50	
<i>S. infantis</i>	1	1/10/19/21/36/38	
	2	1/10/36/38	
	3	1/8/11/36/38	
	4	1/21/36/38/48	
<i>S. jerusalem</i>	1	5/10/19/31/36/47	
	2	5/10/47	
<i>S. montevideo</i>	1	19/21/36	
	2	1/10/17/18/19/21/22/25/35/36/38/45/48/50	
	3	9/16/21/35/36	
	4	9/36	
	5	10/15/35/36	
<i>S. oranienburg</i>	1	21	
	2	1/9/10/18/25/35/36/38/45/50	
	3	10/22/25/35/36/38/45/50	
	4	10/12/18/19/21/25/35/36/38/45/50	
	5	10/18/21/25/35/36/38/45/50	
<i>S. paratyphi</i> C	1	10/12/13/14	
<i>S. tennessee</i>	1	9/11/14/17/19/20/21/47	
	2	1/9/36	
	3	1/8/10/19/21/38	
<i>S. thompson</i>	9	10/12/13/14/15/17/18/21/22/25/35/36/38/45/50	
	10	12/13/14/17/18/21/25/35/36/38/45/50	
	11	10/12/13/14/15/18/36/38/48/49	

TABLE 3—Continued

Salmonella type strain	Culture no.	Pattern
<i>S. thompson</i>	12	9/10/12/13/14/15/18/25/35/36/38/45/50
	13	12/14/15/16/36
	14-26 <sup>d</sup>	10/12/13/14/15/18/21/25/35/36/38/45/50
	27	10/12/13/14/15/18/20/21/25/35/36/38/45/48/50
	28	10/12/13/14
	29	1/10/12/13/14/15/18/20/21/25/35/36/38/45/48/49/50
	30 <sup>e</sup>	24/25
Group C <sub>2</sub>		
<i>S. blockley</i>	1	1/5/11/17/19/22/28/31/34/36/37/40/41/47
	2	5/11/17/18/19/20/22/28/31/34/35/36/37/40/41/47
	3	5/6/8/10/11/17/18/19/22/25/26/28/31/32/34/35/36/37/40/41/45/47/50
	4	1/11/19/36
<i>S. kottbus</i>	1	1/5/8/11/17/19/20/22/28/31/34/36/37/40/41/47
	2	1/5/8/16/17/22/28/31/34/36/37/40/41/47
	3	5/8/11/17/21/22/28/31/34/36/40/47
<i>S. newport</i>	4-12 <sup>b</sup>	5/11/16/17/19/20/22/28/31/34/36/37/40/41/47
	23	1/5/8/19/20/22/28/31/34/36/37/38/40/41/47/48
	24	1/5/8/11/14/17/18/19/21/22/28/31/36/38/40/47
	25	8/17/22/28/31/34/36/38/40/41/47
	26	1/5/10/18/28/38/40
	27	21/36/38
	28	1/19/20
	29	1/5/22/28/33/34/36/38/40
	30	1/5/17/21/28/31/34/36/38/40
31 <sup>e</sup>	38/40	
Group D		
<i>S. berta</i>	1	3/5/6/7/20/21/23/26/27/28/32/34/36/37/38/39/40/41/46/47/48/49
<i>S. dar-es-salaam</i>	1	20/21/31/36/38/47/48
<i>S. dublin</i>	1	3/5/6/7/10/17/18/19/23/24/25/26/27/28/29/30/31/32/33/34/35/37/39/40/41/45/46/47
<i>S. eastbourne</i>	1	1/10/18/20/21/23/24/25/28/30/31/32/33/34/35/36/45
	2-4 <sup>c</sup>	1/10/18/19/20/23/24/25/26/28/29/30/31/32/33/34/35/36/40/41/45/46/47/50
<i>S. enteritidis</i>	30	1/5/7/10/18/19/20/24/25/26/28/30/31/32/33/34/36/37/38/39/40/41/43/46/47
	31	5/7/19/21/24/26/28/30/31/32/33/34/36/37/38/39/40/41/46/47
	32	5/7/24/26/27/28/31/34/39/40/41/46/47
	33	5/7/20/24/26/28/29/30/31/34/36/37/39/40/41/45/46/47
<i>S. gateshead</i>	1	5/8/10/18/20/21/24/25/26/28/30/31/32/33/34/35/36/37/38/39/40/41/42/44/45/46/47/50
<i>S. miami</i>	1	4/5/6/7/8/10/15/17/18/22/23/24/25/26/28/30/31/32/33/34/35/36/37/38/39/40/41/45/46/47/50
<i>S. panama</i>	1	5/6/7/19/20/21/23/25/26/28/30/31/32/33/34/35/36/37/38/39/40/41/46/47/48
	2	5/6/8/10/18/19/20/21/24/25/26/28/30/31/32/33/34/35/36/37/38/39/40/41/45/46/47/48/50
<i>S. pullorum</i>	1	5/7/19/20/23/26/27/28/30/31/32/33/34/37/39/40/41/46/47
<i>S. typhi</i>	2	3/5/7/19/20/27/28/30/31/32/33/34/37/40/41/47
	1	1/5/6/8/10/17/22/24/25/26/28/30/31/32/33/34/37/39/40/41/45/46/47/50
	2	7/23/24/26/32/33/46
	3	1/5/6/7/8/10/17/22/24/25/26/28/30/31/33/34/35/36/38/39/40/46/47/50
4	1/5/6/7/8/10/17/18/22/24/25/26/28/30/31/34/37/39/40/41/46/47	
Group E <sub>1</sub>		
<i>S. amsterdam</i>	1	23/36/38/44/49
	2	20/21/22/23/36/38/44
	3	17/20/21/22/23/31/36/38/44
	4	17/21/22/23/31/38/44
	5	17/21/22/23/30/31/32/33/36/38/47/49
	6	17/22/23/31/36/38/44/47
	7	17/22/23/30/31/36/38/44/47
	8	20/21/22/23/36/38/44/47
	9	17/23/36/38/44/47



TABLE 3—Continued

Salmonella type strain	Culture no.	Pattern	
<i>S. amsterdam</i>	10	36/38/47	
	11	5/17/23/31/38/44/47	
	12	17/22/23/30/38/44	
	13	17/20/21/22/23/31/36/38/44/47	
	14	5/17/20/21/22/23/31/36/348/44/48/49	
	15	17/20/21/22/23/31/36/38/44/49	
	<i>S. anatum</i>	36	5/7/19/21/23/26/27/28/30/31/32/33/34/36/37/38/39/40/41/43/46/47/49
		37	1/5/17/22/23/26/28/31/32/34/35/36/37/38/39/40/41/43/44/46/47/49
		38	5/10/17/22/23/25/28/31/32/34/35/36/37/38/40/41/44/47/49
		39	23/36/48/49
		40	1/5/10/17/18/22/23/28/30/31/32/34/35/36/37/38/40/41/44/47/49
		41 <sup>e</sup>	26
		42 <sup>e</sup>	23/38/44
		43	1/5/10/16/17/22/23/26/28/30/31/32/33/34/35/36/37/38/39/40/41/43/44/46/47/49
		44	5/16/22/23/28/31/32/34/35/36/37/40/41/44/47/48/49
45		5/10/17/22/28/30/31/32/33/34/36/37/38/40/41/42/44/49	
46		1/5/17/22/23/26/28/30/31/32/33/34/36/37/38/39/40/41/43/44/46/47/49	
47		5/8/10/17/18/22/23/28/31/32/34/35/37/38/40/41/44/47	
48		5/10/17/18/22/23/24/25/28/31/32/33/34/35/36/37/38/40/41/44/45/47/49	
49		5/6/8/10/17/18/22/23/24/26/28/30/31/32/33/34/35/36/37/38/39/40/41/43/44/45/46/47/49	
<i>S. give</i>	50	5/10/17/22/23/24/25/28/30/31/32/33/34/36/37/38/40/41/44/45/47/50	
	51	5/17/22/23/28/31/32/34/35/36/37/38/40/41/44/47/49	
	1	17/21/22/23/28/31/36/38/44	
	2	17/19/20/21/22/23/28/31/36/38/44	
	3	5/17/22/28/31/34/36/37/38/40/41/47	
	4	5/8/17/19/20/21/22/23/26/28/31/32/34/35/36/37/38/40/41/43/46/47/48/49	
	5	17/20/21/23/36/38/44	
	6	17/19/20/21/23/36/38/44/49	
	7	10	
	8	5/17/20/21/22/23/26/28/31/32/34/36/37/38/40/41/43/46/47/48/49	
	9	5/10/17/18/19/20/21/22/23/24/25/28/30/31/32/33/34/35/36/37/38/40/43/44/45/47/48/49/50	
<i>S. lexington</i>	10	17/20/21/36/38	
	11	1/17/23/28/32/36/38/44	
<i>S. london</i>	1	36	
<i>S. meleagridis</i>	2	2/6/17/22/23/31/34/36/38/41/48/49	
<i>S. muenster</i>	1	5/17/20/21/22/26/28/31/32/34/35/36/38/40/41/42/46/47	
	1	5/17/22/23/28/31/34/37/38/40/41/44/47	
<i>S. uganda</i>	1	1/5/17/22/23/28/31/32/34/35/36/37/38/40/41/47	
	2	1/5/17/22/23/28/30/31/32/34/35/36/37/38/40/41/44/47	
	3	5/17/20/21/22/23/28/31/32/33/34/36/37/38/40/41/44/47/49	
	4	1/5/17/22/23/28/30/32/34/35/37/40/41/44/47	
	5	6/17/22	
	6	5/17/22/23/28/30/31/32/34/35/37/38/40/41/44/47	
	7	5/17/22/23/28/30/32/33/34/35/37/38/40/41/44/47	
	8	5/6/10/17/18/22/23/24/25/26/28/30/31/32/33/34/35/36/37/38/39/40/41/43/44/45/46/50	
<i>S. westhampton</i>	1	5/8/16/17/22/23/26/28/31/32/34/35/36/37/38/39/40/41/43/46/47/48/49	
Group E <sub>2</sub>	1	23	
	2	23/47/49	
	<i>S. binza</i>	42	1/5/8/17/22/26/28/31/34/35/36/37/39/40/41/42/43/46/47
		43	1/5/8/17/22/23/26/28/30/31/32/33/34/36/37/38/39/40/41/42/43/44/46/47
		44	1/5/8/23/26/28/31/34/36/37/39/40/41/42/43/46/47
		45	1/5/8/10/17/18/22/24/25/26/28/31/34/35/36/37/39/40/41/43/45/46/47/50
		46	1/5/8/17/22/26/28/31/34/36/37/40/41/42/46/47
47		5/8/17/23/26/28/31/34/37/39/40/41/42/43/46/47	
48		38	
49	28/42		

TABLE 3—Continued

Salmonella type strain	Culture no.	Pattern
<i>S. binza</i>	50	1/5/8/17/22/26/28/31/34/36/37/40/41/42/46/47
	51	1/5/6/8/10/17/22/24/25/26/28/31/34/35/36/37/39/40/41/43/45/46/47/48
	52	1/5/6/8/10/17/22/24/25/26/28/31/34/36/37/39/40/41/43/45/46/47/50
	53	1/5/8/17/22/26/28/31/34/35/36/39/40/41/42/43/46/47
	54	5/8/10/17/22/23/24/25/26/28/31/34/35/36/37/39/40/41/42/43/45/46/47
	55 <sup>c</sup>	5/23/28
	56 <sup>c</sup>	5/17/28/47
	57 <sup>c</sup>	1/5
<i>S. drypool</i>	1	5/20/21/28/36/38
	2	5/23/28/31/36/38/47
	3	20/21/28/31/36/38/47/48
	4	5/28/38
	5	20/21/28/31/36/38
	6	5/20/21/23/28/36/38/48
	7	21/23/28/38/48
	8	23/38/48
	9	21/28/31/36/38/41/47
	10	28/31/36/38
	11	21/23/28/31/36/38
	12	28/38
	13	5/20/21/28/31/36/38/41/47
	14	21/28/32/33/35/36/38
	15	23/28/36/38/47
<i>S. halmstad</i>	1	31
	2	26/31/43/46
<i>S. manila</i>	1	36
<i>S. newington</i>	1	4/20/21/23/28/31/34/36/37/38/39/40/41/47
	2	5/17/28/34/38
	3	28/31/36/38/41/49
	4	5/23/28/31/34/36/37/38/40/41/47/49
	5	5/20/21/28/31/34/36/37/38/39/40/41/47/48
	6	5/17/23/24/25/28/30/31/34/35/36/37/38/40/41/47/48/49/50
	7	5/17/22/28/31/34/36/37/40/41/47/49
	8	5/17/23/28/30/31/34/36/37/40/41/47/49
	9	5/8/10/17/18/24/25/28/30/31/34/35/36/37/38/40/41/45/47/50
	10	20/21/23/28/31/36/38/48
	11	5/19/28/31/34/36/37/40/41/47
	12	5/19/28/31/34/36/37/40/41/47/50
Group E <sub>3</sub>		
<i>S. illinois</i>	1	31/36
	2	5/31/36
	3	5/17/22/28/31/34/36/37/38/40/41/47
	4	5/28/31/36/38/41/47
	5	36
	6	5/31/36/38/41/47
	7	5/22/28/31/36/41/47
	8	5/6/10/16/17/19/20/21/23/24/25/27/29/30/31/32/33/34/35/36/37/38/39/40/45/46/47/50
<i>S. minneapolis</i>	1	5/17/23/26/28/30/31/34/36/37/38/39/40/41/43/46/47/49
	2	1/17/36/38
	3	6/10/17/18/22/24/25/26/28/30/31/32/33/34/35/36/37/38/40/41/45/46/47/48/49/50
	4	6/10/17/26/28/30/31/34/35/36/37/38/39/40/41/44/45/46/47/49/50
	5	6/10/17/22/26/28/30/31/34/36/37/38/39/40/41/43/46/47/49
	6	6/10/17/26/28/30/31/34/35/36/37/38/39/40/41/45/46/47/49/50
<i>S. thomasville</i>	1	36/38/42/47/48
	2	3/4/8/10/17/18/22/23/24/25/26/28/31/32/34/35/36/37/38/39/40/41/42/43/44/45/46/47/49/50
	3	31/34/36/42/47
	4	1/4/5/18/19/23/26/27/28/30/31/32/33/34/35/36/37/39/40/41/43/46/47
	5	4/5/18/19/24/25/26/31/34/35/36/37/39/40/41/42/45/46/47/50

TABLE 3—Continued

Salmonella type strain	Culture no.	Pattern	
<i>S. thomasville</i>	6	36	
	7	3/4/8/10/17/18/24/25/28/31/31/34/35/37/40/41/42/45/46/47/50	
	8	3/17/28/31/36/42/47	
	9	3/4/8/10/16/17/28/30/31/34/36/37/38/40/41/42/46/47/48/49	
	10	1/4/5/18/19/23/26/27/28/30/31/32/33/34/35/36/37/39/40/41/43/46/47	
	11	3/4/8/10/17/18/24/25/28/31/36/42/45/46/47/48/49/50	
	12	3/4/8/10/17/18/22/23/24/25/26/28/31/34/36/37/38/39/40/41/42/43/44/45/46/47/49/50	
	13	1/5/16/17/23/26/27/28/30/31/32/33/34/35/36/37/39/40/41/43/46/47	
	14	1/3/5/6/8/10/17/18/22/23/24/25/26/28/31/32/34/35/36/37/38/39/40/41/42/43/44/45/46/47/49/50	
	Group E <sub>4</sub>		
	<i>S. chittagong</i>	1	5/17/19/20/21/24/26/28/30/31/32/33/34/36/37/38/39/40/41/44/46/47/48/49
		2	5/17/19/20/21/22/23/24/26/28/30/31/34/36/37/38/40/41/44/46/47/48/49
		3	5/11/17/19/20/21/22/24/26/28/31/34/36/37/38/40/41/46/47/48/49
		4	5/17/19/20/21/22/26/28/31/34/36/37/38/40/41/44/46/47/48/49
<i>S. senftenberg</i>	48	17/22/31/44	
	49	30/36	
	50	17/22/26/31/36/44/48	
	51	17/22/26/31/36/39/44/46/47/48	
	52	17/22/31	
<i>S. taksony</i>	1	1/5/17/22/28/31/34/36/37/40/41	
	2	5/17/22/28/30/31/34/37/40/41/47	
	3	17/22/31/44	

<sup>a</sup> Only strong reactions (+++ or above) are recorded.

<sup>b</sup> Cultures were isolated from hospital outbreaks.

<sup>c</sup> Cultures were isolated from family outbreaks.

<sup>d</sup> Cultures were isolated from a campus outbreak.

<sup>e</sup> Cultures were previously untypable with serotype-specific phages.

TABLE 4. Number of patterns observed for serovars typed

Serovar	Group	Isolates typed	Different lysis patterns
<i>agona</i>	B	25	7
<i>amsterdam</i>	E <sub>1</sub>	47	15
<i>anatum</i>	E <sub>1</sub>	39	22
<i>bareilly</i>	C <sub>1</sub>	10	4
<i>berta</i>	D	2	1
<i>binza</i>	E <sub>2</sub>	35	22
<i>blockley</i>	C <sub>2</sub>	9	4
<i>braenderup</i>	C <sub>1</sub>	4	3
<i>bredeney</i>	B	5	1
<i>california</i>	B	6	3
<i>chester</i>	B	7	3
<i>chittagong</i>	E <sub>4</sub>	6	4
<i>cholerae-suis</i>	C <sub>1</sub>	3	1
<i>cholerae-suis</i> var. <i>kunzensdorf</i>	C <sub>1</sub>	2	1
<i>dar-es-salaam</i>	D	2	1
<i>decatur</i>	C <sub>1</sub>	5	2
<i>derby</i>	B	10	5
<i>drypool</i>	E <sub>2</sub>	39	15
<i>dublin</i>	D	5	1
<i>eastbourne</i>	D	4	2
<i>eimsbuettel</i>	C <sub>1</sub>	11	7
<i>enteritidis</i>	D	20	11
<i>gateshead</i>	D	5	1
<i>give</i>	E <sub>1</sub>	17	11
<i>halmstad</i>	E <sub>2</sub>	6	2
<i>heidelberg</i>	B	27	19
<i>illinois</i>	E <sub>3</sub>	19	8
<i>infantis</i>	C <sub>1</sub>	10	4
<i>jerusalem</i>	C <sub>1</sub>	6	2
<i>kottbus</i>	C <sub>2</sub>	13	4
<i>lexington</i>	E <sub>1</sub>	5	2
<i>london</i>	E <sub>1</sub>	4	1
<i>manila</i>	E <sub>2</sub>	4	1
<i>meleagridis</i>	E <sub>1</sub>	5	1
<i>miami</i>	D	4	1
<i>minneapolis</i>	E <sub>3</sub>	15	6
<i>montevideo</i>	C <sub>1</sub>	11	5
<i>muenster</i>	E <sub>1</sub>	17	8
<i>newington</i>	E <sub>2</sub>	29	12
<i>newport</i>	C <sub>2</sub>	27	16
<i>oranienburg</i>	C <sub>1</sub>	11	5
<i>panama</i>	D	4	2
<i>paratyphi A</i>	A	6	3
<i>paratyphi A</i> var. <i>durazzo</i>	A	7	2
<i>paratyphi C</i>	C <sub>1</sub>	4	1
<i>pullorum</i>	D	6	2
<i>saint paul</i>	B	11	5
<i>schwarzengrund</i>	B	7	3
<i>senftenberg</i>	E <sub>4</sub>	20	11
<i>taksony</i>	E <sub>4</sub>	9	3
<i>tennessee</i>	C <sub>1</sub>	8	3
<i>thomasville</i>	E <sub>3</sub>	25	14
<i>thompson</i>	C <sub>1</sub>	32	18
<i>typhi</i>	D	9	4
<i>typhimurium</i>	B	44	28
<i>typhimurium</i> var. <i>copenhagen</i>	B	3	1
<i>uganda</i>	E <sub>1</sub>	3	1
<i>westhampton</i>	E <sub>1</sub>	6	2
Totals		735	347

and include contributions from our own diagnostic unit. A summary of these results appears in Table 3.

We were able to characterize all cultures used in this study, including isolates that we were unable to type or delineate previously with serovar-specific phages. In addition, we were able to relate and confirm the derivation of a number of blind specimens submitted for our inspection. Patterns were reproducible and epidemiologically significant. The isolates obtained from a campus incident and from two hospital and seven family outbreaks, as an example, produced identical patterns of lysis even though the *Salmonellae* originated from different individuals. Representative serovars and their phage patterns are listed in Table 4.

Approximately 95% of all the *Salmonellae* isolated belong to groups A, B, C<sub>1</sub>, C<sub>2</sub>, D, E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, and E<sub>4</sub>. Hundreds of serovars are represented by these serological groups, and any single serovar can suddenly assume a position of prominence. Under the circumstances, it is inconceivable that serovar-specific phages will always be available for laboratory use. Consequently, the convenience of a single, general-purpose phage-typing set has definite advantages.

Given the widespread distribution of *Salmonella*, the frequency of isolation, and the variety of serovars in existence, a wide-spectrum phage-typing potential appears most desirable.

#### ACKNOWLEDGMENTS

Appreciation is expressed to Jacqueline Hunter for her most valuable laboratory assistance, to E. S. Anderson and J. D. H. de Sa of the Central Public Health Laboratory, Colindale, England, for their instruction in phage typing,

and to Billie O. Blackburn of the National Animal Disease Laboratory, Ames, Iowa, and L. Le Minor of the Pasteur Institute, Paris, France, for their assistance in securing cultures for this project.

#### LITERATURE CITED

1. Anderson, E. S. 1962. The genetic basis of bacteriophage typing. *Br. Med. Bull.* 18:64-68.
2. Anderson, E. S. 1964. Phage typing of *Salmonella* other than *S. typhi*, p. 89-110. *In* E. Van Oye (ed.), *The world problem of salmonellosis*. Junk, The Hague.
3. Craigie, J., and C. H. Yen. 1938. The demonstration of types of *B. typhosus* by means of preparations of type II Vi phage. I. Principles and technique. *Can. J. Public Health* 29:448-484.
4. Craigie, J., and C. H. Yen. 1938. The demonstration of types of *B. typhosus* by means of preparations of type II Vi phage. II. The stability and epidemiological significance of V form types of *B. typhosus*. *Can. J. Public Health* 29:484-496.
5. Gershman, M. 1972. Preliminary report: a system for typing *Salmonella thompson*. *Appl. Microbiol.* 23:831-832.
6. Gershman, M. 1974. A phage-typing system for *Salmonella anatum*. *Avian Dis.* 18:565-568.
7. Gershman, M. 1974. A phage-typing system for *Salmonella newport*. *Can. J. Microbiol.* 20:769-771.
8. Gershman, M. 1976. Phage typing set for group C<sub>1</sub> and C<sub>2</sub> salmonellae. *J. Clin. Microbiol.* 3:214-217.
9. Gershman, M. 1976. A phage-typing system for *Salmonella binza*. *Public Health Lab.* 34:97-99.
10. Gershman, M. 1976. Phage typing system for *Salmonella enteritidis*. *Appl. Environ. Microbiol.* 32:190-191.
11. Okey, C. H. 1967. Salmonellosis. *J. Maine Med. Assoc.* 58:85-94.
12. Sayamov, R. M. 1963. Treatment and prophylaxis of cholera with bacteriophage. *Bull. W.H.O.* 28:361.
13. Sonnenschein, K. 1925. Die Verwendbarkeit der Bakteriophage für die bakteriologische Diagnose. *Muench. Med. Wochenschr.* 72:1443-1444.
14. Sonnenschein, K. 1928. Bakteriendiagnose mit Bakteriophagen. *Dtsch. Med. Wochenschr.* 54:1034-1036.
15. Swanstrom, M., and M. H. Adams. 1951. Agar layer method for the production of high titer phage stocks. *Proc. Soc. Exp. Biol. Med.* 7:372-375.