Bacteriophage Typing of Clinically Isolated
Serratia marcescens

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A bacteriophage-typing scheme for the differentiation and classification of clinically isolated strains of Serratia marcescens was developed. Thirty-four Serratia bacteriophages were isolated from sewage and used to type 185 of 204 isolates (90.6%) of S. marcescens into 23 bacteriophage groups representing 71 types. Different bacteriophage types occurred at different intervals, suggesting that particular strains of S. marcescens are found at certain times. A correlation was found between inositol fermentation and bacteriophage type and between susceptibility to carbenicillin and bacteriophage type. However, there was no relationship between source of isolate and bacteriophage type. Bacteriophage typing of S. marcescens should provide a system which will aid in determining the origin of nosocomial Serratia infections.

Serratia marcescens was first reported as causing human infection by Woodward in 1913 (26). Since that time, infections due to S. marcescens have been reported with increasing frequency (2, 5, 9, 10), and many have been shown to be of a nosocomial nature (3, 13, 19, 25). Consequently, the origin of these strains of S. marcescens causing infections is of extreme importance.

Several different schemes have been described for differentiating strains of S. marcescens. Serological typing (11) now includes 15 somatic and 13 flagellar antigens, and has been used effectively in many epidemiological studies (7, 8, 18, 24). Bacteriocin typing has also been reported as an effective method for strain differentiation within the genus Serratia. Two independent bacteriocin-typing schemes, based either on production of or sensitivity to bacteriocins (14, 15, 20, 21), have been described for the differentiation of clinical isolates of S. marcescens. Bacteriophage typing has been suggested for differentiation within the genus Serratia, but has not yet been applied to the differentiation of clinical isolates of S. marcescens (17, 23). The purpose of this study was to devise a bacteriophage-typing scheme to differentiate and classify those strains of S. marcescens which are causing human infections.

MATERIALS AND METHODS

Cultures. From December 1969 to March 1971, 204 clinical isolates of S. marcescens were obtained from Detroit General Hospital. These isolates were identified as S. marcescens by biochemical reactions which we have previously described (16). Stock cultures were maintained on nutrient agar (Difco) slants and stored at 4 C. In addition, the following American Type Culture Collection (ATCC) strains were used: S. marcescens 14764 and 13880, Escherichia coli 12795 and 14948, Edwardsiella tarda 15947, Shigella dysenteriae 13313, S. flexneri 12661, S. boydii 9207, S. sonnei 9290, Salmonella typhi 19430, S. enteritidis 13076, Arizona hinshawii 13314, Citrobacter freundii 8090, Klebsiella pneumoniae 13883, Enterobacter aerogenes 13048, E. cloacae 13017, E. liquefaciens 14460, Proteus vulgaris 13315, and Providencia alcalifaciens 9886. These cultures were maintained in the same manner as the clinical isolates.

Isolation of phages. Bacteriophages were isolated from nonchlorinated sewage obtained from the influent pipe of the Wayne County Sewage Disposal System, Wyandotte, Mich. Amounts of 5 ml of the sewage were added to four flasks, each containing 50 ml of brain heart infusion broth (Difco). Two of the four flasks were inoculated with one strain of S. marcescens and incubated for 24 hr at 35 C. After incubation, the cultures were centrifuged for 10 min at 1,000 x g, and the supernatant fluids were filtered through 0.22-µm pore size membrane filters (Falcon Plastics, Division of Bioquest, Los Angeles, Calif.). Phage activity was determined by spotting the four filtered suspensions onto nutrient agar plates containing lawns of each of 106 clinically isolated indicator strains of S. marcescens. The indicator strains were grown in nutrient broth (18 hr, 35 C), and cotton swabs were used to inoculate the nutrient agar plates. These plates were incubated for 18 hr at 35 C, and bacteriophage action was revealed by zones of complete or semicomplete lysis or by discrete plaques.
Purification and propagation of phages. Lytic areas, as described above, were picked and transferred to 3 ml of nutrient broth. Propagating strains (PS) of Serratia were inoculated into the respective nutrient broth phage suspensions, and the mixtures were incubated for 18 hr at 35 C. After incubation, the broth cultures were centrifuged for 15 min at 3,500 × g and the supernatant fluids were collected. Three successive single-plaque pickings from soft-agar overlays were used to isolate the Serratia phages (1). Phage-resistant bacteria present in the lytic area of the second and third pickings were eliminated by filtering through 0.22-μm membrane filters.

The phages were propagated by the soft-agar overlay method with a modification of the technique described by Adams (1). A 0.1-ml amount of a 10-fold dilution of the purified phages and 0.4 ml of overnight PS broth cultures were added to 6 ml of nutrient broth containing 0.5% agar. The suspensions were mixed and poured onto nutrient agar plates, which were then incubated for 24 hr at 35 C. The propagated phages were recovered by adding 2 ml of nutrient broth to the incubated plates, and the respective soft-agar overlays from each propagation were pooled. The pooled suspensions were centrifuged for 10 min at 5,000 × g, and the supernatant fluids were filtered through 0.45- and 0.22-μm membrane filters (Gelman Instrument Co., Ann Arbor, Mich.). The filtered phage suspensions were the stock phage preparations and were stored at 4 C.

Determination of phage patterns. The routine test dilution (RTD) of each bacteriophage was determined prior to each typing and was defined as the greatest 10-fold dilution of the stock phage still giving confluent lysis (Fig. 1). The typing of Serratia (Fig. 2) was a modification of the procedure described by Brown and Parisi (6) for bacteriophage typing of E. coli. Isolates of Serratia to be typed were cultured in 3 ml of nutrient broth (18 hr at 35 C) and streaked with a swab onto dried nutrient agar plates. After the inocula absorbed into the agar, one drop of the RTD of each of the phages used in the study was placed onto each plate with a tuberculin syringe with a 25-gauge needle. The drops were placed consecutively in rows across the plate. The drops were allowed to absorb into the agar, and the plates were incubated for 18 hr at 35 C. Lytic reactions were recorded as 4 (confluent lysis), 3 (75% lysis), 2 (50% lysis), 1 (isolated plaques to 25% lysis), and 0 (no observed lysis). Reactions of 1 through 4 were scored as positive.

RESULTS

Bacteriophage typing of Serratia. Sixty bacteriophages which lysed S. marcescens were isolated from raw sewage. However, of these 60 phages, many had similar lytic patterns on 140 clinical isolates of Serratia; therefore, 34 phages were selected for the typing scheme. This selection was based on the unique host range of these phages. These 34 phages were then used to type clinical isolates of Serratia as well as to determine their host range on representative type strains within the Enterobacteriaceae.

Computer analysis (UWPGM program [4] run on an IBM 360 model 67 at Wayne State University Computing and Processing Center) of the host-range data obtained from 140 clinical isolates of Serratia tested against the 34 Serratia phages was used to construct a bacteriophage-typing scheme for Serratia. Sixty-four additional isolates of Serratia were obtained and tested against the 34 phages. These results
TABLE 1. Bacteriophage-typing scheme for Serratia marcescens

| Group | Grouping phage | Type | Typing phage | No. of Serratia isolates | Percent Serratia isolates |
|-------|----------------|------|--------------|--------------------------|--------------------------|
| 1     | 1, 3, 4, 5, 9, 10, 13, 16, 18, 21, 22, 30, 31, 33 | A    | 23, 26, 29   | 4                        | 4.0                      |
|       |                | B    | 25, 29, 34   | 3                        | 1.5                      |
|       |                | C    | 23, 24, 25, 34 | 1                      | 0.5                      |
| 2     | 1, 3, 4, 5, 16, 17, 18, 19, 21, 22, 23, 29, 30, 31, 33 | A    | —           | 4                        | 4.5                      |
|       |                | B    | 26           | 3                        | 1.5                      |
|       |                | C    | 6, 7, 8, 11, 24, 25 | 1                    | 0.5                      |
|       |                | D    | 6, 8, 11, 24, 25, 26, 27, 28, 34 | 1              | 0.5                      |
| 3     | 1, 3, 4, 5, 13, 16, 18, 19, 21, 22, 25, 30, 33 | A    | 26           | 4                        | 4.0                      |
|       |                | B    | —           | 4                        | 2.0                      |
| 4     | 1, 2, 3, 4, 5, 9, 10, 13, 16, 18, 19, 21, 22, 23, 24, 25, 27, 29, 30, 31, 33 | A    | 17, 26       | 17                       | 26.5                     |
|       |                | B    | 26, 28       | 11                       | 5.5                      |
|       |                | C    | 17, 28       | 7                        | 3.5                      |
|       |                | D    | 20, 26       | 6                        | 3.0                      |
|       |                | E    | —           | 10                       | 5.0                      |
|       |                | F    | 11, 17, 26, 28, 34 | 1            | 0.5                      |
| 5     | 1, 2, 3, 4, 5, 6, 7, 9, 10, 13, 16, 18, 19, 21, 22, 23, 24, 25, 27, 28, 29, 30, 33, 34 | A    | —           | 2                        | 8.5                      |
|       |                | B    | 8           | 5                        | 2.5                      |
|       |                | C    | 8, 17       | 10                       | 5.0                      |
| 6     | 1, 2, 3, 4, 5, 9, 10, 13, 16, 17, 18, 19, 21, 22, 23, 29, 30, 31, 33 | A    | 20           | 4                        | 5.0                      |
|       |                | B    | 26           | 1                        | 0.5                      |
|       |                | C    | 26, 34      | 1                        | 0.5                      |
|       |                | D    | 24, 26      | 1                        | 0.5                      |
|       |                | E    | 27           | 3                        | 1.5                      |
| 7     | 20             | A    | 12, 18, 19  | 1                        | 7.0                      |
|       |                | B    | 12, 19      | 1                        | 0.5                      |
|       |                | C    | 12, 32      | 4                        | 2.0                      |
|       |                | D    | 12, 18, 32  | 1                        | 0.5                      |
|       |                | E    | 12, 18, 19, 32 | 2            | 1.0                      |
|       |                | F    | —           | 4                        | 2.0                      |
|       |                | G    | 19, 32     | 1                        | 0.5                      |
| 8     | 15             | A    | —           | 2                        | 4.5                      |
|       |                | B    | 14           | 3                        | 1.5                      |
|       |                | C    | 14, 19     | 1                        | 0.5                      |
|       |                | D    | 14, 24     | 3                        | 1.5                      |
| 9     | 19             | A    | —           | 4                        | 4.0                      |
|       |                | B    | 26           | 1                        | 1.5                      |
|       |                | C    | 11, 17, 18 | 1                        | 0.5                      |
| 10    | 1, 2, 6, 7, 8, 9, 10, 11, 13, 16, 17, 18, 19, 23, 24, 25, 26, 27, 28, 29, 30, 34 | A    | 5, 14, 15, 22, 33 | 1                   | 2.0                      |
|       |                | B    | 32, 33    | 1                        | 0.5                      |
|       |                | C    | —           | 2                        | 1.0                      |
| 11    | 1, 2, 3, 5, 6, 7, 8, 9, 13, 16, 17, 18, 19, 23, 24, 25, 26, 27, 28, 29, 33, 34 | A    | 4, 10, 21, 22 | 3                     | 8.0                      |
|       |                | B    | 4, 10, 21  | 2                        | 1.0                      |
|       |                | C    | 10, 20, 21 | 8                        | 4.0                      |
|       |                | D    | 4, 10, 22  | 1                        | 0.5                      |
|       |                | E    | 4           | 1                        | 0.5                      |
|       |                | F    | 4, 21, 22  | 1                        | 0.5                      |
| 12    | 8, 17, 18, 23, 24, 25, 26, 27, 28, 29 | A    | 6, 16, 19, 33 | 1                   | 2.0                      |
|       |                | B    | 16, 19, 32, 33 | 1              | 0.5                      |
|       |                | C    | 16, 21, 22, 33 | 1              | 0.5                      |
|       |                | D    | 1, 34      | 1                        | 0.5                      |
| 13    | 11, 17, 18, 23, 25, 26, 27, 28, 34 | A    | —           | 4                        | 2.5                      |
|       |                | B    | 6, 30, 31, 33 | 1               | 0.5                      |
| 14    | 5, 16, 19, 21, 22, 25, 30, 31, 33 | A    | 13, 23, 27, 29 | 1                   | 2.0                      |
|       |                | B    | 18           | 3                        | 1.5                      |
| 15    | 4, 22, 23, 29, 30, 31, 33 | A    | 21           | 1                        | 2.0                      |
|       |                | B    | 3, 5, 16, 18, 19, 20 | 1             | 0.5                      |
|       |                | C    | 10, 13, 16, 17, 18, 19, 21 | 1             | 0.5                      |
|       |                | D    | 5, 11, 16, 17, 18, 19, 34 | 1             | 0.5                      |

*NL = not lysed by one or more Serratia phages.*
were then added to the bacteriophage-typing scheme. The proposed bacteriophage-typing scheme for *Serratia* (Table 1) was based on the combined results of 204 clinical isolates of *Serratia* tested against the 34 *Serratia* phages. As shown in Table 1, an isolate was first grouped according to susceptibility to a certain set of phages referred to as grouping phages. In order for an isolate to be placed within a particular group, that strain had to be susceptible to all of the phages of that group. Further differentiation into types was made within the group by a second set of computer-determined phages referred to as typing phages. It should be stressed that typing phages of one group may be grouping phages in another group; their designation as grouping and typing phages refers only to the particular group and type in question. A total of 23 groups representing 71 different types are described. The number and percentage of isolates which belonged to any particular group and type are also shown in Table 1. In all, 90.6% of the *Serratia* isolates were typed by phages in the bacteriophage-typing scheme.

The reproducibility of the results was tested by selecting at random 25 *Serratia* isolates and testing a second time against the 34 typing phages. The second set of typing results were comparable to those found during the first host-range study.

**Correlations of bacteriophage type with time of isolation, source, and metabolic reactions.** The results of bacteriophage typing were correlated with other characteristics as shown in Table 2. Certain phage types occurred at certain time intervals. This was demonstrated by the occurrence at the same times of all or most of the isolates in a particular phage type. In 19 instances when multiple isolates were obtained from the same patient, they were of the same phage type. In addition, strains in certain phage groups were susceptible to the antibiotic carbenicillin, whereas strains belonging to other phage groups were resistant. In group 7, 12 of the 14 isolates were susceptible to carbenicillin, group 8 had 3 of 9 isolates susceptible, group 9 had 7 of 8 isolates susceptible, and in group 13 all 5 isolates were susceptible to carbenicillin. Furthermore, 13 groups representing 45 types were resistant to carbenicillin. In all, 37 of 185 typable isolates of *Serratia* were susceptible to carbenicillin. There was also a correlation between phage type and fermentation of inositol (Table 2). Twenty-four isolates were inositol-negative, and with one exception all of the inositol-negative isolates belonged to bacteriophage types containing only inositol-negative isolates. However, as also shown in Table 2, a correlation could not be made between source of the isolate and phage type.

**Host range of Serratia phages within Enterobacteriaceae.** The 34 typing phages were tested against other members of the *Enterobacteriaceae* (Table 3). Of the ATCC strains tested, only *E. coli* K-12, *S. dysenteriae*, *S. flexneri*, *A. hinshawii*, and two *S. marcescens* strains were lysed by one or more of the *Serratia* phages. Higher dilutions of phage produced only isolated plaques rather than complete lysis, suggesting susceptibility to phage action rather than bacteriocins or bacteriophage inhibition.

**DISCUSSION**

The results of this study show that clinical isolates of *S. marcescens* can be differentiated...
| Group | Type | Month isolated | Source* | Carbenicillin* | Inositol† |
|-------|------|----------------|---------|---------------|----------|
| 1     | A    | 5/70, 5/70 | U, U, U, U | R, R, R, R | +, +, +, + |
| B     | 7/70 | 7/70, 7/70 | ? , ?  | R, R, R | + , +, + |
| C     | 7/70 |           | ?  | R | + |
| 2     | A    | 2/70, 1/70 | M, U, U, U | R, R, R, R | +, +, +, + |
| B     | 1/70, 12/69 | U, U, U, U | R, R, R | +, +, + |
| C     | 5/70 | U | R | + |
| D     | 5/70 | U | R | + |
| 3     | A    | 11/70, 11/70 | B, B, B, U | R, R, R, R | + , +, + , + |
| B     | 11/70, 11/70, 9/70, 11/70 | S, U, U, U | R, R, R, R | +, +, +, + |
| 4     | A    | 5/70, 6/70, 1/71, 1/71, 1/71, 1/71, 2/71, 1/71, 12/70, 11/70, 12/70, 1/71, 1/71 | U, U, U, U, U, U, U | R, R, R, R, R | + , +, +, +, + |
| B     | 11/70, 10/70, 11/70, 11/70, 10/70, 11/70 | U, U, U, U, U, U, U, U, U | R, R, R, R, R, R | +, +, +, +, + |
| C     | 11/70, 11/70, 10/70, 11/70 | U, U, U, U, U, U, U, U, U | R, R, R, R, R, R, R | +, +, +, +, +, +, +, + |
| D     | 11/70 | U | R | + |
| E     | 10/70, 10/70, 11/70, 11/70, 11/70, 11/70 | M, U, U, U, U, U | R, R, R, R, R, R | +, +, +, +, +, + |
| F     | 1/71 | U | R | + |
| 5     | A    | 9/70, 9/70 | U | R | + |
| B     | 9/70, 9/70, 9/70, 9/70, 9/70, 2/71 | U | R | + |
| C     | 6/70, 6/70, 7/70, 7/70, 7/70, 7/70 | U, U, U, U, U, U | R, R, R, R, R, R | +, +, +, +, +, +, +, + |
| 6     | A    | 9/70, 9/70, 9/70, 9/70 | U, U, U | R, R, R | + , +, + |
| B     | 5/70 | U | R | + |
| C     | 5/70 | U | R | + |
| D     | 5/70 | U | R | + |
| 7     | A    | 9/70 | U | R | + |
| B     | 9/70 | U | R | + |
| C     | 9/70, 9/70, 9/70, 9/70 | U | R | + |
| D     | 9/70 | U | R | + |
| E     | 9/70, 9/70 | U | R | + |
| F     | 5/70, 5/70, 5/70, 6/70 | U | R | + |
| G     | 9/70 | U | R | + |
| 8     | A    | 1/70, 7/70 | U | S, S, S, S | +, +, +, + |
| B     | 2/70, 8/70, 7/70 | U | S | + |
| C     | 5/70 | U | S | + |
| D     | 8/70, 8/70, 8/70 | U | S, S, S, S | +, +, +, + |
| 9     | A    | 12/69, 2/70, 5/70, 9/70 | U | S | + |
| B     | 5/70, 6/70 | U | S, S, S, S | +, +, +, +, + |
| C     | 1/70 | U | S | + |
| 10    | A    | 1/70 | U | S, S | +, + |
| B     | 5/70 | U | S | + |
| C     | 11/70, 11/70 | U | S, S, S, S | +, +, +, + |
| 11    | A    | 8/70, 8/70, 8/70 | U | S, S, S | +, +, + |
| B     | 2/70, 2/70 | U | S, S, S, S | +, +, +, + |
| C     | 9/70, 9/70, 9/70, 9/70, 9/70, 9/70, 9/70 | U, U, U, U, U, U, U U | R, R, R, R, R, R, R | +, +, +, + |
| D     | 1/71 | U | R | + |
| E     | 5/70 | U | R | + |
| 12    | A    | 5/70 | U | S | + |
| B     | 2/70 | U | S | + |
| C     | 5/70 | U | S | + |
| D     | 7/70 | U | S | + |

*U, urine; B, blood; M, wound and central nervous system; ?, unknown; S, sputum.
*R, resistant; S, susceptible.
†Positive, +; negative, –.
‡NL, not lysed by any of the Serratia typing phage.
### Table 2—Continued

| Group | Type | Month isolated | Source* | Carbenicillin* | Inositol* |
|-------|------|----------------|---------|----------------|----------|
| 13    | A    | 1/71, 1/71, 1/71 | S, B, U, B | S, S, S | +, +, +, + |
|       | B    | 1/71 | S         | S         | +         |
| 14    | A    | 1/71 | ?         | R         | +         |
|       | B    | 1/71, 1/71, 1/71 | U, U, B | R, R, R | +, +, + |
| 15    | A    | 5/70 | U         | R         | +         |
|       | B    | 9/70 | B         | R         | -         |
|       | C    | 5/70 | U         | R         | +         |
|       | D    | 5/70 | U         | R         | +         |
| 16    | A    | 11/70 | U         | S         | +         |
|       | B    | 1/71 | S         | R         | +         |
|       | C    | 1/70 | ?         | R         | +         |
| 17    | A    | 5/70 | U         | R         | +         |
|       | B    | 5/70 | U         | R         | +         |
| 18    | A    | 9/70 | U         | S         | +         |
|       | B    | 9/70 | U         | R         | +         |
| 19    | A    | 1/70 | ?         | S         | +         |
| 20    | A    | 11/70 | U         | R         | +         |
| 21    | A    | 7/70 | S         | S         | +         |
| 22    | A    | 1/70 | S         | R         | +         |
|       | B    | 5/70 | ?         | S         | +         |
| 24    | NL†  | 7/70, 7/70, 7/70, 7/70, 8/70, 8/70, 8/70, 9/70, 9/70, 9/70, 9/70, 9/70, 9/70, 10/70, 9/70, 11/70, 11/70, 8/70, 9/70, 1/71 | U, U, U, S, S, S, S, S | R, R, R, S, S, S, S | +, +, +, +, +, +, +, +, + |

### Table 3. Host range of Serratia bacteriophages within the Enterobacteriaceae

| Organism                        | Lysed by phage no. |
|---------------------------------|-------------------|
| *Escherichia coli* 12795        | NL†               |
| *E. coli* K-12 14948            | 14, 15            |
| *Shigella dysenteriae* 13313    | 32                |
| *S. flemeri* 12661              | 12, 20, 32        |
| *S. boydii* 9207                | NL                |
| *S. sonnei* 9290                | NL                |
| *Escherichia tarda* 15947       | NL                |
| *Salmonella typhi* 19430        | NL                |
| *S. enteritidis* 13076          | NL                |
| *Arizona hinsawii* 13314        | 19                |
| *Citrobacter freundii* 8090     | NL                |
| *Klebsiella pneumoniae* 13883   | NL                |
| *Enterobacter aerogenes* 13048   | NL                |
| *E. cloacae* 13047               | NL                |
| *E. liquefaciens* 14460         | NL                |
| *Serratia marcescens* 14764     | 19                |
| *S. marcescens* 13880           | 14, 15, 22, 24    |
| *Proteus vulgaris* 13315         | NL                |
| *Providencia alcalifaciens* 9886| NL                |

* NL = not lysed by any of the *Serratia* typing phages.
† ATCC number.

The results also agree with those reported by Pillich, Hradecna, and Kocur (17) for the bacteriophage typing of *Serratia*. They found that 10 culture collection bacteriophages lysed 87.9% of 107 culture collection strains of *Serratia* tested. However, their bacteriophage-typing scheme was based on only 6 of the 10 phages. Those phages which were too discriminatory (lysed only one strain of *Serratia*) were eliminated from the system. This resulted in only seven bacteriophage groups which could be used for strain differentiation. Furthermore, only 77% of their *S. marcescens* strains were subsequently typable.

There are two disadvantages of the bacteriophage typing scheme proposed by Pillich, Hradecna, and Kocur: the scheme was based on culture collection strains of *Serratia*, and it contained only seven phage groups. These disadvantages were overcome in our bacteriophage-typing scheme, which was based on the host-range data from 204 clinical isolates of *S. marcescens* tested against 34 phages. The proposed typing scheme consists of 23 groups representing 71 distinct types as established by computer analysis of the host-range data. A higher degree of strain differentiation and classification can be obtained with the larger number of typing phages. The results have shown 71 different phage types or strains within the 204 isolates of *S. marcescens* tested. The high
number of phage types points out the need for a high degree of strain differentiation before any initial epidemiological study could be undertaken. The 204 clinical isolates have been differentiated into a number of phage types by use of 34 phages. It is possible to limit further the number of typing phages, but to do so would result in a loss in the degree of strain differentiation. Further studies, therefore, are needed to determine the number of phages and consequently the number of phage types which are required to maintain a high degree of strain differentiation among clinical isolates of S. marcescens and still be of epidemiological value.

The bacteriophages used in this study were found to be stable upon storage in nutrient broth at 4 °C for 1 year. No decrease in titer was observed under these conditions. However, minor variations in the lytic activity of some of the bacteriophages were observed after repeated propagation in the host strains. This was generally demonstrated in that a few bacteriophages gave lytic reactions of 1 rather than 0 against certain strains of S. marcescens. The significance of this observation is the subject of further investigation.

The results showed that bacteriophage typing of Serratia is reproducible. When 25 cultures were retested against the 34 typing bacteriophages, only minor variations were observed in the lytic patterns. This was attributed to an increase in the number of bacteriophage-resistant organisms or to a slight variation in the number of bacteria plated in the lawn. The reproducibility of the system is further supported because identical lytic reactions were observed with duplicate platings. In 19 instances when multiple isolates were obtained from the same patient, they were of the same phage type. This clinical evidence suggests that bacteriophage typing can be used as a tool in hospital epidemiology.

The reliability of bacteriophage typing is demonstrated by the correlation of certain reactions to specific phage types. Specifically, all isolates in certain phage types were found to be resistant to the antibiotic carbenicillin, whereas all isolates in other types were found to be susceptible to the antibiotic. Of the 71 different phage types, 51 types were resistant and 17 types were susceptible. The remaining three types comprising 10 isolates were found to contain both carbenicillin-susceptible and carbenicillin-resistant strains.

The inositol fermentation was a second reaction to show a relationship to bacteriophage typing. Our results showed that 88.3% of the Serratia isolates were positive for the inositol reaction, which is in agreement with the results reported by Ewing (12). Of the 11.7% inositol-negative isolates, 23 of the 24 belonged to phage types containing only inositol-negative isolates. This further indicates that the described bacteriophage-typing scheme is discriminatory and can effectively identify types within this group of organisms.

It can also be seen from the results that particular phage types occurred at certain time intervals. This is demonstrated by the occurrence at the same time of most of the isolates in one phage type. This suggests that certain strains of Serratia are found at certain times. Of the isolates tested, only 2 of the 71 phage types showed strains of Serratia occurring at different time intervals. The explanation of these observations should be the subject of further epidemiological research.

To determine the specificity of the typing phages, the 34 phages were tested against selected ATCC strains of Enterobacteriaceae. Only E. coli, S. dysenteriae, S. flexneri, and A. hinshawii were lysed by the typing bacteriophages. In 1953, Wassermann and Seligmann (23) reported that Serratia phages lysed certain strains of other genera within the Enterobacteriaceae. These results are similar in that some Serratia phage lyse bacteria other than Serratia, but this does not detract from the Serratia typing scheme. The fact that an isolate is lysed by a typing phage does not mean that the isolate is a member of the genus Serratia. This system was designed to differentiate strains within the genus Serratia, but not for identifying an isolate as Serratia.

The origin of most nosocomial Serratia infections is still unknown. The E. coli bacteriuri strains were shown to be endogenous infections (22). However, since Serratia is rarely identified from feces and yet many urinary tract infections have been described, further epidemiological studies are needed. Bacteriophage typing should provide a method which will aid in such studies. We encourage others to use our system, which we will supply on request.

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