Epidemiological Differentiation of *Serratia marcescens*: Typing by Bacteriocin Production

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A new method for comparing and differentiating strains of *S. marcescens* is described which has proved useful in determining the epidemiology of hospital infections. Strains were grown in Trypticase soy broth, and bacteriocin production was induced with mitomycin C for 5 hr. The bacteriocin lysates were then spotted onto nine standard indicator strains, which were chosen with the aid of computer analysis from the 118 indicators tested. After 24 hr at 37°C, zones of inhibition due to bacteriocins were recorded. One hundred twenty-nine strains were differentiated into 72 different bacteriocin production patterns, but 11 strains were nontypable. None of the 45 other strains of *Enterobacteriaceae* produced bacteriocins. Bacteriocin production was a stable epidemiological marker. Colonial mutants always had identical patterns, as did the same strain which has passed from patient to patient through cross-infection. The new technique does not require any specialized equipment and can be used in laboratories with limited budgets. The applications of the new method in cross-infection studies and as a supplement to serological typing are discussed.

Infections due to gram-negative bacteria continue to be a major hazard of hospitalization. *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella*, and *Proteus* often cause nosocomial infections. Recently, *Serratia marcescens* has been added to this list of hospital pathogens, and several epidemics and cases of cross-infection have been documented (5, 24).

Several epidemiological techniques have been used to differentiate strains of *S. marcescens*. Ewing, Davis, and Reavis (4, 5) reported a method for determining 15 O antigens and 13 H antigens, and serological differentiation based on this scheme has been used in epidemiology (5, 15, 20, 24). Differentiation based on bacteriophage susceptibility (16, 23) and bacteriocin production or sensitivity (11, 12, 17, 22) has been described, but only the recent method of Traub, Raymond, and Startxman (22) was designed for routine typing.

Farmer and Herman (8) recently developed a sensitive typing method for *P. aeruginosa* by testing a large number of bacteriocin indicator strains and choosing only those most useful for differentiation. The purpose of the present study was to develop a similar method for comparing and differentiating hospital strains of *S. marcescens*.

**MATERIALS AND METHODS**

**Media.** Trypticase soy broth (TSB) and Trypticase soy agar (TSA) were from BBL, and were prepared according to the instructions on the label. Special media were prepared as follows. Medium A contained: Difco Brain Heart Infusion (BHI), 18.5 g; Oxoid Ionagar no. 2 (Colab Laboratories, Inc., Chicago, Ill.), 9.3 g (or 17.4 g of Difco agar); streptomycin sulfate (Sigma Chemical Co., St. Louis, Mo.), 0.5 g; and distilled water, 500 ml. BHI agar, and water were combined in a 1-liter flask, a Teflon stirring bar was added, and the flask was autoclaved for 15 min at 121°C. The contents were cooled to 50°C, and streptomycin (although nonsterile powder was used, contamination was not observed) was added as the flask was stirred on a mechanical stirrer. The medium was then poured into petri plates (100 by 20 mm), 25 ml per plate. Medium B contained: Oxoid Ionagar no. 2, 4 g (or 7 g of Difco agar); and distilled water, 1,000 ml. The medium was heated to boiling, dispensed into screwcap test tubes (100 by 13 mm), 3 ml per tube, and autoclaved.

**Strains of S. marcescens.** The strains were from the following sources: 4 strains were obtained from I. L. Roth, University of Georgia, Athens, who had obtained them from R. P. Williams (10), Baylor University Medical School, Houston, Tex.; strain ATCC
14756 was obtained from M. T. Kenny (14), Dow Chemical Co., Zionsville, Ind.; strain 8 UK was obtained from R. J. Heckly (13), University of California, Berkeley; 23 different strains were obtained from J. A. Currie, University of Michigan, Ann Arbor, who had obtained them from W. H. Ewing (4), Center for Disease Control (CDC), Atlanta, Ga.; 12 different patient isolates were obtained from M. J. Ramirez (18), Emory University Hospital, Atlanta, Ga.; 10 different patient isolates were obtained from G. P. Bodey (2), M. D. Anderson Hospital and Tumor Institute, Houston, Tex.; 6 different patient isolates were obtained from J. N. Wilfert (24), Boston City Hospital, Boston, Mass.; 3 patient strains were obtained from C. H. Zierdt, Clinical Center, National Institutes of Health (NIH), Bethesda, Md.; 47 different patient isolates were obtained over 18 months from hospitals in the Washington, D.C., area; 9 marine and 10 river isolates were obtained from F. Denis, Laboratoire Le Dantec, Poitiers, France; and 3 environmental isolates were obtained from Leo Hart, Environmental Services Branch, NIH. The original identification as *S. marcescens* was confirmed for each strain. Among the criteria used were colonial morphology, positive reaction on deoxyribonuclease-toluidine blue-agar (19), biochemical reactions as described by Ewing, Davis, and Reavis (4), reaction to antisera prepared against the 15 O antigens described by Ewing (4, 5), and production of and sensitivity to bacteriocins and bacteriophages of known *S. marcescens* strains.

**Stock and working cultures.** Each strain was spread on a TSA plate and incubated at 37°C for 16 hr. A small amount of the growth was spread on the slant and stabbed into the butt of a TSA slant in a screw-cap tube (100 by 15 mm). The tube was incubated at 37°C for 16 hr and sealed with paraffin. This was designated the stock culture and was stored at room temperature in the dark without transfer (40 tubes are conveniently stored in a divided cardboard box available from De Freest Inc., Albany, N.Y.). The remainder of the growth on the TSA plate was removed with a cotton swab and mixed with 10 ml of 10% (w/v) skim milk in a screw-cap tube. This was designated the working culture and stored in the dark at 4°C without transfer. The latter storage method prevented the selection of new colonial types.

**Other strains of the family Enterobacteriaceae.** A typical strain of each of the other species of *Enterobacteriaceae* was obtained from V. R. Dowell, Enteric Bacteriology Unit, CDC. The species were: *E. coli*, *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*, *Edwardsiella tarda*, *Salmonella cholera-suis*, *S. typhi*, *S. enteritidis*, *Arizona hinshawii*, *Citrobacter freundii*, *K. pneumoniae*, *K. ozrenae*, *K. rhinoscleromatis*, *Enterobacter cloacae*, *E. aerogenes*, *E. hafniae*, *E. liquefaciens*, *Pectobacterium carotovorum*, *Proteus vulgaris*, *P. mirabilis*, *P. morganii*, *P. rettgeri*, *Providencia alcalifaciens*, and *P. stuartii*. Ten additional strains of *K. pneumoniae*, eight of *E. aerogenes*, and two of *E. cloacae* were obtained from different hospital patients. Klebsiella and Enterobacter are closely related to *Serratia* and were considered most likely to cross-react in the *Serratia* typing system.

**Mutants in colony type.** Stock cultures were made from isolated colonies grown on TSA plates. When these clonal cultures were streaked after storage at room temperature for several months, new colonial types were present. These new colonial types were called colonial dissociants, and were stored as skim milk working cultures.

**Bacteriocin production.** A number of methods were evaluated for bacteriocin production. The highest titers of bacteriocin were obtained when strains were grown on a reciprocal shaker and incubated with mitomycin C. However, previous experience from *P. aeruginosa* typing (8) had indicated that many laboratories do not have a mechanical shaker. Therefore, bacteriocins were produced in screw-cap test tubes without agitation (see standard method for details). Bacteria remaining after mitomycin induction were killed by 0.5 ml of chloroform which was added to the tube. All of the bacteriocins of *S. marcescens* were resistant to chloroform and were presumably similar to the type A bacteriocins described by Prinsloo (17).

**Bacteriocin assays.** Bacteriocins were diluted twofold in TSB by microtiter (Cooke Engineering Co., Alexandria, Va.). Each dilution was spotted (0.025 ml) onto bacteriocin indicator strains (see standard method for preparation), which were then incubated at 37°C for 18 to 24 hr. The end point was defined as the highest dilution that inhibited the indicator more than a control spot of TSB, and an arbitrary unit(s) of bacteriocin activity was defined as the reciprocal of this end point. If bacteriophage plaques were present, they were counted and converted to the number of plaque-forming units per milliliter.

**Bacteriocin indicator strains.** One hundred eighteen different *S. marcescens* indicators were screened against 56 bacteriocin producers. Most of the trial indicators were eliminated because they (i) differentiated the producer strains poorly, (ii) had too many zones of partial inhibition which could lead to difficulty in reading and reproducing results, or (iii) had too many zones due to bacteriophage lysis. The 23 best indicators were chosen for further evaluation.

**Streptomycin-resistant indicators.** The 23 trial indicators were then made streptomycin-resistant (str-r). Each indicator was spread on a TSA plate and incubated at 37°C for 18 hr. The growth was removed with a cotton swab and harvested into medium A, melted, and cooled to 50°C, to give 10¹⁴ to 10¹⁵ cells per plate. After 96 hr at 37°C, str-r mutants were picked and streaked on medium A. Stock and working cultures were then prepared as previously described, except that medium A was used instead of TSA. The indicators were made str-r so that the chloroform treatment could be eliminated. Traces of chloroform partially killed the indicator strains and made slight zones of inhibition which were difficult to distinguish from bacteriocin inhibition. The few viable cells remaining after mitomycin induction did not form colonies on medium A because they were streptomycin-sensitive. This procedure was modified from that of Prinsloo (17).

**Standard set of bacteriocin indicators.** Each of the 23 str-r trial indicators was then tested against
bacteriocins from 96 strains of *S. marcescens*, randomly selected from the collection (see standardized typing method for details of testing). The 2,208 bits of host-range data were assembled into an array and analyzed by an electronic computer which utilized a program (Farmer, Logan and Hutson, *to be published*) written to choose the nine indicators which gave the best differentiation of the 96 bacteriocin producers. The final set of indicators was coded 1 1 to 1 9, and was used to type all of the strains included in this report. Each of the indicators has been lyophilized and will be furnished in this form upon request.

**Turbidity standard.** *S. marcescens* strain 1 2 was inoculated into a screw-cap tube of TSB until it was just turbid. The tube was then incubated with the cap loose at 37 C for 24 hr. The turbidity standard was prepared by mixing 0.8 ml of the 24-hr culture with 9 ml of TSB. This turbidity standard has an optical density (OD) of 0.10 at 550 nm when the light path is 13 mm.

**Standardized method for typing S. marcescens by bacteriocin production.** Mitomycin lysates which contained bacteriocins (called bacteriocin lysates) were prepared in the following way. A small amount of growth was removed from a TSA plate with a straight wire and rubbed against the side of a screw-cap tube containing 4 ml of TSB. The tube was incubated in a water bath at 32 C (if an incubator is used, it must be set to insure a tube temperature of 32 C) for 24 hr. Then 0.7 ml was removed (conveniently done with a 1-ml Biopette from Becton, Dickinson and Co., Orangeburg, N.Y.) and added to 3.3 ml of TSB. The contents of tube were mixed, and the tube was returned to 32 C. After 1 hr, 1 ml of 25 μg/ml mitomycin C (Sigma Chemical Co.), dissolved in TSB, was added. The contents of the tube were vigorously mixed, and the tube was returned to 32 C for an additional 5 hr. During this period, the cells lysed and liberated bacteriocins (the curves are very similar to those in previously published figures (8)).

Lawns of each str-r indicator I 1 to I 9 were prepared as follows. A loopful of the litmus milk working culture was spread in a 1-cm diameter circle on medium A, which was then incubated at 37 C for 24 hr. Some growth was removed with a cotton swab and agitated with 9 ml of TSB until the turbidity was the same as the turbidity standard. Then 0.1 ml was removed and added to 3 ml of medium B which had been melted and cooled to 50 C. The contents of the tube were mixed and then overlaid onto a plate of medium A. The top was replaced and the overlay was allowed to solidify for 10 min. The top was removed, and the plate was dried for 3.5 to 4 hr at room temperature.

The bacteriocins (plus one control containing TSB and mitomycin) were then applied to each indicator strain plus a control plate. The bacteriocin lysates can be applied manually with disposable tuberculin syringes; however, 23 bacteriocins and one control can be added simultaneously with the ACCU-DROP bacteriophage-bacteriocin applicator (Sylvana Corp., Millburn, N.J.) as modified by Farmer (6). There was considerable time saving and convenience when the latter method was used. After the drops had dried, the plates were incubated at 37 C for 18 to 24 hr. The plates were read against a black background (the reader shown in Fig. 1 is available from the Sylvana Corp.) as follows: each bacteriocin zone was compared to the control zone (position 24 in Fig. 1), and any inhibition greater than the control was defined as positive. I also find it useful to record the degree of inhibition in the zone: for example, + = completely clear, + (50) = clear zone with about 50 colonies in it, etc. Thus, nine positives or negatives were recorded for each bacteriocin producer. It is difficult to write and discuss long rows of pluses or minuses, so a simplified method was devised for reporting bacteriocin types (7). Nine bacteriocin reactions were converted into a three-digit number according to the notation shown in Table 1. For example, a strain with a bacteriocin pattern "++- - - + + + -" would be coded 375 (+ + + = 3, - - + = 7, and ++ - = 5). Nonproducers were coded "888" and were considered nontypable.

**Serological typing.** A number of CDC strains had been typed by the Enteric Bacteriology Unit there. Several strains furnished by J. N. Wilfert (24) included serotype and clinical history. J. A. Currie determined the O antigens of the remainder of the strains with antisera prepared against O 1 to O 15 of Ewing (4, 5).

**RESULTS**

**Effect of temperature on bacteriocin production.** Since most laboratories have a 37 C incubator and water bath, this temperature was tested first for bacteriocin production. However, Table 2 shows that bacteriocin production was diminished at 37 C. There was a reduction in the number of positive reactions by about 75% when 180 reactions were considered. At 32 C, bacteriocin production was better, and this temperature was chosen for the final typing method.

**Effect of mitomycin induction on the amount of bacteriocin produced.** Table 3 shows that strains produced about 100 times as much bacteriocin when they were induced with 5 μg of mitomycin C/ml. Mitomycin induction

| Reaction against three indicator strains | Notation |
|-----------------------------------------|----------|
| ++ +                                   | 1        |
| ++ -                                   | 2        |
| + - +                                  | 3        |
| - + +                                  | 4        |
| + - -                                  | 5        |
| - + -                                  | 6        |
| - - +                                  | 7        |
| - - -                                  | 8        |
usually increased the amount of bacteriocin produced; however, SM 7 was changed from a nonproducer to a producer against SM 22. Mitomycin also induced phage production of SM 23 against SM 30, as none was produced without treatment. Zones of inhibition were due to bacteriophages about 3% of the time, and due to bacteriocins the remaining 97%.

Results from typing by bacteriocin production. Figure 1 shows clear zones due to bacteriocins on one of the indicators. Zones 1, 2, 3, 7, 8, 11, 15, 16, 22, and 23 are completely clear, but zone 13 has about 50 colonies growing in it. The remaining zones are identical to control 24, and are thus defined as negative.

Table 4 shows the sensitivity of typing by bacteriocin production in differentiating 129 different strains of S. marcescens. The strains were well distributed into different types, which is essential if the technique is to be sensitive in comparing strains. There were 11 strains (9%) which did not inhibit any of the nine indicators. None of the other patterns comprised more than 5% of the total.

Application in studying hospital-acquired infections. Table 5 shows the sensitivity of the method in establishing cross-infection or a single source outbreak. Study A comprised strains isolated over a 6-month period from patients on the same ward. There was no reason to suspect cross-infection or a single-source outbreak, and this hypothesis was confirmed when the bacteriocin production patterns were compared. Each patient clearly had a different strain. Study B comprised strains isolated from patients on closely related wards within 6 weeks. The incidence of S. marcescens had risen significantly, and there was reason to suspect cross-infection or a single-source outbreak. This hypothesis was confirmed, as seven of the eight patients had strains with identical bacteriocin production patterns. These seven were also the same serotype and had identical antibiotic-susceptibility patterns. However, patient B3 had a strain which was not a result of this outbreak.

Stability of bacteriocin production as an

| Producer | Temp (°C) | Bacteriocin production against indicator |
|----------|----------|----------------------------------------|
|          | 11       | 23                                      |
|          |          | 5                                        |
|          |          | 6                                        |
|          |          | 8                                        |

Table 2. Effect of growth temperature on bacteriocin production.

| Producer | Mitomycin addition | Units of bacteriocin activity against |
|----------|--------------------|--------------------------------------|
|          | SM 22              | SM 30 | SM 33 | SM 35 | SM 39 |
| SM 7     | -                  | 0     | 0     | 8     | 8     | 4     |
|         | +                  | 400   | 0     | 1,600 | 800   | 400   |
| 15       | -                  | 2     | 1     | 0     | 0     | 1     |
|         | +                  | 200   | 100   | 0     | 0     | 800   |
| 23       | -                  | 1     | 0     | 4     | 1     | 4     |
|         | +                  | 100   | 0 (phage) | 400   | 200   | 200   |

* In this experiment the end point was defined to be the last dilution that gave complete inhibition, rather than any inhibition.

* Present at $10^6$ plaque-forming units per ml.
epidemiological marker. In several experiments, as many as 10 colonies were picked from the same plate and typed, and bacteriocin production patterns were identical each time. Stock cultures tested over several months also had identical patterns. Table 5 shows that different colony types derived from the same strain had identical bacteriocin production patterns. Table 5 shows that the bacteriocin production of an epidemic strain was stable even though it had passed from patient to patient. Multiple isolates from the same patient over a period of months also typed identically. None of the experiments designed to test the stability of bacteriocin production indicated any variation, even though these were considerable ecological pressure both in vivo and in vitro.

Bacteriocin production as a supplement to serological typing. Table 7 illustrates that

### Table 4. Bacteriocin production patterns of 129 strains

| Pattern | No. of strains | Pattern | No. of strains | Pattern | No. of strains |
|---------|----------------|---------|----------------|---------|----------------|
| 111     | 3              | 411     | 1              | 721     | 1              |
| 114     | 1              | 413     | 2              | 722     | 1              |
| 121     | 1              | 414     | 1              | 727     | 1              |
| 131     | 4              | 421     | 1              | 742     | 1              |
| 134     | 3              | 422     | 1              | 758     | 1              |
| 136     | 1              | 431     | 1              | 788     | 1              |
| 137     | 1              | 433     | 2              |         |                |
| 138     | 1              | 437     | 3              | 824     | 1              |
| 141     | 1              | 481     | 1              | 837     | 3              |
| 143     | 1              |         |                | 857     | 1              |
| 211     | 2              | 512     | 1              | 866     | 2              |
| 213     | 1              | 537     | 1              | 868     | 1              |
| 214     | 1              | 564     | 2              | 877     | 2              |
| 221     | 1              | 577     | 4              | 885     | 1              |
| 222     | 1              | 586     | 1              | 886     | 1              |
| 231     | 1              | 587     | 1              | 887     | 2              |
| 236     | 2              | 588     | 5              | 888     | 11             |
| 241     | 1              |         |                |         |                |
| 242     | 1              | 612     | 2              |         |                |
| 258     | 1              | 622     | 1              |         |                |
| 265     | 1              | 633     | 1              |         |                |
| 273     | 1              | 637     | 1              |         |                |
| 274     | 2              | 653     | 1              |         |                |
| 311     | 5              | 664     | 1              |         |                |
| 321     | 1              | 677     | 1              |         |                |
| 332     | 1              | 685     | 1              |         |                |
| 341     | 6              | 688     | 4              |         |                |
| 342     | 1              |         |                |         |                |
| 378     | 1              |         |                |         |                |

* Multiple isolates, shown by bacteriocin and serological typing and antibiotic susceptibility to be the same strain, are included only once.

### Table 5. Bacteriocin production in determining the epidemiology of hospital infections

| Study | Isolate from patient | Specimen | Bacteriocin production pattern |
|-------|----------------------|----------|-------------------------------|
| A     | A1                   | Sputum   | 241                           |
|       | 2                    | Urine    | 131                           |
|       | 3                    | Sputum   | 858                           |
|       | 4                    | Urine    | 586                           |
|       | 5                    | Urine    | 141                           |
| B     | B1                   | Blood    | 838                           |
|       | 2                    |          | 838                           |
|       | 3                    |          | 678                           |
|       | 4                    |          | 838                           |
|       | 5                    |          | 838                           |
|       | 6                    |          | 838                           |
|       | 7                    |          | 838                           |
|       | 8                    |          | 838                           |

### Table 6. Bacteriocin production patterns of colonial dissociants

| Test strain | Bacteriocin production pattern |
|-------------|--------------------------------|
| 18-68-1*a  | 136                            |
| 1A          | 136                            |
| 1B          | 136                            |
| 1C          | 136                            |
| 6           | 283                            |
| 6A          | 283                            |
| 7           | 237                            |
| 7A          | 237                            |
| 9           | 541                            |
| 9A          | 541                            |

* The parent colony type is the first of the group, and the dissociants follow with an A, B, or C.

### Table 7. Differentiation of strains undifferentiated by serological typing

| Strain | Serotype  | Bacteriocin production pattern |
|--------|-----------|-------------------------------|
| SM 22  | O 9, H 11 | 282                           |
| 58     | O 9, H 11 | 888                           |
| 21     | O 2, H 4  | 111                           |
| 57     | O 2, H 4  | 277                           |
| 86     | NT*       | 612                           |
| 115    |           | 274                           |
| 154    |           | 221                           |
| 162    |           | 677                           |

*NT: no reaction to antisera against Ewing's O 1 to O 15.
results from serological typing can sometimes be misleading. Strains SM 22 and SM 58 had the same O and H antigens but were shown to be different strains by their bacteriocin production patterns. The latter finding was in agreement with epidemiological data, since SM 22 was from the sputum of a hospital patient and SM 58 was from the nasal swab of an accident patient who had never been in the hospital before. In one instance, erroneous results would have been obtained if serological typing had been the only epidemiological technique used. SM 21 and SM 57 had the same O and H antigens and were isolated from the urines of two different patients who had been hospitalized on the same ward at different times. This had appeared to be cross-infection from the serological evidence alone; however, bacteriocin typing indicated that the strains were different. Two hypotheses could explain this case: (i) the strains were the same (clonal) but one had mutated in bacteriocin production; (ii) the strains were different (not clonal) and had the same serotype only by chance. It was difficult to decide which hypothesis was correct until it was noted that SM 21 had a high back-mutation rate from white to red, whereas SM 57 never mutated from white to red. This evidence strongly supported the hypothesis that the strains were different. Table 7 shows that the strains with undefined O antigens can be differentiated by bacteriocin typing.

Bacteriocin production by strains isolated from nature. Most of the strains described in this paper were nonpigmented isolates from hospital patients, and could have evolved to be quite different from those found in nature. Table 8 shows the bacteriocin production patterns of 10 strains (from F. Denis) isolated from natural waters. All of the strains had bacteriocin production patterns and O antigens quite similar to the strains from patients. However, it is possible that some or all of the strains were of human origin.

Supplementary techniques to compare strains with the same bacteriocin production pattern. When many strains are typed, unrelated strains may have the same bacteriocin production pattern simply by chance (Table 4). These strains can often be differentiated if the conditions of bacteriocin production are changed. For example, Table 2 shows that 18 different reactions are obtained if bacteriocins are produced at both 32 and 37°C. The only limitation to this type of modification is that all strains must be compared under carefully standardized conditions.

Typability of other Enterobacteriaceae. Bacteriocin lysates were prepared (standard production method) from the 45 other strains of Enterobacteriaceae and added to the nine str-r S. marcescens indicator strains. The results were uniformly negative: of 405 possible reactions, only three zones of very weak inhibition (300 colonies or more in the zone) were observed. Providencia stuartii partially inhibited I 7 and I 9, and Enterobacter aerogenes 45 partially inhibited I 7. It appears that this bacteriocin typing method will be limited to S. marcescens.

DISCUSSION

In the past few decades, infections caused by gram-negative bacteria have increased alarmingly. S. marcescens was once considered a harmless saprophyte, but has now been added to the list of bacteria which can cause serious infections, particularly in hospital patients. Evidence has accumulated that institutions which have a high incidence of S. marcescens in patient specimens are having serious cross-infection or a single-source outbreak. However, in many institutions, S. marcescens infections go unreported because the strains are incorrectly identified, or grouped with Klebsiella-Enterobacter. In 1968, the Laboratory Approval Program of the Massachusetts Department of Public Health found that only 40% of 121 laboratories could identify a nonpigmented strain of S. marcescens (24).

Epidemiological surveillance of S. marcescens infections has been limited because there has been no simple technique for comparing strains. The serological method of Ewing has

| Strain | Source | Bacteriocin production pattern | O antigen |
|--------|--------|-------------------------------|-----------|
| SM 115 | Marine | 274                           | NT*       |
| 116    |       | 511                           | 8         |
| 117    |       | 274                           | 12        |
| 118    |       | 727                           | 9         |
| 119    |       | 258                           | 10        |
| 120    |       | 311                           | 8         |
| SM 124 | River  | 311                           | 8         |
| 125    |       | 686                           | 5         |
| 126    |       | 222                           | 5         |
| 127    |       | 622                           | 6         |

*NT: no reaction to antisera produced against Ewing’s O 1 to O 15.
proved useful in differentiating strains, but unfortunately the antisera are not available commercially. Many of the studies which used serological differentiation were done only through the cooperation of the CDC. The reliability of serological typing based on O and H antigens has been unquestioned. However, the present study has shown that two strains can have both O and H in common but still be unrelated epidemiologically. Therefore, bacteriocin typing can be a useful supplement to serological typing.

Bacteriocin production has been used as an epidemiological marker for a number of gram-negative bacteria. Typing methods have been described for Shigella sonnei (1), Proteus (3), Klebsiella (21), and Pseudomonas aeruginosa (6, 9). Hamon and Peron (12) found that 86% of their 85 Serratia strains produced bacteriocins. Prinsloo (17) found that Serratia produced two kinds of inhibitory substances. Group A bacteriocins were heat-stable, resistant to chloroform and trypsin, and active on Serratia, Salmonella, Escherichia, and Enterobacter. Group B bacteriocins were inactivated by heat, chloroform, and trypsin, and were active on Escherichia and Enterobacter but inactive on Serratia. Of the 139 strains examined by Prinsloo, 36 produced group A bacteriocins, 19 produced group B, 35 produced groups A and B, and 49 were nonproducers.

The typing method described in the present report was designed for routine typing. Ultraviolet light has been used to stimulate bacteriocin production, but its use is time-consuming and severely limits the number of strains which can be typed reproducibly. Farmer and Herman (8) showed that mitomycin C, which also stimulates bacteriocin production, is applicable to routine typing. Bacteriocin production in broth is preferable to production on agar surfaces (9) for a number of reasons: (i) mitomycin C is easily added to stimulate bacteriocin production, (ii) the bacteriocins can be added quickly to indicator strains, (iii) inhibition zones are easily compared when two isolates appear to be the same strain. The size and shape of zones from duplicate strains are identical and the same number of colonies develop in zones of partial inhibition. These similarities are particularly obvious if the indicator is prepared as a soft-agar overlay. The stability of bacteriocin production as an epidemiological marker for S. marcescens is in agreement with numerous studies with other bacteria (1, 3, 8, 9).

This new method for typing S. marcescens has proved useful in tracing hospital infections. The high degree of sensitivity was obtained because the set of indicator strains was chosen with the aid of computer analysis. With these indicators, 91% of the strains were typable, and additional indicators will be added to increase the number of typable strains and to increase sensitivity. The typing procedure has been described in sufficient detail in this report to eliminate most variables that have existed in previous bacteriocin typing procedures. The technique in its simplest form requires a minimum of special equipment and should be applicable in laboratories with a limited budget.

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