Bacteriocin (Marcescin) Typing of Clinical Isolates of Serratia marcescens

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A simple, reproducible technique is described for bacteriocin typing of clinical isolates of Serratia marcescens. With 10 marcescin-producer strains, a total of 46 of 50 isolates (92%) of S. marcescens could be typed and categorized into 16 provisional types according to their sensitivity or tolerance to marcescins. The potential significance of this procedure with regard to delineation of outbreaks of nosocomial infection is discussed on the basis of preliminary epidemiological data.

Bacteria produce proteinaceous antibiotics, known as bacteriocins, that kill sensitive bacteria (5, 7). Bacteriocins have been used for typing clinical isolates of Shigella sonnei (1) and Pseudomonas aeruginosa (3, 10). Almost a decade ago, Hamon and Peron (6) reported that 76 of 85 isolates of Serratia marcescens produced bacteriocins, referred to as marcescins. It was noted that many of the detected bacteriocins were trypsin-sensitive and active against strains of Escherichia coli as well; subsequently, Prinsloo (8) was able to demonstrate that S. marcescens produced two types of bacteriocins: group A bacteriocins were active mainly against S. marcescens, were produced spontaneously, and were resistant to trypsin, chloroform, and heat; group B bacteriocins were not active against S. marcescens and were sensitive to trypsin, chloroform, and heat. Recently Foulds and Shemin (4) described an inducible, trypsin-sensitive bacteriocin elaborated by S. marcescens that was inactive against Serratia, thus corresponding to Prinsloo's group B of marcescins. Prinsloo was able to subdivide her group A marcescins into eight subgroups based on their spectrum of activity and through use of resistant mutants of indicator strains. With this background information at hand, the present study served to determine the feasibility of marcescin typing of 50 clinical isolates of S. marcescens. In this paper, we wish to describe the technique and report preliminary epidemiological findings.

MATERIALS AND METHODS

Bacteria. A total of 50 isolates of S. marcescens from various clinical sources were examined. Of these, 24 isolates had been cultivated in the spring of 1969 and had been frozen and kept stored at −65 C in Brain Heart Infusion broth with 50% added heat-inactivated horse serum; the remaining 26 isolates were procured between 1 June and 31 July 1970. The isolates were coded and identified according to previously published criteria (9) and were maintained on Brain Heart Infusion agar slants at 4 C.

Media. MacConkey broth (MACB), MacConkey agar without crystal violet (MAC), Trypticase soy broth (TSB) and agar (TSA), Mueller-Hinton broth (MHB) and agar (MHA), nutrient broth (NB), and Brain Heart Infusion broth (BHIB) were purchased from Difco; MacConkey agar with added crystal violet (MAC-CV) was bought from Fisher Scientific Co.

Mitomycin C. Nonsterile mitomycin C powder (lot 99B-0090) was obtained from Sigma Chemical Co., St. Louis, Mo. The powder was dissolved in sterile distilled water to yield 10 μg/ml. The solution was passed through 0.2-μm membrane filters (Nalge Sybron Corp., Rochester, N.Y.) and dispensed in 2-ml amounts into sterile screw-capped vials which were frozen and kept stored at −15 C in the dark. The vials were not refrozen after they had been thawed.

Bacteriocin (marcescin) typing of S. marcescens. Through trial and error, the following procedure was developed for induction of marcescins and bacteriocin typing of S. marcescens: at least five colonies of each of 10 marcescin producers were picked from MAC plates (control for viability and purity) and inoculated into 2.5 ml of TSB in tubes (13 by 100 mm) which were incubated at 33 C for 6 hr. Next, the growths were transferred to screw-capped tubes (15 by 125 mm) containing 9 ml of TSB to yield 1.5 × 10^6 organisms/ml (McFarland barium sulfate standard 0.5), which were incubated at 33 C for 2 hr, after which 1 ml of 10 μg of mitomycin C per ml was added to each tube (final mitomycin C concentration, 1 μg/ml); control tubes received 9 ml of TSB and 1 ml of mitomycin C stock solution. The tubes were incubated at 33 C for 8 hr in the dark; after induction, 1 ml of chloroform (certified A.C.S., Fisher Scientific Co.) was added to each tube. The tubes were shaken violently 20 times and centrifuged at
1,000 × g for 10 min. The supernatant fluids were carefully removed and transferred to sterile disposable petri dishes (15 by 100 mm); these were aerated for at least 10 min. Isolates of S. marcescens to be typed had been pregrown in 2.5 ml of TSB at 33 C overnight. The turbidity of the organisms was adjusted to that of McFarland standard 0.5 (corresponding to 1.5 × 10⁹ organisms/ml) in saline; these suspensions were diluted 10-fold further in TSB. Large MAC (or MAC-CV) plates (15 by 150 mm, 80 ml of medium per plate) were divided into appropriate numbers of sectors, including a sector for the TSB-mitomycin C control; the plates were streaked with the bacterial inocula in three planes and subsequently were dried for 10 min. Finally, one drop (0.05 ml) of each marcescin preparation, including the control, was delivered to respectively designated sectors. The drop inocula were allowed to “dry in” for 20 min, after which the plates were incubated at 33 C overnight. Next morning, the plates were examined for the presence or absence of marcescin-produced plaques. Sectors were scored as positive if there were completely clear plaques or if the areas of inhibition contained less than 50 marcescin-resistant variant colonies against a clear background. Those plaques that revealed greater than 50 variant colonies and those sectors that showed no inhibition or trace inhibition of growth only were interpreted as negative.

**Trypsin.** A 1% (w/v) solution of trypsin (1:250; Difco certified) in phosphate-buffered saline (PBS), pH 7.4 (Grand Island Biological Co., Grand Island, N.Y.), served to test marcescins for trypsin sensitivity. To 1.0 ml of marcescin was added 0.2 ml of the 1% trypsin solution; controls received 0.2 ml of PBS. The tubes were incubated for 1 hr at 37 C, after which the trypsin-treated and untreated marcescin preparations were examined for activity against appropriate indicator strains of S. marcescens.

**“Titration” of marcescins.** Supernatant fluids were serially diluted twofold in TSB over the range of 1:2 through 1:256; 0.05 ml of each dilution was delivered to corresponding sectors of indicator strain-streaked MAC plates. “Titers” were defined as the highest dilutions of marcescins yielding positive responses.

**RESULTS**

Relatively few of the S. marcescens isolates spontaneously elaborated marcescins. Therefore, all isolates were induced with mitomycin C at a final concentration of 1 µg/ml. Incubation at 33 C was superior to that at 37 or 25 C. It was found that growth on MAC yielded larger and more clearly outlined marcescin-produced zones of clearing than appeared on either MHA or TSA; MAC and MAC-CV agar could be used interchangeably, although the former medium tended to give somewhat larger plaques. Of the broths tested, NB and MACB proved unsatisfactory for marcescin induction. None of the detected marcescins was active against their respective producer strains. Marcescins were produced by pigmented as well as nonpigmented isolates of S. marcescens. The presence of bacteriophage was ruled out by failure to transfer activity (8). The marcescin preparations had to be well aerated after addition of chloroform and centrifugation. The “titers” of marcescins amounted to 1:128 in most instances. Peak activity occurred at 6 to 8 hr after induction; “titers” remained at peak levels for 24 hr after induction. The marcescins were inactive against E. coli and proved resistant to trypsin; thus, they presumably correspond to Prinsloo’s (8) subgroup A of Serratia bacteriocins.

After these preliminary experiments, all 50 isolates of S. marcescens were simultaneously induced and examined for bacteriocin production and susceptibility in a checkerboard fashion (2,500 sectors, plus 50 control sectors). A total of 37 isolates (74%) regularly produced marcescins after induction. None of the control sectors revealed evidence of growth inhibition. All but four strains (no. 2, 22, 30, and 44) proved susceptible to one or more of the marcescins. A significant number of isolates produced marcescins with identical host ranges. Computer analysis (model 3200, Control Data Corp., Minneapolis, Minn.) of the data allowed one to limit the number of marcescin producers to 10 isolates (strains no. 5, 10, 12, 16, 17, 18, 31, 33, 43, and 46) yet maintain the extent of typability of S. marcescens isolates at the same level as before (46/50 = 92% typed). A total of 16 marcescin types could be differentiated under our experimental conditions (Table 1).

It was observed that a number of S. marcescens strains (among isolates no. 3 to 26) isolated during 1969 belonged to the same marcescin types as some of those that had been isolated during the summer of 1970, suggesting extended prevalence or reintroduction of certain strains of S. marcescens within our hospital environment. Inspection of Table 2, which summarizes a limited number of epidemiological data (isolates no. 1, 2, and 27 to 50), discloses several patients whose sputum or wound exudate cultures repeatedly yielded S. marcescens over periods of 2 to 4 weeks, all of which belonged to the same marcescin type, respectively. In one instance, nosocomial infection within the same room of a ward was demonstrable (marcescin type IX, isolates 28, 29, and 34 from two patients in room 225). On the other hand, S. marcescens isolates no. 46 (marcescin type IV) and 42 (marcescin type XIV) were isolated from two patients both of whom were in the intensive care unit on the date (7/12/70) that their sputum
TABLE 1. Results of bacteriocin typing of 50 clinical isolates of Serratia marcescens with 10 marcescin-producing strains

| Marcescin type | Host range of 10 marcescin producer strains$^a$ | Isolates yielding specified marcescin patterns | Total (50) |
|----------------|-----------------------------------------------|----------------------------------------------|------------|
| I              | $+$ $-$ $-$ $+$ $-$ $-$ $-$ $-$ $-$ $-$       | 1, 48                                        | 2          |
| $+$            |                                              | 2, 22, 30, 44                               | 4          |
| II             | $+$ $-$ $-$ $-$ $+$ $-$ $-$ $-$ $-$ $-$       | 3                                            | 1          |
| III            | $+$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$       | 4, 6, 31, 50                                | 4          |
| IV             | $-$ $-$ $-$ $-$ $+$ $-$ $-$ $-$ $-$ $-$ $-$   | 5, 13, 17, 18, 23, 39, 40, 46               | 8          |
| V              | $+$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$       | 7, 20, 26                                   | 3          |
| VI             | $-$ $+$ $-$ $+$ $-$ $+$ $-$ $-$ $-$ $-$ $-$  | 8, 9                                        | 2          |
| VII            | $-$ $-$ $-$ $-$ $+$ $-$ $-$ $-$ $-$ $-$ $-$  | 10, 16                                      | 2          |
| VIII           | $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$  | 11                                           | 1          |
| IX             | $-$ $-$ $-$ $-$ $+$ $-$ $-$ $-$ $-$ $-$ $-$  | 12, 14, 19, 21, 24, 25, 28, 29, 34, 36, 41 | 11         |
| X              | $+$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$  | 15                                           | 1          |
| XI             | $+$ $+$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$  | 27                                           | 1          |
| XII            | $+$ $-$ $-$ $-$ $-$ $+$ $-$ $-$ $-$ $-$ $-$  | 32, 37, 38                                  | 3          |
| XIII           | $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$  | 33                                           | 1          |
| XIV            | $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$  | 35, 42, 43, 47                              | 4          |
| XV             | $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$  | 45                                           | 1          |
| XVI            | $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$ $+$  | 49                                           | 1          |

$^a$ Symbols: $+$, sensitive; $-$, resistant to respective marcescin.

$^b$ Nontypable.

TABLE 2. Clinical sources of Serratia marcescens isolates of nosocomial significance

| Marcescin type | Isolate code no. | Date isolated | Source$^a$ | Location$^b$ | Service | Patient (initials) |
|----------------|------------------|---------------|------------|--------------|---------|-------------------|
| I              | 1                | 7/8/70        | Ex         | 340          | Med-S   | L.B.              |
|                | 48               | 7/20/70       | Sp         | 333          | Med-P   | G.B.              |
| III            | 31               | 6/26/70       | Eye        | NBN          | Ped-S   | Baby S.           |
|                | 50               | 7/24/70       | Sp         | 363          | Med-P   | V.H.              |
| IV             | 39               | 6/18/70       | Sp         | 417          | Med-P   | C.A.              |
|                | 40               | 6/21/70       | Sp         | 417          | Med-P   | C.A.              |
|                | 46               | 7/12/70       | Sp         | 265          | Surg-P  | E.S.              |
| IX             | 28               | 6/28/70       | Ex         | 225          | Surg-S  | D.R.              |
|                | 29               | 6/13/70       | Ex         | 225          | Surg-S  | D.R.              |
|                | 34               | 6/21/70       | Sp         | 225          | Urol-S  | M.S.              |
|                | 36               | 7/3/70        | Sp         | 317          | Med-P   | S.G.              |
|                | 41               | 7/10/70       | Sp         | 265          | Surg-P  | C.R.              |
| XII            | 32               | 6/18/70       | Ex         | 420          | Surg-S  | N.M.              |
|                | 37               | 7/7/70        | Ex         | 420          | Surg-S  | N.M.              |
|                | 38               | 6/10/70       | Ex         | 420          | Surg-S  | N.M.              |
| XIV            | 35               | 6/9/70        | Sp         | 352          | Med-P   | G.S.              |
|                | 42               | 7/12/70       | Sp         | 352          | Surg-P  | P.P.              |
|                | 43               | 7/16/70       | Sp         | 352          | Surg-P  | P.P.              |
|                | 47               | 7/20/70       | Blood      | 559          | Surg-P  | P.P.              |

$^a$ Ex, exudate; sp, sputum.

$^b$ NBN, newborn nursery; ICU, intensive care unit.
specimens were submitted for culture, indicating that two different strains of *S. marcescens* were prevalent within that unit on that particular date. Further inspection of Table 2 reveals also that certain strains of *S. marcescens* were ubiquitous within our hospital, i.e., were retrieved from different patients located in various wards (e.g., strains of marcescin types IV, IX, and XIV).

**DISCUSSION**

The described procedure for bacteriocin (marcescin) typing of clinical isolates of *S. marcescens* appears to be a simple and reproducible procedure; repeated typing experiments yielded identical results. Furthermore, all strains of *S. marcescens* isolated from four patients over extended periods of time were demonstrated to belong to the same marcescin type, respectively, a finding further attesting to the reproducibility of this technique.

It should be emphasized that the categorization of the 46 typable isolates of *S. marcescens* into 16 marcescin types is tentative. It is expected that future outbreaks of nosocomial infection in our hospital might very well be caused by newly introduced strains of *S. marcescens* that are of different sensitivity/tolerance to marcescins.

Our preliminary findings indicate that infections due to various marcescin types of this organism can be detected by this procedure. This information is important for hospital infection control committees in their efforts to discover outbreaks of infection as quickly as possible so as to promptly institute appropriate control measures (2).

Very recently, Wilfert et al. (11) serotyped 95 isolates of *S. marcescens* and found four serotypes (02:H4, 04:H1, 011:H4, and 011:H13) to be the most prevalent at Boston City Hospital. It would be of interest to determine whether serotypes of *S. marcescens* can be further subdivided through marcescin typing, or vice versa. Unfortunately, specific anti-O and anti-H *S. marcescens* hyperimmune sera were not available.

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