Use of Molecular Typing To Study the Epidemiology of Serratia marcescens

A. McGEER,¹ D. E. LOW,¹ J. PENNER,² J. NG,¹ C. GOLDMAN,¹ and A. E. SIMOR³*

Department of Microbiology, Mount Sinai Hospital, Toronto, Ontario M5G 1X5,¹ and Department of Microbiology, University of Toronto, Toronto, Ontario M5S 1A1,² Canada

Received 25 May 1989/Accepted 2 October 1989

Although Serratia marcescens is a well-known nosocomial pathogen, investigation of its hospital ecology has been limited by the lack of available typing techniques. During an investigation of the occurrence of this organism in a neonatal intensive care unit, we evaluated a number of such techniques. Using a selective medium, we conducted prospective surveillance of neonatal rectal colonization and environmental contamination with S. marcescens. In 8 months of surveillance, 5.1% (20 of 394) of the infants admitted to the unit became colonized. Most sink surfaces and drains were also culture positive. Differences between isolates could not be detected in biotypes from a commercial identification system (MicroScan) or by antibiograms, total protein fingerprints, or plasmid profiles. Serogrouping and genomic DNA restriction endonuclease analysis revealed the presence of six strains that colonized infants and a similar number of environmental strains. These two methods were concordant, with the exception that genomic DNA analysis demonstrated lack of relatedness between some strains within the same serogroup. DNA restriction endonuclease analysis was practical and reliable. The differences this method detected between environmental and neonatal strains provided strong evidence that the environment was not an important reservoir for S. marcescens in our neonatal intensive care unit.

Serratia marcescens is a well-known cause of nosocomial pneumonia, meningitis, and septicemia in critically ill neonates (1, 5). Studies during the 1970s suggested that neonatal colonization and infection were rare events (12, 13, 15, 23, 27). However, from 1982 to 1987, at least eight publications documented epidemics of serious S. marcescens infections in neonatal intensive care units (NICUs) (8-10, 17, 22, 28, 31, 36).

Numerous methods have been proposed for typing S. marcescens. Antibiograms and commercially available biotyping schemes have been used, but published opinions on their reliability vary, and they appear to lack sensitivity (1, 26). Phage typing, bacteriocin typing, and serotyping are available in only a few reference laboratories, and their performance characteristics have infrequently been tested. Protein fingerprinting by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis with or without [35S]methionine labeling has been evaluated in three reports with conflicting conclusions (4, 10). Several studies have shown plasmid profiling to be of use for typing S. marcescens (19, 30, 33), but none have compared this technique with other methods. Data on the role of restriction endonuclease analysis (REA) of genomic DNA are limited to a single abstract, in which a unified typing scheme consisting of both plasmid profiling and genomic DNA REA is proposed (H. Białkowska-Hobrzanska, D. McClory, V. Harry, H. Richardson, and A. Simor, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C-204, p. 357).

We conducted an 8-month study of the occurrence of S. marcescens in our NICU. The multiple neonatal and environmental isolates identified during the study allowed us to evaluate the utility of various typing techniques in the investigation of the hospital epidemiology of this organism. (Presented in part at the 88th Annual Meeting of the American Society for Microbiology, Miami, May 1988 [Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, L-7, p. 412].)

MATERIALS AND METHODS

Background. The NICU and special care unit are 31 and 20 isolette units, respectively. Each unit is open, with a single isolation room with its own anteroom and sinks located at the unit entrance and between isolettes (eight in the NICU and six in the special care unit). Critically ill neonates are admitted to the NICU; less ill babies are admitted to the special care unit. Infants may be moved from one unit to the other during their hospitalization and may be discharged from either unit. The combined units average 600 admissions per year, with an average length of stay of 20 days.

Surveillance. Prevalence screening of neonates using rectal and pharyngeal swabs was carried out on two occasions in January and February of 1987. Surveillance rectal swabs from all infants were collected biweekly from mid-February until April and then weekly until the end of October 1987. During March, extensive environmental sampling was done in both the NICU and the special care unit. Previously described methods (35) were used to collect 600 specimens from incubators, respiratory care equipment, suction jars, various medical solutions (including ointments, emoliants, and hand soap), sinks (including surfaces, drains, and aerators), and medical equipment shared by neonates (e.g., blood pressure cuffs and thermometer bases). Weekly surveillance of sites found to be positive on initial surveys was continued until October.

During unannounced visits to the units, 79 hand washes of medical and nursing staff were collected. Individual staff members agitated each hand for 1 min in a sterile bag containing 50 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) which was incubated overnight and cultured as described below.

Microbiology. All specimens from patients and the environment were incubated at 37°C on MacConkey agar (Oxoid

* Corresponding author.
colonized infants were also isolated from damp areas on sink surfaces and from sink drains. Five additional strains were isolated only from the environment: four from sink drains and one from both sink surfaces and drains. No other environmental cultures were positive, and none of the handwash cultures of unit staff members were positive.

Strain A (Table 1) colonized 11 infants: 3 identified in the prevalence surveys and 8 who acquired the organism during the surveillance period. This strain was also isolated intermittently from the wet surfaces and drains of 10 of 14 sinks in the units. Strains B, C, D, and F were isolated from rectal swabs of single infants. Strain E was first isolated simultaneously from rectal swabs of three infants in June. Five additional infants became colonized with this strain within the next 5 weeks. Concern about the rapid dissemination of this strain resulted in collection of all colonized infants into a cohort; this measure was associated with the disappearance of both strains A and E from specimens from infants and strain A from environmental specimens. Late in July, after the last infant colonized had acquired strain E, this strain was first identified in an environmental culture (from a sink surface). Strain E was then isolated intermittently from the surfaces of 4 of 14 sinks through August and September.

Eighty-seven percent (20 of 23) of infants who became colonized with *S. marcescens* remained colonized for the rest of their stay in the unit.

**Microbiologic investigation.** The phenotypic characteristics of the six strains of *S. marcescens* isolated from neonates are shown in Table 1. All but one had identical MicroScan biotypes, and that one differed by a single reaction. Most of the isolates from other sources at our hospital were also of this biotype. All strains were resistant to cephalothin but susceptible to piperacillin, cefotaxime, gentamicin, amikacin, tobramycin, and trimethoprim-sulfamethoxazole.

None of the neonatal isolates contained plasmids, with the exception of strain B, which contained a single low-molecular-weight plasmid. Total protein fingerprinting yielded identical patterns for all isolates from infants, with the exception of strain F, which differed by a single protein band (data not shown).

Seroserotyping distinguished five of the six strains (Table 1). However, *REA* provided the most sensitive means of typing. There were clear and reproducible differences among all strains with each of four endonucleases (Fig. 1 and 2). The visual patterns of the different strains were easily distinguished. Different DNA preparations from the same isolates were identical, and different gels were sufficiently reproducible to be compared to each other. The results of seroserotyping and *REA* were concordant, except that *REA* found
differences between strains B and C and between strain F and two other epidemiologically unrelated hospital isolates within the same serogroup (Fig. 2).

**DISCUSSION**

Until very recently, epidemiologic investigation of nosocomial infection with *S. marcescens* has been limited by the lack of availability of typing techniques. Often most of the strains from one hospital have the same biotype according to commercial identification systems (18) and, as in this study, similar antibiograms. Thus, these characteristics cannot be relied on to discriminate between strains within a hospital. More discriminatory methods are either lengthy and labor-intensive (the biotyping system of Grimont et al. [14, 29]) or available through only a few reference laboratories (serotyping, phage typing, and bacteriocin typing [24, 26, 34]).

However, molecular and electrophoretic methods for finger-printing protein and DNA are now feasible in many laboratories. Determination of phenotype by protein finge-printing by SDS-polyacrylamide gel electrophoresis has been used to examine genetic relatedness among strains of a number of bacterial species, but in general, it appears to lack sufficient sensitivity for use as an epidemiologic tool (2, 20, 21, 25, 32). This typing method distinguished between an outbreak strain and a reference strain of *S. marcescens* in one study (10) but could not differentiate among the five strains of another (Bialkowska-Hobrzańska et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987). In a third study, [35S]methionine and computerized densitometry were used (4). Again, differences were evident when strains from different hospitals were compared, but strains of differing serotypes from the same hospital had very similar profiles. Our results are in concordance with these reported findings: protein finger-printing is insufficiently sensitive to discriminate between different strains from one hospital. There is good evidence for a number of species that genetic analysis of plasmid and chromosomal DNAs is more accurate than systems dependent on analysis of gene products. Plasmid profiling has been shown in several studies (19, 30, 33) to be a valuable epidemiologic tool for typing *S. marcescens*, but the strains from our NICU lacked plasmids. However, the isolation and REA analysis of chromosomal DNA provided a reliable, practical method of typing strains. When compared with the combination of epidemiologic evidence, biotyping with a commercial identification system, antibiogram, and serogrouping, the method appeared to be both sensitive and specific. REA analysis of total genomic DNA thus enables optimal discrimination of isolates with a single test.

*S. marcescens* grows well in moist environments, and solutions and damp surfaces have been implicated as reservoirs in several nosocomial outbreaks involving this organism (1, 3, 18, 37, 38). Until the results of typing studies were available, there was considerable concern in our NICU that the surfaces of sinks in the unit were a significant reservoir. However, in the final analysis, there are several lines of evidence to suggest that any environmental reservoir was insignificant. Only two of the seven strains found in the environment were ever isolated from infants. For the second of these two (strain E), the organism appeared in the environment only after all infants had been colonized; its persistence in the environment for 3 months was not associated with its acquisition by newly admitted infants. Formation of cohorts of infants, rather than any environmental manipulation, was associated with termination of nosocomial spread of strains A and E. The major reservoir of NICUs for endemic, as well as epidemic, *S. marcescens*...
appears to be the gastrointestinal tracts of infants (1, 9, 28, 36, 38).

Our results emphasize the need for care in the interpretation of epidemiologic studies of S. marcescens which are unaccompanied by data on typing. Our evaluation of the various methods suggests that genomic DNA typing by REA is the optimal method for differentiation of strains in epidemiologic investigations and that it may be used alone for this purpose.

ACKNOWLEDGMENTS

We are grateful to R. Poon and L. Kurjanczyk for technical assistance and L. Ford-Jones for critical review of the manuscript.

LITERATURE CITED