Letters to the Editor

Temporally Overlapping Nosocomial Outbreaks of *Serratia marcescens* Infections: An Unexpected Result Revealed by Pulsed-Field Gel Electrophoresis

To the Editor:

Serratia marcescens is a well-known cause of serious hospital-acquired infections, and several outbreaks in surgical intensive-care units (SICUs) have been described. The remarkable resistance of *S marcescens* to disinfectants seems to play an important role in the establishment of such nosocomial infections.[1-3] Unfortunately, it is often impossible to detect the sources leading to outbreaks, so that cross-infection can be assumed only.

Numerous methods have been described for typing *S marcescens*, eg, antibiotic resistance pattern, serotyping, biotyping, plasmid analysis, electrophoretic analysis of isoenzymes, ribotyping, and polymerase chain reaction (PCR)-fingerprinting. Two study groups reported pulsed-field gel electrophoresis (PFGE) to be a powerful tool for discriminating isolates of *S marcescens*.[4,5] We report an outbreak of nosocomial infections due to *S marcescens* where PFGE typing revealed unexpected results that prompted us to do further investigation, leading to the identification of previously unrecognized environmental sources of the organism.

In our 1,500-bed university hospital, each first *S marcescens* isolate recovered from any specimen of newly admitted surgical patients (n=59) was collected during an 18-month outbreak. Forty-nine isolates originated from patients treated in the same SICU and 10 isolates from patients in surgical wards who formerly were in the SICU. *S marcescens* isolates revealed by environmental screening included one from an hexetidine solution used for oral hygiene, one from an air-conditioning filter, and two from washing effluents of bronchoscopes. *S marcescens* was identified using the API 32 E system (bioMérieux, Nürtingen, Germany). Antimicrobial susceptibility was determined by disk diffusion according to National Committee for Clinical Laboratory Standards performance standards. Minimum inhibitory concentrations for ceftazidime and the expression of extended-spectrum β-lactamases were determined by E-test (AB-Biodisc, Solna, Sweden). Pulsed-field gel electrophoresis (CHEF-DR II; Bio-Rad Laboratories, Munich, Germany) was performed according to recommended methods.[4-7]

All surgical patients involved in the outbreak (n=53) had been colonized or infected with *S marcescens* no earlier than 5 days after admission to the hospital, thus confirming the nosocomial origin according to Centers for Disease Control and Prevention guidelines.[8]

The distribution of the first positive clinical specimens of the patients was as follows: sputum and bronchial lavage, 35; blood, 7; wounds, 3; and others, 8. Thus, in 35 patients the bronchial system was colonized first, leading to pneumonia in 31. Two clonal types (each with identical banding patterns) were found by PFGE, indicating two temporally overlapping nosocomial outbreaks. Unrelated isolates (including American Type Culture Collection 8100) showed distinct PFGE patterns according to definitions previously published.[9] The Figure shows the resulting frequency of isolation of the genotypically related isolates from specimens of patients involved in the two outbreaks. All isolates with PFGE banding pattern A or B were resistant to ampicillin, Augmentin, cefotiam, cefotaxime, azlocillin, and piperacillin, and were sensitive to ciprofloxacin, imipenem, gentamicin, tobramycin, and amikacin. No isolate with expression of extended-spectrum β-lactamases was detected. Reevaluation of biotyping by prolongation of incubation time up to 4 days revealed two biotypes consistent with the two PFGE genotypes. This phenomenon was due to several weak reactions in the API 32 E system when read after 18 hours (data not shown).

Since our surveillance program during previous years had revealed isolation frequencies for patients infected with *S marcescens* of between 50 and 70 cases in the entire hospital and of 7 to 10 cases per year in the surgical department, the marked increase of isolation frequency from specimens of surgical patients was striking (Figure, months 3 and 4). Assuming patient-to-patient or patient-to-environment transmission via hands, all staff were instructed to adhere strictly to our guidelines for hand disinfection (3 mL of an antimicrobial handwashing product containing 70% alcohol to be rubbed intensively into the hands for 2-3 minutes). Environmental screening performed to detect transmission paths in the SICU covered all known sources and transmission paths or factors facilitating the spread of *S marcescens* described previously, eg, chlorhexidine handwashing solution, chloroxylenol soap solution,[3] saline solution reservoirs of nebulizers, dialyzer units, tap water, damp areas on sink surfaces, faucets, drains, transducers, handwashing brushes, hands, blood transfusion bags, and bronchoscopes. *S marcescens* was isolated from four sources implicated in previously described outbreaks.[10,11]

One of our environmental isolates, derived from hexetidine solution diluted 1:4 with tap water, showed banding pattern A; it was concluded that this solution was involved in the spread of the first nosocomial outbreak. Because a high percentage of patients in SICUs have to be ventilated mechanically, the bronchial system seems to be the most susceptible site for these nosocomial infections. Mouth irrigation is a common practice in our SICU for reducing bacterial colonization and thus lowering the risk of ventilated patients acquiring pneumonia. Upon testing, the isolate from the hexetidine solution survived incubation in a 1:2 dilution of 0.1% vol/vol hexetidine for at least 18 hours, but did not grow in undiluted solution (data not shown). The intention underlying the use of the "improper" weaker dilution was avoidance of the mucos-

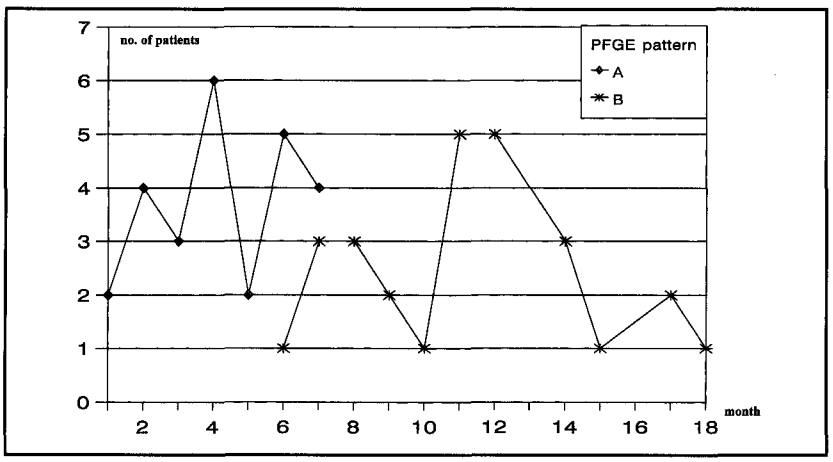

FIGURE. Isolation frequency of outbreak-related *Serratia marcescens* from newly colonized or infected patients, by month.

al irritation regularly observed when using 0.1% vol/vol hexetidine. The preparation of this solution was performed in a corner of a room also used for waste disposal. Since no stock solution was prepared and only disposable cups were used, the previously reported means of transmission[10] by contamination of stock solution and the cups for the diluted hexetidine solution could be excluded in our case. We conclude that primary colonization of patients led, via contaminated hands, to inoculation of the hexetidine solution.

At first glance, reinforcement of hand disinfection measures before preparing this solution seemed to be effective (Figure, month 5). The subsequent increased isolation of *S marcescens* (Figure, months 6 and 7) was thought to be due to compliance problems, understaffing, and continuous rapid rotation of staff. Monthly analysis of PFGE with each new isolate recovered from different specimens of surgical patients revealed that a second outbreak, with banding pattern B, was masking the decline of the first outbreak (Figure, month 7).

Subsequent environmental screening revealed bacterial contamination of bronchoscopes with *S marcescens* of banding pattern B. Bronchoscopies are performed regularly in the SICU for diagnosing pneumonia and for removing mucous. Use of bacterially contaminated bronchoscopes obviously was involved in the second outbreak, whose isolates exhibited banding pattern B. Due to an insufficient number of bronchoscopes, these instruments sometimes were used again after semiautomated reprocessing before being dried completely. Since disinfection is not as effective as sterilization, which is not applicable to flexible bronchoscopes, regrowth of surviving *S marcescens* cells within the storage period of wet instruments may occur. The deployment of a fully automated reprocessor (month 13), which ensured that bronchoscopes were dried completely, brought this second outbreak in the SICU under control.

Isolation of *S marcescens* with typing pattern B from an air-conditioning filter may indicate the ability of *S marcescens* to survive in relatively dry environments, and suggests the extent to which droplets can spread during bronchial toilet in ICUs. Thus, airborne transmission in the case of *S marcescens* cannot be excluded. The discriminatory power of antibiogram typing and biotyping is not always sufficient for epidemiological investigations. In our case, the discriminatory power of PFGE uncovered a second, temporally overlapping outbreak; the banding patterns of the different isolates studied could be readily discriminated visually. Pulsed-field gel electrophoresis is an easy-to-perform method and therefore is advocated for laboratories not experienced in PCR-based methods, which may be error-prone due to cross-contamination problems.

In conclusion, we recommend regular analysis of epidemiologically related bacteria by PFGE to reveal transmission paths in nosocomial outbreaks in order to establish more effective infection control.

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The authors thank Sigrid Klik for her excellent technical assistance.

Another Disinfectant for Enterococci

To the Editor:

In the past year, I have read several excellent reports about environmental disinfectants for vancomycin-resistant enterococci. These studies have examined quaternary ammoni-