Genotyping of Clinical Methicillin-Susceptible *Staphylococcus aureus* Isolates in a Dutch Teaching Hospital

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Methicillin-susceptible *Staphylococcus aureus* isolates, recovered from 204 patients in our hospital in a 22-month period, were characterized by pulsed-field gel electrophoresis. Among the multiple *S. aureus* types six clonal lineages dominated, comprising isolates from 158 patients. Despite the limited genetic variation, cross-transmission was made plausible only sporadically.

*Staphylococcus aureus* is an important causative agent of nosocomial infections, including surgical site infections and catheter-related bacteremia (5, 12). Consequently, microbiologists are frequently asked to determine the relatedness of staphylococcal isolates collected during the investigation of an outbreak or as part of an ongoing surveillance system.

From earlier studies it has been concluded that pulsed-field gel electrophoresis (PFGE) is well suited for genetic analysis and monitoring of nosocomial spread of *S. aureus* (1, 10). Most attention has been focused on the characterization of methicillin-resistant *S. aureus* (MRSA), but not much is known about the structure of methicillin-susceptible *S. aureus* (MSSA). Due to the search-and-destroy policy, MRSA is not endemic in The Netherlands (11). In order to increase the understanding of the molecular epidemiology of MSSA strains in our hospital, we collected all *S. aureus* isolates recovered from clinical specimens between November 1997 and September 1999 and subjected them to PFGE.

The Diakonessenhuis is a 378-bed teaching hospital in Utrecht, The Netherlands, with approximately 11,500 admissions and 7,000 clinical surgical procedures each year. Between November 1997 and September 1999, the ward, date, and site of isolation were recorded for each hospitalized patient with a positive *S. aureus* culture. *S. aureus* isolates were defined as catalase-producing gram-positive cocci which were positive for coagulase. Antibiograms were determined by disk diffusion on Mueller-Hinton agar according to the National Committee for Clinical Laboratory Standards (NCCLS) (6). The antimicrobial agents tested included penicillin, oxacillin, gentamicin, clindamycin, erythromycin, and vancomycin. PFGE typing by Smal macrorestriction was performed as described previously (10). Patterns were not subjected to the guidelines for interpretation of PFGE based on differences in banding patterns posed by Tenover et al., because these are intended to be used to examine relatively small sets of isolates (<30). For larger collections of isolates, equipment to perform computer-based image acquisition and analysis is recommended (9). The PFGE patterns were analyzed by Gel-Compar (Applied Maths, Kortrijk, Belgium). A cutoff value of 70% of genetic similarity was chosen for discrimination between distinct clusters of strains, while confirmation of genetic similarity or difference was performed by visual interpretation of the gels.

Two hundred and twenty-six *S. aureus* isolates recovered from 204 hospitalized patients were available for antimicrobial susceptibility tests and PFGE typing. Most staphylococci were recovered from wounds, pus, drains, and indwelling catheters; 16 isolates were derived from blood and 36 were derived from respiratory specimens. Isolates from 35 patients were susceptible to all antibiotics tested, while all isolates showed in vitro susceptibility to oxacillin and vancomycin. High-level resistance against gentamicin was not observed. The percentages of isolates resistant to penicillin, erythromycin, and clindamycin amounted to 72, 11, and 7%, respectively.

After analysis of PFGE patterns we were able to discriminate 19 main types dominated by 6 clusters comprising 177 isolates from 158 patients, which were designated A through F (Fig. 1). From 22 patients of whom more than one isolate was available for typing, successive isolates were identical in all cases. The genetic diversity observed is in agreement with the limited data available in the literature (2–3). Blumberg et al. identified 15 different ribotypes among a selected collection of 13 MSSA and 37 MRSA isolates (2), while Couto et al. found 23 distinct main types among 54 MRSA and 93 MSSA isolates from a Portuguese hospital by using PFGE (3). Within the six clusters, small variations in genetic profile could be distinguished. We are reluctant to ascribe small variations in profile for isolates to different genetic background or to variations within one strain, since it is not possible to make certain whether isolates with two to six fragment differences are related or not (9).

In agreement with other studies, no consistent correlation between antibiograms and genotype patterns was seen (2–3). Antibiograms would have erroneously identified a large number of MSSA isolates with distinct PFGE types as homogeneous strains. Similar findings have been reported previously for MRSA isolates (3).
The clusters comprised the most frequently encountered types in the hospital, accounting for 158 out of 204 patients (77%). These six clusters were all identified during a prolonged period of at least 15 months. The number of wards where isolates belonging to one of these clusters were recovered varied from 11 to 15. The fact that six clusters were identified next to unique genotypes suggests that staphylococcal strains may vary considerably in epidemiological potential. It seems likely that, among the different PFGE types, certain clusters of \textit{S. aureus} spread easily and remain genotypically relatively constant. The minor differences in PFGE patterns among the isolates belonging to the same strain might thus be clarified.

Obviously, the occurrence of cross-infection in our hospital was of minor importance, since outbreaks with an epidemic \textit{S. aureus} strain were not elicited. In addition, clear epidemiological linkages between patients with an isolate belonging to one of the clustered pulsotypes could generally not be demonstrated. There were seven episodes of repetitive isolation of staphylococci belonging to the same cluster in patients on the same ward within a 1-month period at five wards, involving a total of only 21 patients (Fig. 1). In these cases, cross-transmission between hospitalized patients could not be excluded and had possibly occurred via the hands of health care workers (8). Moreover, medical equipment, such as surgical instruments, catheters, ventilators, stethoscopes, and ultrasound instruments, can be reservoirs for \textit{S. aureus} (7).

Otherwise, it might be speculated that the genetic variation between \textit{S. aureus} isolates among nonhospitalized individuals in the population is limited. Thus, the six clustering genotypes could be highly endemic in the Utrecht area and subsequently be represented in our hospital. Accordingly, the PFGE results suggest that most staphylococcal infections arise endogenously. Correspondingly, several epidemiological studies indicated that nasal carriers of \textit{S. aureus} have an increased risk for the
development of surgical site infection (5, 11). Whereas the majority of MSSA cases in this study could not be explained by cross-infection or a common source, MRSA infections are acquired predominantly by hospital cross-infection (2, 4).

In conclusion, this study was designed to gain insight into the population characteristics of the resident MSSA strains and nosocomial transmission in our hospital. A considerable variation in the genetic background was detected, but six clusters were found to be dominant. PFGE patterns suggest that nosocomial MSSA infections differ from MRSA infections in that most arise endogenously. Cross-transmission which may occur now and then had not resulted in the dissemination of an epidemic MSSA strain in our hospital.

REFERENCES