Molecular Epidemiology of Methicillin-Resistant Staphylococcus aureus in 12 New York Hospitals

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Consecutive single-patient methicillin-resistant Staphylococcus aureus (MRSA) isolates (270) from 12 hospitals (8217 beds) in metropolitan New York City were collected during May 1996. In 11 of 12 hospitals, MRSA was most frequent in the general medical services. DNA typing (“fingerprinting”) revealed that mecA:Tn554:PFGE (pulsed-field gel electrophoresis) type I:A:A accounted for 113 (42%) of 270 isolates, was detected in all hospitals, and was the predominant clone in 9. Thirteen of 15 I:E:F isolates were from 1 hospital, and the remaining 2 were from another hospital of the same health system. Type V:NHE was isolated from 22 (79%) of the 28 patients with AIDS, including 8 of 9 patients from an additional hospital. Subtype V:NHE:2 was recovered from 11 patients, 9 of whom had AIDS, including all 5 AIDS patients from one floor of a nursing home affiliated with a third hospital. By using both mecA:Tn554 probes and PFGE, MRSA clusters and outbreaks may be detected and provide a rationale for appropriate infection control intervention.

Methicillin-resistant Staphylococcus aureus (MRSA) remains the most prevalent nosocomial pathogen in the United States [1]. Furthermore, the percentage of MRSA among all hospitals in the National Nosocomial Infection Surveillance (NNIS) System rose from 2.1% in 1975 to 29% in 1991 and to 35% in 1996 [2, 3]. In New York City and surrounding counties, ∼13,550 patients hospitalized in 1995 had S. aureus infections; MRSA accounted for 29% of these infections and 50% of associated deaths (The Lewin Group, unpublished data).

An initiative to investigate the prevalence and transmission of antibiotic resistance in metropolitan New York City was launched in 1994. The goal of this initiative was to use molecular typing (“fingerprinting”) techniques for epidemiologic and surveillance investigations of antibiotic resistance. Initially, 30 MRSA and 30 vancomycin-resistant Enterococcus faecium isolates collected over a 3-week period from 6 hospitals were examined [4]. Nineteen of the 27 confirmed MRSA isolates were closely related strains carrying the same mecA and Tn554 polymorph and pulsed-field gel electrophoresis (PFGE) subtypes of a single pattern (mecA:Tn554:PFGE type I:A:A), suggesting the possible wide distribution of this MRSA clone in New York City. More recently, an MRSA outbreak over an 18-month period in a New York hospital burn center was investigated [5]. Ninety-nine (97%) of 102 MRSA isolates had the mecA:Tn554:PFGE genotype I:E:F. PFGE also identified eight subtypes, of which 60 isolates were subtype F2. This MRSA “Iberian” clone has previously been reported from both Spain and Portugal [6–9].

The present study was therefore designed to confirm the prevalence of MRSA clone I:A:A throughout metropolitan New York, to detect the MRSA clone I:E:F in other New York hospitals, and to begin to introduce a high-resolution tracking system to assist hospitals and chronic care facilities in controlling the transmission of MRSA infections.

Materials and Methods

Hospital network. Twelve hospitals participated in this collaborative study. Hospitals were located in each of the five New York City boroughs (Manhattan, 3; Brooklyn, 2; Queens, 2; Bronx, 2; Staten Island, 1) as well as Suffolk (Long Island; 1) and Westchester counties (1). At each institution, collaborating investigators included both a member of the clinical infectious disease service and the director or designee of the clinical microbiology laboratory.

Patient demographics. Medical records were reviewed for the following clinical data: age, sex, location (inpatient, outpatient, emergency department, nursing home), clinical inpatient service, specific clinical unit, intensive care unit, transfer from another hospital or nursing home, underlying clinical condition, prior surgery, whether the isolate was associated with infection or colonization, and whether the isolate was epidemiologically related to a suspected outbreak. The presence of infection was determined by appropriate clinical signs and symptoms and by recovery of the isolate from a sterile site.

Microbiologic demographics. The following data were collected with each MRSA isolate: specimen source, days from admis-
to compare proportional differences between patients with MRSA clone I:A:A and all other clones combined.

**Results**

**Collaborating hospitals.** The 12 collaborating hospitals were widely dispersed throughout metropolitan New York (figure 1). Five institutions were university medical centers, 6 were community hospitals, and 1 was a Department of Veterans Affairs hospital. Hospitals IV and VI, located in Brooklyn and Queens, respectively, were members of the network of university hospital I. As shown in table 1, the total number of beds in the 12 hospitals was 8212, ranging from 261 to 1475 beds in each hospital. In addition to the 638 beds in hospital X, the hospital and its clinical microbiology laboratory serviced two nursing homes with 628 beds, which were also included in the study.

**Clinical demographics.** MRSA was isolated from 281 patients. Two hundred seventy single-patient isolates were available for DNA typing, and the medical records from these 270 patients were reviewed. The age of patients ranged from 1 month to 97 years (mean, 62 years); 58% of the patients were >60 years. Two hundred eleven patients (78%) were on an inpatient service, whereas 28 (10%) were from an outpatient clinic, 23 (8.5%) from a nursing home, and 1 from an emergency department. The majority (147/211) of inpatients (41%–92%; mean, 71%) were on a medical service in all but 1 hospital, where 7 of 12 MRSA patients were on the surgical service. Other clinical services included general surgery (35 patients), a surgical specialty (15 patients), pediatrics (6 patients), and obstetrics/gynecology (1 patient). Eighty-nine inpatients (42.8%) were in an intensive care unit at the time of the MRSA culture.

<table>
<thead>
<tr>
<th>Hospital</th>
<th>No. of beds</th>
<th>MRSA isolates</th>
<th>S. aureus isolates (% MRSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>818</td>
<td>27</td>
<td>164 (16)</td>
</tr>
<tr>
<td>II</td>
<td>1475</td>
<td>20</td>
<td>207 (14)</td>
</tr>
<tr>
<td>III</td>
<td>813</td>
<td>35</td>
<td>112 (31)</td>
</tr>
<tr>
<td>IV</td>
<td>560</td>
<td>37</td>
<td>NA</td>
</tr>
<tr>
<td>V</td>
<td>324</td>
<td>15</td>
<td>39 (38)</td>
</tr>
<tr>
<td>VI</td>
<td>487</td>
<td>28</td>
<td>102 (27)</td>
</tr>
<tr>
<td>VII</td>
<td>261</td>
<td>4</td>
<td>40 (12)</td>
</tr>
<tr>
<td>VIII</td>
<td>580</td>
<td>23</td>
<td>236 (10)</td>
</tr>
<tr>
<td>IX</td>
<td>458</td>
<td>18</td>
<td>NA</td>
</tr>
<tr>
<td>X</td>
<td>1266*</td>
<td>27</td>
<td>87 (31)</td>
</tr>
<tr>
<td>XI</td>
<td>536</td>
<td>19</td>
<td>106 (18)</td>
</tr>
<tr>
<td>XII</td>
<td>639</td>
<td>17</td>
<td>71 (24)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8217</td>
<td>270</td>
<td>1164 (18)</td>
</tr>
</tbody>
</table>

**NOTE.** NA = data not available.

* Includes 628 beds in 2 nursing homes.
Patients had a vast array of underlying clinical conditions. These included 62 patients with respiratory disorders, 52 with hematologic-oncologic disorders, including 28 patients with AIDS, 40 patients with vascular infections or sepsis of unknown etiology, and 28 patients with cutaneous lesions and a similar number with severe renal dysfunction, including end-stage renal disease.

**Microbiologic demographics.** The number of single-patient MRSA isolates from each hospital varied from 15 to 37 (mean, 24) except those from hospital VII, which recorded only 4 patients with MRSA during the study month. The total number of single-patient *S. aureus* isolates from 10 of the 12 hospitals was 1164, of which 215 (18%) were resistant to methicillin. The percentage of MRSA isolates at each of these 10 hospitals ranged from 10% to 38% (table 1).

MRSA was isolated from respiratory specimens in 118 patients (43.7%) and from skin and subcutaneous lesions in 80 patients (29.6%). In 47 patients (17.4%), MRSA was recovered either from blood or from an intravascular device. Nineteen and 5 isolates were recovered from urine and intraabdominal specimens, respectively. MRSA was recovered from blood, lower respiratory tract, or peritoneal fluid in 72 patients (27%), from other sites in 178 patients (66%), and from urine in 19 patients (7%). For inpatients, the mean number of days from hospital admission to culture was 27 (range, 0–126). On the basis of clinical signs and symptoms, recovery from a sterile site, and vancomycin use, the MRSA isolate was associated with infection in 129 patients, 62% of all hospitalized patients.

For antimicrobial susceptibility testing, 5 laboratories used the Kirby-Bauer method and 7 used automated techniques. All MRSA isolates were sensitive to vancomycin; 75% of isolates were sensitive to rifampin and 64% to trimethoprim-sulfamethoxazole. However, only 160 isolates from 7 of the 12 hospitals were tested for rifampin susceptibility. The majority of isolates were resistant to gentamicin (58%), clindamycin (88%), erythromycin (94%), and ciprofloxacin (96%).

**Genomic DNA typing patterns.** The resolution of the molecular typing methods used in this study is shown in figure 2. Both mecA and Tn554 hybridization patterns and PFGE subtypes combined to provide 97 different genomic patterns, compared with 42 mecA:Tn554:PFGE types and 23 mecA:Tn554 genotypes. Of 270 MRSA isolates, 163 carried mecA polymorph 1 and 132 had Tn554 type A. The most frequent mecA polymorph and Tn554 type were V (41/270) and NH (no hybridization; 59/270), respectively. Of the three molecular typing techniques used, PFGE had by far the highest resolution; this was further increased by the combination with the mecA and Tn554 polymorphism data, which together provided a clonal assignment for the isolates.

The distribution of the five major MRSA clones among the 12 hospitals, defined as ≥10 isolates, is listed in table 2. Genotype I:A:A accounted for 113 (41.9%) of the 270 isolates, was detected in all hospitals, and was the predominant clone in 9 of the 12 hospitals. The I:A:A clone consisted of 22 PFGE subtypes, of which the A1 subtype was identified in 50 of the 113 isolates. This subtype was detected in all but 1 hospital (hospital XII in Westchester County) and, as shown in figure 3, was also the predominant subtype among the 27 MRSA isolates from 6 New York hospitals in 1994. When patients I:A:A were compared to patients with other major clones (90 patients), there was no difference in recovery from a sterile site (P > .1), association with infection versus colonization (P > .5), location in an intensive care unit (P > .5), or location on a medical versus a surgical service (P > .5).

Genotype I:E:F was recovered from 15 patients, accounting for 5.6% of all isolates (table 2). Thirteen of these patients were in hospital I. This clone accounted for 48% (13/27) of all MRSA isolates recovered at this institution. Although associated with an MRSA outbreak that commenced in a 46-bed burn center in January 1995, only 5 of the 13 I:E:F isolates were recovered from burn patients at the time of this study (May 1996). Genotype I:E:F was also recovered from 2 patients on the medical service at hospital IV. The I:E:F clone consisted of five PFGE subtypes. Subtype I:E:F2 was recovered from each of 2 patients at both hospital I and hospital IV (figure 4).

In contrast to the restricted location of clonal type I:E:F, genotype IV:M:B was detected in each of the 12 hospitals and accounted for 9.6% of all isolates (26/270). This clonal type consisted of eight closely related PFGE subtypes. Two subtypes, IV:M:B5 and IV:M:B11, were recovered from 5 patients
Table 2. Distribution of major clones of MRSA.

| Hospital | No. of MRSA isolates | I:A:A | | | I:D:C | | | I:E:F | | | IV:M:B | | | V:NH:E | | | Others* | |
|----------|----------------------|------|---|---|------|---|---|------|---|---|------|---|---|------|---|---|------|---|---|
| I        | 27                   | 5    | 18.5 | 2 | 7.4 | 13 | 48.1 | 1 | 3.7 | 3 | 11.1 | 3 (3) | 11.1 |
| II       | 20                   | 5    | 25.0 | 2 | 10.0 | 0  | 0    | 1 | 5.0 | 6 | 30.0 | 6 (6) | 30.0 |
| III      | 35                   | 10   | 28.6 | 1 | 2.9 | 0  | 0    | 2 | 5.7 | 14 | 40.0 | 8 (8) | 22.9 |
| IV       | 37                   | 16   | 43.2 | 2 | 5.4 | 2  | 5.4 | 6 | 16.2 | 1 | 2.7 | 10 (8) | 27.0 |
| V        | 15                   | 9    | 60.0 | 0  | 0   | 0  | 0    | 5 | 33.3 | 1 | 6.7 | 0 (0) |      |
| VI       | 28                   | 15   | 53.6 | 1 | 3.6 | 0  | 0    | 1 | 3.6 | 1 | 3.6 | 10 (6) | 35.7 |
| VII      | 4                    | 2    | 50.0 | 0  | 0   | 0  | 0    | 1 | 25.0 | 0  | 0   | 1 (1) | 25.0 |
| VIII     | 23                   | 15   | 65.2 | 0  | 0   | 0  | 2    | 8.7 | 2 | 8.7 | 4 (4) | 17.4 |
| IX       | 18                   | 5    | 27.8 | 0  | 0   | 0  | 2    | 11.1 | 5 | 27.8 | 6 (6) | 33.3 |
| X        | 27                   | 9    | 33.3 | 1  | 3.7 | 0  | 2    | 7.4 | 6 | 22.2 | 9 (9) | 33.3 |
| XI       | 19                   | 14   | 73.7 | 0  | 0   | 1  | 5.3  | 0  | 4   | 3 (3) | 21.1 |
| XII      | 17                   | 8    | 47.1 | 1  | 5.9 | 0  | 2    | 11.8 | 0 | 6   | 6 (6) | 35.3 |
| Total    | 270                  | 113  | 41.9 | 10 | 3.7 | 15 | 5.6  | 26 | 9.6 | 39 | 14.4 | 67 | 24.8 |

* Parentheses indicate no. of minor clones of total of 37.

on two clinical units in hospital V and accounted for one-third of the 15 isolates from this institution.

Genotype V:NH:E was isolated from 39 patients, accounting for 14.4% of isolates, and consisted of eight PFGE subtypes. These 39 patients resided in 8 of the 12 hospitals and in one nursing home. This genotype was recovered from 22 (79%) of the 28 patients with AIDS who were hospitalized in 5 widely dispersed hospitals (I, II, III, IX, X [see figure 1]). As shown in table 2, this genotype was isolated from 14 (40%) of 35 patients in hospital III, including 8 of 9 patients with AIDS. Subtype V:NH:E2 was recovered from 11 (28%) of the 39 patients, 9 of whom had AIDS. This subtype was also isolated from all 5 AIDS patients residing on the same floor of a nursing home affiliated with hospital X (figure 5).

Genotype I:D:C was recovered from 10 patients at 7 different hospitals throughout metropolitan New York. A cluster of this genotype, or one of the three PFGE subtypes, was not identified. Thirty-seven minor clones (<10 isolates) were detected among the remaining 67 isolates. Collectively, these clones accounted for 11%–36% of MRSA isolates at each institution but were distributed throughout the 12 hospitals (table 2).

Figure 3. Pulsed-field gel electrophoresis pattern of clone I:A:A1 isolated from patients in 9 hospitals (I, II, III, IV, V, VII, VIII, IX, XI), and 6 hospitals in 1994 (LHH, QNS, WMC, MMC, SH, NYH) [4]. Strain VI-3 differs by 1 band (subtype A36) and strain X-7 differs by 3 bands (unrelated subtype). White bars in each band represent computer digitization. Strain 8325 is control MRSA.
However, PFGE combined with hybridization using mecA and Tn554 probes appears to provide the most useful discrimination [10, 21, 22].

In the present study, consecutive single-patient MRSA isolates were collected at 12 hospitals throughout metropolitan New York City. The hospitals were geographically widely dispersed and were both university- and community-based. The percentage of staphylococcal isolates that were MRSA ranged from 10% to 38%, with a mean of 18%. The percentage of MRSA often correlates with bed size of the hospital [1, 3]. However, in our relatively small sample of hospitals, this does not seem to be the case (see table 1).

Review of patient medical records revealed that the majority of patients were hospitalized and were located on a general medical service. Although patients tended to be older and have serious underlying conditions, only 43% of patients were in an intensive care unit at the time of specimen collection. Admission to intensive care has been a well-recognized risk factor.

Figure 4. mecA:Tn554:E:PFGE subtype 2 (lanes 4–6) from 2 patients in hospital I and in hospital IV. Subtypes F3 and F6 from 2 patients in hospital I differ from F2 by 3 and 2 bands. Strain 8325 is control MRSA.

Discussion

*S. aureus* is a significant cause of hospital-acquired infections, accounting for 12% of all nosocomial infections in the United States [1]. The percentage of MRSA has risen dramatically over the past 2 decades and accounted for 35% of all staphylococcal isolates reported to NNIS hospitals in 1996 [3]. MRSA is now considered endemic in many hospitals [14] and is associated with sporadic outbreaks of serious infections as well [15, 16]. Conventional methods of tracking (antibiotype and/or phage type) are becoming increasingly difficult with multiresistant strains of MRSA [6, 17]. Molecular typing techniques now provide a sensitive and specific method for the detection and tracking of outbreaks. Thus, the spread of MRSA clones has been detected within and between hospitals [5, 7, 9, 18] as well as over wide geographic areas [6, 19, 20]. Various molecular techniques have been used to detect MRSA-related strains [17]. However, PFGE combined with hybridization using mecA and Tn554 probes appears to provide the most useful discrimination [10, 21, 22].

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Figure 5. mecA:Tn554:NH:PFGE subtype E2 (lanes 4–8) isolated from 5 AIDS patients on single clinical unit in nursing home. Subtype E7 differs from E2 by 2 bands. Strain 8325 is control MRSA.
for MRSA acquisition [22] and has been correlated with the prevalence of MRSA infections in NNIS system hospitals [2]. Our data suggest that in New York hospitals, MRSA is prevalent on general medical services (47%), which has important infection control implications. A point prevalence study to determine the percentage of MRSA and the correlation of MRSA with clinical service, bed size, and intensive care unit status in all metropolitan New York hospitals would be of interest.

The respiratory tract was the most common source of MRSA, accounting for 44% of all clinical specimens. MRSA pneumonia was suspected in about one-half of patients with positive sputum cultures. However, this number is uncertain, since it may also include colonization. The lung is the most frequent nosocomial infection site on a medical service, and S. aureus accounts for 20% of these infections [1]. In our study, 49% and 52% of those patients with positive sputum cultures were on a general medical service and in a medical intensive care unit, respectively.

Of the five major clones, genotype I:A:A was the predominant clonal type, and subtype A1 was detected in 11 of the 12 hospitals. mecaA:Tn554:A was the most common hybridization pattern observed in a survey of 472 isolates from the 1980s [12] and was one of three patterns associated with MRSA clusters over a 3-year period (1988–1991) in a Brooklyn hospital [10]. As mentioned previously, pattern I:A:A was the most common genotype identified among 27 MRSA isolates from 6 widely dispersed New York hospitals in 1994 [4]. The basis for the widespread geographic distribution of this predominant New York clone is currently unknown.

By integrating epidemiologic and clinical information with DNA typing, three distinct MRSA clusters were detected. Genotype I:E:F, also referred to as the “Iberian” MRSA clone [6–9], was detected in only 2 hospitals, and the majority of isolates were isolated from patients hospitalized in hospital I. This MRSA clone has been associated with an outbreak in this hospital’s 46-bed burn center since January 1995 and accounted for 97% of all clinical isolates from that clinical unit during the subsequent 18 months [5]. In the study described here, the majority of I:E:F isolates from this hospital were recovered from non-burn patients, suggesting transmission of this clone from the burn center to other clinical areas of the hospital. Previous reports have suggested that burn units may be the source of MRSA transmission to non-burn patients [24].

Subtype I:E:F2 was isolated from 4 patients, 2 in hospital I and 2 in hospital IV, the latter hospital being a network institution of hospital I. This subtype was also the most prevalent of the eight I:E:F subtypes in the ongoing burn center outbreak [5]. Epidemiologic investigations revealed that neither of the 2 patients in hospital IV had been hospitalized in hospital I, although both had had recent surgery at hospital IV. Surgical residents from hospital IV rotate for 1 month on the cardiothoracic, pediatric transplantation, and burn services at hospital I. During the 9 months before May 1996, five hospital IV surgical residents rotated on these hospital I services, including two residents on the burn service. Three of these five residents subsequently participated in the surgical care of the 2 hospital IV patients from March to June 1996. Nasal cultures from the five residents were negative for MRSA, although surveillance was done 16 months later. The interhospital transmission of MRSA by surgeons has been reported previously [25–27] and suggests that both patients and health care workers should be considered in the infection control policies of hospital network affiliates.

While genotype IV:M:B was only isolated from small numbers of patients in 11 of the 12 hospitals, this clone was responsible for one-third of all MRSA in 1 specific hospital, hospital V. Two subtypes, IV:M:B5 and B11, were recovered from patients hospitalized on two clinical units at this hospital. In addition to this genotype, clone I:A:A was isolated from 9 of the 15 patients, and both genotypes were isolated from 6 surgical patients on a single clinical unit. MRSA accounted for 38% of all staphylococci at this Department of Veterans Affairs 324-bed hospital. Previous surveys have emphasized the prevalence of MRSA in VA hospitals and outbreaks of MRSA infections in these clinical facilities [26, 28–30]. The highly prevalent MRSA with clonal IV:M:B may be endemic to VA Hospital V.

Staphylococcal infections occur frequently in patients with AIDS [31]. Underlying immunologic factors, such as impaired B cell function, neutrophil function, and chemotaxis, certainly play a role in these infections [32–35]. Dermatologic problems also occur in 80% of human immunodeficiency virus (HIV)–infected patients, which may contribute to the acquisition of MRSA [36]. Staphylococcal bacteraemia is frequently observed in AIDS patients with a history of drug abuse or placement of indwelling venous catheters [37, 38]. In our study, MRSA genotype V:NH:E was closely associated with AIDS patients who were hospitalized in 5 widely dispersed hospitals in three New York City boroughs. This pattern was also recovered from 8 of 9 patients in hospital III, suggesting intrahospital transmission. Similar findings have been reported from a small Brazilian hospital for AIDS patients, in which the same MRSA clone (III:B:B) was isolated from 9 patients and 3 health care workers [39]. Subtype V:NH:E2 was recovered from 9 AIDS patients, 7 of whom had bacteremia, and from all 5 AIDS patients residing on the same floor of a nursing home affiliated with hospital X. Epidemiologic investigations revealed that the 8 AIDS patients hospitalized in hospital III, 2 of whom had subtype pattern V:NH:E2, had never been admitted to the nursing home affiliated with hospital X. These data suggest that genotype V:NH:E may be an AIDS-associated MRSA clone among HIV–infected patients in metropolitan New York. Additional surveillance studies to confirm these observations are warranted.

In summary, these investigations demonstrate that hybridization with mecaA and Tn554 probes and PFGE attain a high resolution that provides discrimination between MRSA clones. Combining these molecular typing methods with epidemiologic
and clinical information allows for the detection of MRSA clusters and outbreaks and therefore provides a rationale for appropriate infection control intervention.

MRSA Collaborative Study Group

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References


