Mupirocin Resistance among Consecutive Isolates of Oxacillin-Resistant and Borderline Oxacillin-Resistant *Staphylococcus aureus* at a University Hospital

MARCELLE C. LAYTON† and JAN EVANS PATTerson†,‡

*Section of Infectious Diseases, Department of Medicine,¹ and Department of Laboratory Medicine,² Yale University School of Medicine, New Haven, Connecticut 06520*

Received 19 October 1993/Accepted 25 April 1994

Mupirocin resistance was determined in consecutive oxacillin-resistant and borderline oxacillin-resistant *Staphylococcus aureus* clinical isolates collected over 14 months at a university hospital during 1991 and 1992. Twenty of 86 (23%) oxacillin-resistant and borderline oxacillin-resistant *S. aureus* isolates were mupirocin resistant; 80% were high-level resistant. Prior mupirocin use was a significant risk factor (relative risk, 6.08; 95% confidence interval, 3.7 to 9.99). Seven of 20 resistant isolates were distinct strains, as determined by pulsed-field gel electrophoresis typing. Two instances of clonal dissemination of a single strain occurred, but several other distinct mupirocin-resistant strains were documented. Mupirocin resistance was unexpectedly common among these isolates.

Mupirocin, formerly known as pseudomonic acid A, is a naturally occurring antibiotic produced by fermentation cultures of *Pseudomonas fluorescens*. Its unique mode of action involves reversible inactivation of the bacterial enzyme isoleucyl tRNA synthetase, with inhibition of protein synthesis (19). For staphylococci, including oxacillin-resistant *Staphylococcus aureus* (ORSA), the MICs of mupirocin are usually less than 0.1 μg/ml (7).

Mupirocin has been used for therapy of impetigo and secondarily infected skin disorders (5, 25, 37) and for the prevention of *Staphylococcus aureus* bacteremia in hemodialysis patients and *S. aureus* colonization of central venous catheters (4, 16, 18). Mupirocin has also been used increasingly against ORSA infections and colonizations and has been used for outbreak control (3, 8, 11, 15, 17, 30, 33). Reports of resistance in these studies have usually been less than 2%, with MICs of less than 32 μg/ml.

Mupirocin resistance was initially reported in Great Britain (2, 6, 10, 21, 26, 31, 34, 35, 36, 38) and has now been reported in the United States as well (20). We have recently reported an outbreak of mupirocin-resistant *S. aureus* on a dermatology ward associated with an environmental reservoir in a university hospital in the United States (23). Low-level resistance is defined as an MIC of between 4 and 64 μg/ml and is of questionable clinical significance. High-level resistance is defined as an MIC of greater than 500 μg/ml, is extrachromosomally mediated (12, 13, 29, 32), and has been associated with therapeutic failures (26, 31, 34).

After a 14-month prospective epidemiologic survey of ORSA and borderline oxacillin-resistant *S. aureus* (BORA) (22) and identification of the causative organism in the dermatology outbreak during the survey (23), a retrospective analysis of the prevalence of mupirocin resistance among consecutive ORSA and BORA isolates was conducted. Susceptibility and epidemiologic data for 11 mupirocin-resistant isolates are presented in this report, and the data are compared with those obtained for the 9 isolates from the dermatology ward outbreak to allow analysis of consecutive isolates from the 14-month survey. The risk factors for mupirocin resistance determined by a case-control study for all 20 patients infected with these isolates are reported here.

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The Clinical Microbiology Laboratory at Yale-New Haven Hospital identifies isolates as *S. aureus* by colony morphology, catalase positivity, and coagulase positivity (BBL Coagulase [BBL Microbiology Systems, Cockeysville, Md.] and Staph aurex LA [Wellcome Diagnostics, Dartford, England]). Oxacillin resistance is determined after 24 h of incubation at 35°C with a 1-μg oxacillin disk. Resistance is reported if there is growth within a zone of inhibition (<10 mm, according to the standards of the National Committee for Clinical Laboratory Standards [NCCLS] [28]). Cases were identified from a daily list of all ORSA or BORA isolates identified by microbiology reports. Isolates were collected and stored at −70°C; duplicate isolates from the same patient were excluded. Mupirocin resistance (*Mupr*) was defined as growth within a 14-mm zone of inhibition by using a 5-μg disk, with *S. aureus* reference strain 25923 used as a susceptible control (14). Mupirocin disks and powder were provided by SmithKline Beecham. Oxacillin resistance was confirmed by methicillin agar dilution screening at 12.5 μg/ml as described previously by Archer and Pennell (1); this method correlates well with the mec probe.

The medical records of patients identified to be infected with ORSA or BORA were reviewed for demographic data, including age, gender, race, medical service, bed location, and admitting and underlying illnesses. Any isolate obtained 48 h after admission or in direct relation to a hospital procedure was defined as being nosocomially acquired; other isolates were defined as being present on admission to the hospital. In a case-control study to determine risk factors for *Mupr*, cases were patients infected with *Mupr* ORSA or BORA and controls were patients infected with mupirocin-susceptible ORSA or BORA. The risk factors studied included receipt of...
antibiotics in the preceding 3 months, any prior mupirocin use, hospitalization within the previous 12 months, and nursing home residence. Statistical analysis was performed by using EpilInfo 5.0 (Centers for Disease Control and Prevention). Antibiotic disk susceptibility tests to erythromycin, clindamycin, ciprofloxacin, gentamicin, and tetracycline were performed according to the standards of NCCLS (28). The isolates were further characterized by the contour-clamped homogeneous electrical field modification of pulsed-field gel electrophoresis (PFGE) of whole-cell DNA. Genomic DNA was prepared as described previously (23). Whole-cell DNA was digested with Smal (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Repeat digestion with the restriction endonuclease EagI (New England BioLabs, Beverly, Mass.) was done to confirm homogeneity. Levels of Mup were defined as follows: low level, MIC, 4 to 64 µg/ml; intermediate, MIC, 128 to 256 µg/ml; and high level, MIC, $\geq 512$ µg/ml. Antibiotic solutions were prepared from a mupirocin stock solution diluted in a 0.1 M phosphate buffer adjusted to pH 5.5 as described by Caswell and Hill (9) to approximate the pH of the skin. Microdilution plates were prepared according to the standards of NCCLS (27). S. aureus ATCC 25923 was used as a susceptible control.

Twenty of the 86 ORSA or BORS isolates (23.3%) collected over a 14-month period were Mup by disk susceptibility testing. Seven of the 20 Mup isolates were confirmed to be ORSA; the other 13 isolates were BORS. The characteristics of the Mup isolates are summarized in Table 1. The demographic data for the patients revealed a mean age of 59.1 years (range, 24 to 89 years). Thirty percent of the patients were female; 70% were male. The primary medical services involved were dermatology (60%), internal medicine (25%), and the surgical subspecialties (15%). The major sites of involvement included the skin (50%), bloodstream (20%), the respiratory tract (20%), wounds (6%), catheter tips (10%), the urinary tract (5%), and the gastrointestinal tract (5%). Many patients had more than one site of involvement. All of the patients on the dermatology service had severe, exfoliating skin disorders, such as cutaneous T-cell lymphoma. Nine of the 12 isolates from dermatology patients represented the clonal dissemination of one strain on the dermatology ward, as described previously (23). Antibiotics revealed that 6% of the isolates were susceptible to erythromycin, 59% were susceptible to clindamycin, 71% were susceptible to gentamicin, 82% were susceptible to tetracycline, and 68% were susceptible to ciprofloxacin.

Mup-resistant isolates were nosocomially acquired in six patients (30%); 14 of the isolates (70%) were present on admission. Among the 14 patients infected with Mup isolates on admission, 10 had been hospitalized within the past 3 months and were dermatology patients. One patient was from a nursing home. The other four patients had not been hospitalized in over 1 year. In both sets of isolates, most caused infections rather than colonization (Table 1). One death of a patient in the group in which ORSA or BORS was present on admission to the hospital was directly related to S. aureus sepsis. Prior mupirocin use was the only risk factor for patients infected with Mup isolates that was significantly associated with Mup in comparison with patients infected with mupirocin-susceptible isolates. The relative risk for mupirocin resistance, given a history of prior mupirocin use, was 6.08 (95% confidence interval, 3.7 to 9.99; $P < 0.0001$). However, only 7 patients had been treated previously with mupirocin, leaving 13 patients (70%) infected with Mup isolates who had never received prior therapy with mupirocin.

Whole-cell DNA PFGE typing showed seven distinct patterns among the 20 isolates (Fig. 1). Pattern 1 was seen in isolates from 11 patients; 9 of these patients were from the dermatology ward outbreak (23). The remaining two isolates were nosocomially acquired, and neither of the patients infected with these isolates was on the dermatology ward. Pattern 2 was seen in isolates from four patients. Three of those patients were present on admission, one of the patients was from a nursing home, and the fourth patient was infected with a hospital-acquired isolate. One patient each had isolates with patterns 3, 4, 5, 6, and 7; three of these patients had

### Table 1. Characteristics of mupirocin-resistant isolates

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Date of isolation (mo/yr)</th>
<th>Oxacillin susceptibility</th>
<th>PFGE pattern</th>
<th>Service</th>
<th>Site of infection</th>
<th>Source</th>
<th>Hospitalization (time [mo])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/91</td>
<td>BORS</td>
<td>1</td>
<td>Derm</td>
<td>Skin</td>
<td>POA</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>3/91</td>
<td>BORS</td>
<td>1</td>
<td>Derm</td>
<td>Skin, sputum</td>
<td>POA</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>6/91</td>
<td>BORS</td>
<td>1</td>
<td>Derm</td>
<td>Blood</td>
<td>POA</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>7/91</td>
<td>BORS</td>
<td>1</td>
<td>Derm</td>
<td>Wound</td>
<td>POA</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>7/91</td>
<td>BORS</td>
<td>1</td>
<td>Derm</td>
<td>Skin</td>
<td>POA</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
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<td>BORS</td>
<td>1</td>
<td>Derm</td>
<td>Skin</td>
<td>POA</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>1/92</td>
<td>BORS</td>
<td>1</td>
<td>Derm</td>
<td>Toe</td>
<td>POA</td>
<td>1</td>
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<td>Skin</td>
<td>POA</td>
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</tr>
<tr>
<td>9</td>
<td>2/91</td>
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<td>1</td>
<td>Derm</td>
<td>Wound</td>
<td>NOSO</td>
<td></td>
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<tr>
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<td>Neurosurgery</td>
<td>Catheter tip</td>
<td>NOSO</td>
<td></td>
</tr>
<tr>
<td>11</td>
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<td>1</td>
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<td>Sputum</td>
<td>NOSO</td>
<td></td>
</tr>
<tr>
<td>12</td>
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<td>ORSA</td>
<td>2</td>
<td>MICU</td>
<td>Sputum</td>
<td>POA</td>
<td>1</td>
</tr>
<tr>
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<td>ORSA</td>
<td>2</td>
<td>ER</td>
<td>Sputum</td>
<td>POA (NH)</td>
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<tr>
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<td>1/92</td>
<td>ORSA</td>
<td>2</td>
<td>ER</td>
<td>Urine, wound</td>
<td>POA</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>1/92</td>
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<td>2</td>
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<td>Blood</td>
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<tr>
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<td>BORS</td>
<td>3</td>
<td>Derm</td>
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<tr>
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<td>MICU</td>
<td>Sputum, stool, skin</td>
<td>NOSO</td>
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<td>Derm</td>
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<td>9/91</td>
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<td>Derm</td>
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<td>POA</td>
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</tr>
<tr>
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<td>12/91</td>
<td>ORSA</td>
<td>7</td>
<td>ER</td>
<td>Blood</td>
<td>POA</td>
<td>No</td>
</tr>
</tbody>
</table>

*Abbreviations: PFGE, pulsed-field gel electrophoresis; POA, present on admission; NOSO, nosocomial; NH, nursing home; Derm, dermatology; MICU, medical intensive care unit; ER, emergency room; BORS, borderline oxacillin-resistant S. aureus; ORSA, oxacillin-resistant S. aureus.*

* Isolates 1 to 9 and 16 were previously reported in a dermatology ward outbreak (23).
isolates that were present on admission and two acquired their isolates in the hospital.

For all 11 Mupr isolates with pattern 1, MICs were greater than 2,048 μg/ml. For two isolates with pattern 2, MICs were greater than 2,048 μg/ml; however, the other two isolates were low-level resistant (MICs, 16 and 32 μg/ml, respectively). The isolate with pattern 3 was high-level resistant (MIC, ≥2,048 μg/ml). Isolates with patterns 4 and 6 had low and intermediate levels of resistance (MICs, 16 and 256 μg/ml, respectively). Isolates with patterns 5 and 7 had high-level resistance.

The topical antibiotic mupirocin became available in 1985, and by 1988 it was widely used for the management of both infection and colonization with ORSA. Early studies demonstrated low-level Mupr S. aureus in vitro by medium selection, but the majority of S. aureus were susceptible (MICs, less than 0.1 μg/ml) (7). An early study of ORSA isolates from 21 different countries showed no Mupr (24). Low-level Mupr was first cultured in S. aureus in 1987 in a dermatology ward (3). The majority of isolates were methicillin resistant, and only two patients had therapeutic failures. Kavi et al. (21) documented an increase in low-level Mupr (MIC, >8 μg/ml) in S. aureus from 3.0% in 1986 to 6.0% in 1987. Methicillin resistance was uncommon, and the isolates were heterogeneous by antibiograms and phage typing. Smith et al. (35) documented a patient with toxic epidermal necrolysis who developed low-level mupirocin resistance after only 21 days of therapy. A large survey in Great Britain found Mupr in 0.3% of S. aureus and 3% of coagulase-negative staphylococci. Most were low-level resistant, but for four isolates, MICs were >512 μg/ml, and most patients infected with Mupr S. aureus had received prior mupirocin therapy (10). Finally, Wise and Johnson (38) compared a 0.23% rate of Mupr at a district hospital in the United Kingdom with an 8.3% Mupr rate at a dermatology hospital which used 10 times as much mupirocin.

High-level resistance was first reported in the United Kingdom in 1987 on two dermatology wards (31), and other reports in dermatology patients followed (26, 34). In the United States, a recent report by Kaufman et al. (20) documented a 10.8% rate of Mupr ORSA in residents of a Veterans Affairs long-term-care facility where maintenance mupirocin was used for 1 year in an attempt to eradicate ORSA colonization.

In our study, two instances of clonal Mupr strain dissemination occurred. One strain (PFGE pattern 1) accounted for 11 of the 20 isolates. Although most of these (9 of 11 isolates) were isolated on the dermatology ward, as reported previously (23), 2 were not. Dermatology ward patients are transferred to the acute-care areas of the hospital intermittently, however, and are a potential reservoir for intrahospital spread. The second demonstration of clonality was the four isolates with PFGE pattern 2. Two of the patients whose isolates were pattern 4 were infected with hospital-acquired isolates and were on different wards but were both on the medicine service. The other two patients had their isolates on admission, but one patient had been hospitalized in the previous month and the other patient was from a nursing home, so there was the potential for exposure to common reservoirs.

Two of the pattern 2 isolates had high-level mupirocin resistance and two did not. One explanation for the fact that the strains were the same by PFGE typing, which resolves large whole-cell DNA fragments, but were distinct by an antibiotic resistance phenotype which has been transferable in other studies could be the dissemination of a plasmid or transposon among high-level-resistant strains; however, documentation of this would require further study. Likewise, the distinct patterns of the other five strains (patterns 3 to 7) indicate that the prevalence of mupirocin resistance was not solely due to clonal strain dissemination, even though clonal dissemination of a transposon or a plasmid among distinct strains might have occurred.

The prevalence of Mupr that we found among ORSA and BORSA is higher than those described in other reports of which we are aware. Mupirocin resistance may not have been as prevalent among oxacillin-susceptible S. aureus isolates obtained during the same time period, but we cannot assess this because these were not saved for study during the survey of ORSA and BORSA isolates. A large percentage of our patients were on the dermatology ward, so the treatment of severe, longstanding dermatologic disorders. The only significant risk factor for Mupr in these ORSA and BORSA isolates was prior mupirocin use.

In conclusion, we documented mupirocin resistance in ORSA and BORSA isolates which were both nosocomially acquired and present on admission to the hospital. Most of these isolates were high-level resistant and were associated with infections. Although prior therapy with mupirocin was not a risk factor, this was not a prerequisite for the presence of resistance. Results of PFGE DNA typing document that clonal strain dissemination was responsible for some, but not all, of the cases, since seven distinct PFGE types were documented. Mupirocin should be used prudently, and susceptibility testing should be considered prior to instituting mupirocin therapy for S. aureus infection or colonization.

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REFERENCES