Association of Borderline Oxacillin-Susceptible Strains of Staphylococcus aureus with Surgical Wound Infections

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Staphylococcus aureus isolates which produce type A staphylococcal β-lactamase have been associated with wound infections complicating the use of cefazolin prophylaxis in surgery. To further evaluate this finding, 215 wound isolates from 14 cities in the United States were characterized by antimicrobial susceptibility and β-lactamase type and correlated with the preoperative prophylactic regimen. Borderline-susceptible S. aureus isolates of phage group 5 (BSSA-5), which produce large amounts of type A β-lactamase and exhibit borderline susceptibility to oxacillin, comprised a greater percentage of the 120 wound isolates associated with cefazolin prophylaxis than they did of the 95 isolates associated with other prophylactic regimens (25% versus 12.6%, respectively; \( P < 0.05 \)). In contrast, methicillin-resistant S. aureus isolates were distributed evenly between the two groups (8.3% versus 11.6%, respectively). In vitro assays demonstrated that cefazolin was hydrolyzed faster by BSSA-5 strains than by other β-lactamase-producing, methicillin-susceptible strains (1.54 versus 0.50 μg/min/10^8 CFU, respectively; \( P < 0.0001 \)). These data demonstrate that BSSA-5 strains are a distinct subpopulation of methicillin-susceptible S. aureus which frequently cause deep surgical wound infections. Cefazolin use in prophylaxis is a risk factor for BSSA-5 infection.

Despite the almost universal administration of antimicrobial agents with good antistaphylococcal activity for perioperative prophylaxis in patients undergoing clean surgery, Staphylococcus aureus remains the most common cause of surgical wound infection (22). While methicillin-resistant S. aureus (MRSA) accounts for some wound infections, the majority are caused by methicillin-susceptible strains. Although the reasons for breakthrough infections due to apparently susceptible strains are not fully understood, isolates which produce type A staphylococcal β-lactamase have been associated with wound infections complicating the use of cefazolin prophylaxis in surgical patients (9). Type A S. aureus β-lactamase inactivates cefazolin relatively efficiently, conferring a partial resistance to cefazolin that is not easily identified by standard tests for determining antibiotic susceptibility (8–10, 29).

In an effort to correlate patterns of S. aureus resistance with different regimens of perioperative prophylaxis, wound isolates were obtained from 15 hospitals in 14 cities across the United States. These isolates were evaluated by phage typing, MIC determinations, and β-lactamase typing and quantification assays. A subpopulation of methicillin-susceptible staphylococci identified as borderline-susceptible S. aureus typeable with group 5 staphylococcal phages (BSSA-5) and characterized by the production of large amounts of type A staphylococcal β-lactamase and borderline susceptibility to oxacillin were found to be widely disseminated among U.S. hospitals and disproportionately isolated from wound infections of patients who had been given cefazolin prophylaxis. These data suggest that in the perioperative setting, in vivo degradation of cefazolin may enable BSSA-5 strains to survive beyond the time of initial lodgement in wound tissues and, ultimately, to cause infection.

MATERIALS AND METHODS

Bacterial isolates. Between late 1985 and early 1991, a total of 273 isolates of S. aureus associated with deep surgical wound infections that developed after clean surgical procedures were collected by, referred to, or solicited by the authors. A deep wound infection was defined as a postoperative infection requiring surgical incision and drainage for treatment. Of the β-lactamase-producing strains, specific information on the patient’s perioperative antibiotic regimen was available for isolates recovered from 215 deep wound infections. The sources and corresponding numbers of these isolates are shown in Table 1. All isolates were confirmed to be S. aureus by established methods, including colony morphology, Gram stain characteristics, the presence of catalase activity, and the ability to coagulate rabbit serum (11).

Reference strains that produce different variants of staphylococcal β-lactamae were used as controls for β-lactamase typing, as well as in cefazolin degradation assays and time-kill survival studies. These included PCI(pIP254) and NCTC 9789, which produce type A β-lactamase; 22260 and ST79/741, which produce type B β-lactamase; 3804 and RN9(pIP1147), which produce type C β-lactamase; and FAR10 and NCTC 9754, which produce type D β-lactamase (8–10, 12, 17, 19, 20, 29). NCTC 10972, the propagating strain for phage 96, was used as a representative BSSA-5 strain (1, 15), and ATCC 6538P was employed as a non-β-lactamase-producing control.

Antibiotic preparations and media. Standard powders of nitrocefin (BBL Microbiology Systems, Cockeysville, Md.), cephaloridine (Sigma Chemicals, St. Louis, Mo.), methicillin, oxacillin (both from Bristol Laboratories, Syracuse, N.Y.), penicillin G, cefazolin (both from Eli Lilly and Company, Indianapolis, Ind.), and clavulanic acid (SmithKline Beecham Pharmaceuticals, Philadelphia, Pa.) were used to prepare antimicrobial solutions for susceptibility testing, as well as in cefazolin degradation assays and time-kill survival studies. These included PC1(pIP254) and NCTC 9789, which produce type A β-lactamase; 22260 and ST79/741, which produce type B β-lactamase; 3804 and RN9(pIP1147), which produce type C β-lactamase; and FAR10 and NCTC 9754, which produce type D β-lactamase (8–10, 12, 17, 19, 20, 29). NCTC 10972, the propagating strain for phage 96, was used as a representative BSSA-5 strain (1, 15), and ATCC 6538P was employed as a non-β-lactamase-producing control.

Susceptibility determinations. Microdilution MICs were determined in accordance with methods described by the National Committee for Clinical Laboratory Standards (16). Cation-supplemented Mueller-Hinton broth with and without 2% sodium chloride was used for determinations with standard (5 × 10^4 CFU/ml) and large (5 × 10^5 CFU/ml) inocula, respectively. Trays were incubated at 35°C, and the results were recorded at 24 h.
TABLE 1. Sources of S. aureus wound isolates

<table>
<thead>
<tr>
<th>City</th>
<th>Total no. of isolates</th>
<th>No. of MRSA isolates (%)</th>
<th>No. of BSSA-5 isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nashville</td>
<td>64</td>
<td>2 (3.1)</td>
<td>14 (21.9)</td>
</tr>
<tr>
<td>Boston</td>
<td>34</td>
<td>3 (8.8)</td>
<td>4 (11.8)</td>
</tr>
<tr>
<td>Salt Lake City</td>
<td>33</td>
<td>3 (9.1)</td>
<td>10 (30.3)</td>
</tr>
<tr>
<td>Portland, Maine</td>
<td>16</td>
<td>0 (0)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Houston</td>
<td>14</td>
<td>3 (21.4)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Omaha</td>
<td>13</td>
<td>1 (7.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Buffalo</td>
<td>7</td>
<td>1 (14.3)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>Columbia, Mo.</td>
<td>7</td>
<td>4 (57.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Portland, Ore.</td>
<td>7</td>
<td>0 (0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Chattanooga</td>
<td>6</td>
<td>0 (0)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Montgomery, Ala.</td>
<td>5</td>
<td>0 (0)</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>New York City</td>
<td>5</td>
<td>3 (60.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Seattle</td>
<td>3</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Memphis</td>
<td>1</td>
<td>1 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>215</td>
<td>21 (9.8)</td>
<td>42 (19.5)</td>
</tr>
</tbody>
</table>

* Isolates from two Boston hospitals were evaluated.

β-Lactamase typing and quantitation. The type and amount of β-lactamase produced by each strain following β-lactamase induction by growth on agar containing 0.5 μg of methicillin per ml were determined using whole-cell suspensions of bacteria, as previously described (10). Hydrolysis assays were performed with 100 μM solutions of nitrocefin, cefazolin, and cephaloridine and a 500 μM solution of penicillin G at 37°C in 1-cm-light path cuvettes in a DU-70 recording spectrophotometer (Beckman Instruments, Fullerton, Calif). Quantitative rates were corrected for small variations in the absorbance at 272 nm (A272) of different whole-cell preparations and reported as micromoles (or micrograms) of substrate degraded per minute per standard cell mass (A272 = 1.0, or approximately 10^7 CFU).

Phage typing. Bacteriophage typing was performed with the international set of phages at the standard test dilution and 100-fold routine test dilution concentrations (25). The following phages were used: lytic group 1 phages 29, 52, 52A, 70, and 90; lytic group 2 phages 3A, 3C, 55, and 71; lytic group 3 phages 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85; lytic group 5 phages 54 and 96; and nonalcaligenic group 5 phages 94 and 96. Strains typeable with 0.08 log2 of phage concentration (6 isolates). Strains typeable with 0.08 log2 dilution of phage 94 at either the routine dilution (45 isolates) or a 100-fold phage concentration (6 isolates). Strains typeable at the routine dilution were more likely than strains typeable only at a 100-fold phage concentration to exhibit the borderline-susceptible phenotype (41 of 45 versus 1 of 6, respectively; P < 0.0005, two-tailed Fisher's test). Compared to phage group 5 strains, the borderline-susceptible phenotype was observed infrequently among other S. aureus strains; only 4 (2.6%) of the 152 penicillin-resistant, methicillin-susceptible non- phage group 5 isolates met the criteria for borderline susceptibility to the antistaphylococcal penicillins. Overall, 42 (19.5%) of the 215 isolates were typeable with group 5 phages and exhibited borderline susceptibility (i.e., were BSSA-5). At least one MRSA strain was identified among isolates from 10 of the 15 hospitals. Isolates from 10 hospitals, including all 8 of the hospitals from which at least six cefazolin-associated wound isolates were available for evaluation, were identified as being BSSA-5.

Association with prophylactic regimens. One hundred and twenty-five isolates were recovered from wound infections complicating perioperative prophylaxis with cefazolin (Table 2). Ninety-five wound isolates were recovered from patients on other prophylactic regimens, including cefamandole (53 isolates), cefuroxime (20 isolates), vancomycin (6 isolates), ceftazidime (4 isolates), cefonicid (2 isolates), cefotetan (2 isolates), cefoxitin (1 isolate), ceftizoxime (1 isolate), and no prophylaxis (6 isolates). BSSA-5 isolates were recovered more often from cefazolin-associated wound infections than from infections complicating other prophylactic regimens (25.0% versus 12.6%, respectively, P < 0.05, chi-square with Yate's correction). In contrast, MRSA isolates were distributed evenly between the two groups (8.3% versus 11.6%, respectively). If the MRSA isolates are excluded from this analysis, cefazolin prophylaxis and the isolation of BSSA-5 isolates from the wound infection remain significantly associated (P < 0.05, chi-square with Yate's correction). Because of the large number of isolates from Nashville, the association of BSSA-5 isolates with cefazolin prophylaxis was analyzed for hospitals outside of Nashville. Twenty-four percent of isolates associated with cefazolin prophylaxis were BSSA-5, versus 10.9% of isolates associated with other prophylactic regimens (P = 0.068, chi-square with Yate's correction).

Correlation between MICs, rates of cefazolin degradation, and kll-kinetic assays. The MICs and cefazolin hydrolysis rates of the 21 MRSA, 42 BSSA-5, and 152 penicillin-resistant, methicillin-susceptible S. aureus strains were compared (Table 3). BSSA-5 isolates were significantly more resistant to cefazolin than the other methicillin-susceptible strains. The rate of cefazolin hydrolysis was significantly higher for BSSA-5 than for either MRSA or methicillin-susceptible strains.

The capacities of BSSA-5 and representative methicillin-susceptible S. aureus strains to inactivate 50-μg/ml cefazolin

TABLE 2. MRSA and BSSA-5 isolates recovered from surgical wounds

<table>
<thead>
<tr>
<th>Isolate source</th>
<th>Total no. of isolates*</th>
<th>No. of BSSA-5 isolates (%)</th>
<th>No. of MRSA isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound infection of patient on:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazolin prophylaxis</td>
<td>120</td>
<td>30 (25.0)*</td>
<td>10 (8.3)</td>
</tr>
<tr>
<td>Other prophylaxis</td>
<td>95</td>
<td>12 (12.6)*</td>
<td>11 (11.6)</td>
</tr>
<tr>
<td>Any wound infections</td>
<td>215</td>
<td>42 (19.5)</td>
<td>21 (9.8)</td>
</tr>
</tbody>
</table>

* Penicillin-susceptible isolates were excluded from analysis.

b P = 0.036, chi-square, Yate's corrected.
TABLE 3. Cefazolin MICs and degradation of cefazolin by *S. aureus*

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of isolates</th>
<th>MICs of cefazolin with inoculum sizea</th>
<th>Mean hydrolysis rate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Standard</td>
<td>Large</td>
</tr>
<tr>
<td>MRSA</td>
<td>21</td>
<td>256f</td>
<td>256f</td>
</tr>
<tr>
<td>BSSA-5</td>
<td>42</td>
<td>4e</td>
<td>64e</td>
</tr>
<tr>
<td>Otherb</td>
<td>152</td>
<td>1c</td>
<td>4d</td>
</tr>
</tbody>
</table>

a MIC determinations were performed with standard (5 × 10⁵ CFU/ml) and large (5 × 10⁷ CFU/ml) inocula.
b Rates are expressed as micrograms of cefazolin degraded per minute per standard cell mass (A₅₅₀ nm = 1.0) with an initial 100 μM (about 45 μg/ml) concentration of cefazolin. CI, confidence interval.
c MRSA MIC > BSSA-5 MIC, P < 0.0001; BSSA-5 MIC > “Other” MIC, P < 0.0001.d MRSA MIC > BSSA-5 MIC, P < 0.01; BSSA MIC > “Other” MIC, P < 0.0001.e BSSA-5 rate > MRSA rate, P < 0.0001; BSSA-5 rate > “Other” rate, P < 0.0001.

*DISCUSSION*

The term borderline-susceptible (or borderline-resistant) *S. aureus* became popular after the 1986 report by McDougal and Thornberry on *S. aureus* strains with a distinctive phenotype which included all of the following properties: (i) borderline oxacillin MICs, (ii) lowering of oxacillin MICs into the clearly susceptible range by β-lactamase inhibitors, (iii) rapid hydrolysis of the chromogenic cephalosporin nitrocefin, and (iv) high penicillin G MICs (14). These strains produced large amounts of β-lactamase. Subsequently, we have shown that isolates with all of these phenotypic characteristics belong almost exclusively to phage group 5 (i.e., phage 94 and/or phage 96), possess a 17.2-kb plasmid, and produce large quantities of type A staphylococcal β-lactamase (1, 7, 15). Portions of this relationship among phage group, borderline susceptibility, presence of a unique plasmid, and hyperproduction of β-lactamase have been noted by others (5, 13, 21, 23, 28), and these isolates appear to represent a distinct subpopulation of *S. aureus* that is distributed widely among clinical specimens (4, 28). About 85% of phage group 5 strains are borderline susceptible (15). Genetic analyses, including determination of *SmaI* macrorestriction patterns following pulsed-field gel electrophoresis as well as studies on the size and restriction polymorphism of the internal spacer between the 16S and 23S rRNA genes, indicate that there is a high degree of relatedness among phage group 5 isolates (4).

Terms like borderline resistant, low-level resistant, and borderline susceptible have also been applied to other isolates of *S. aureus* that do not exhibit high-level β-lactamase production (24, 27). Most of such strains either have alterations in their normal penicillin-binding proteins, such that they have reduced binding affinity for β-lactams (26), or contain mecA, the gene encoding penicillin-binding protein 2a, but exhibit MICs around the breakpoint between the methicillin-susceptible and methicillin-resistant designations (2, 6) rather than the high MICs exhibited by most MRSA strains. Accordingly, to better distinguish isolates with borderline susceptibility due to different mechanisms, in this study we have used the term BSSA-5 to identify *S. aureus* strains that are typeable with group 5 phages and exhibit all the phenotypic characteristics identified with the high-level β-lactamase-producing isolates of *S. aureus* described by McDougal and Thornberry (14).

This study documents the prevalence of MRSA and BSSA-5 in wound infections and compares the prevalence associated with cefazolin prophylaxis to that associated with other prophylactic regimens. MRSA was found to comprise 11.6% of all *S. aureus* wound isolates, a value comparable to the 11% prevalence of MRSA among nosocomial *S. aureus* isolates from U.S. hospitals during this period of time (18). MRSA isolates comprised a similar proportion of the *S. aureus* wound isolates from patients receiving cefazolin as they did from patients receiving other prophylactic regimens.

BSSA-5 isolates comprised an even larger proportion than MRSA, being recovered from 19.5% of *S. aureus* deep wound infections. We considered several explanations for this finding. BSSA-5 isolates might possess a special tropism for skin and soft tissues or have some other virulence attributes enabling them to cause wound infection. We do not have any evidence to confirm or refute this possibility. However, we found it remarkable that unlike MRSA, the BSSA-5 isolates accounted for twice the proportion of the wound isolates associated with cefazolin prophylaxis compared to the other prophylactic regimens. Furthermore, BSSA-5 isolates are unique among *S. aureus* in their ability to degrade cefazolin. Although cause and effect cannot be determined from these data, the strong association between BSSA-5 wound infection and cefazolin prophylaxis suggests that in vivo hydrolysis of cefazolin may enable BSSA-5 to survive the perioperative period, thereby contributing to the pathogenesis of these infections. We previously have shown that type A β-lactamase-producing strains of *S. aureus*...
are associated with cefazolin-associated wound infections (10). This earlier observation was due, in part, to the common recovery of BSSA-5 isolates from wound infections in Nashville and Salt Lake City without evidence of a common epidemiologic link among the infected patients.

There is an alternative hypothesis to explain the observed association between BSSA-5 and cefazolin prophylaxis that needs to be considered. The isolates evaluated in this study came from multiple hospitals which differed in the type of antibiotic prophylaxis used in surgery. It is possible that cefazolin prophylaxis was used more in hospitals where relatively resistant S. aureus strains were clustered. Although a randomized means of prophylactic regimen assignment would be required to completely rule out this possibility, the observation that the proportion of MRSA was virtually identical in S. aureus wound isolates from patients on cefazolin prophylaxis and in those from patients on other prophylactic regimens suggests that the likelihood of the wound being inoculated with resistant staphylococci was comparable for the two groups. Furthermore, surveillance cultures of specimens from the nares of cardiac surgery patients at the Nashville and Salt Lake City sites yielded 137 S. aureus isolates during this same period of time, only 12 (8.8%) of which were BSSA-5 (10a), suggesting that BSSA-5 was not especially common among patients undergoing surgery at these sites despite the strong association between BSSA-5 and wound infections failing cefazolin prophylaxis.

The observation that staphylococcal resistance may contribute to the pathogenesis of some wound infections has important clinical implications. Resistant subpopulations of staphylococci may account for a significant proportion of apparent prophylaxis failures; one-fourth of the S. aureus isolates recovered from infected wounds associated with cefazolin prophylaxis in this study were BSSA-5. Because of elimination half-life and cost considerations, it would appear reasonable that one of the major determinants of antibiotic prophylaxis used in surgery. It is possible that cefazolin is not only especially resistant staphylococci was comparable for the two groups.

REFERENCES


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