Activity of Selected Antimicrobial Agents Against Strains of *Staphylococcus aureus* Isolated from Bovine Intramammary Infections that Produce β-Lactamase

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ABSTRACT

The activity of selected antimicrobial agents was determined against strains of *Staphylococcus aureus* that were isolated from bovine intramammary infections and that were positive or negative for β-lactamase. A total of 107 *S. aureus* strains (70 that were positive for β-lactamase and 37 that were negative for β-lactamase) were used in the study. Production of β-lactamase was determined using a chromogenic cephalosporin disk method. Minimum inhibitory concentrations (MIC) for each test strain were determined using a commercially available microdilution panel. The following compounds were tested: penicillin, ampicillin, oxacillin, cephapirin, ceftiofur, penicillin plus novobiocin, erythromycin, and pirlimycin. Of the five β-lactam compounds tested, penicillin and ampicillin were most affected by β-lactamase activity, but oxacillin, cephapirin, and ceftiofur were not affected. Penicillin plus novobiocin also demonstrated excellent activity against strains of *S. aureus* that were both positive and negative for β-lactamase. Erythromycin and pirlimycin demonstrated good activity against the *S. aureus* strains that were negative for β-lactamase; 90% of the isolates had an MIC of ≤0.5 μg/ml (MIC90). The MIC90 for erythromycin and pirlimycin for strains that were positive for β-lactamase was >64.0 μg/ml. However, 8 strains, in addition to producing β-lactamase, were also resistant to macrolides and lincosaminides. Recalculation of the MIC90 without these 8 strains yielded equivalent values for both erythromycin and pirlimycin with strains that were positive or negative for β-lactamase (MIC90 ≤ 0.5 μg/ml).

(Key words: minimum inhibitory concentration, β-lactamase, *Staphylococcus aureus*)

Abbreviation key: MRSA = methicillin-resistant *Staphylococcus aureus*.

INTRODUCTION

*Staphylococcus aureus* is frequently isolated from bovine IMI. Elimination of this organism from dairy herds requires treatment of infected mammary glands with antimicrobial agents and aggressive culling of refractory animals (6, 7, 9). Several factors impact the effectiveness of antimicrobial therapy against *S. aureus*, including antimicrobial resistance, scar tissue barriers, and sequestering of bacterial cells in polymorphonuclear leukocytes (5, 6). Of these factors, antimicrobial resistance can be most easily circumvented by selecting antimicrobial agents that are not affected by the specific resistance mechanism.

β-Lactam antimicrobial agents, such as the penicillins and cephalosporins, are often used to treat bovine IMI and inhibit bacteria by interfering with cell-wall synthesis (3, 10,12). Specifically, β-lactam antimicrobial agents inhibit the transpeptidase enzymes that are essential for peptidoglycan synthesis in bacteria (12). β-Lactamases are enzymes produced by *S. aureus* and other bacteria that hydrolyze the β-lactam ring of penicillins and cephalosporins. These enzymes are common mechanisms of resistance to the β-lactams in *S. aureus* strains (3, 10). Jones and Heath (5) reported that 66.1% of *S. aureus* strains isolated from bovine mastitis were positive for β-lactamase production. Owens and Watts (9) reported that only 7.0% of *S. aureus* strains isolated from bovine IMI were resistant to penicillin, but all of these strains were positive for β-lactamase production. Only one antimicrobial agent, cephapirin, is currently approved for use in the treatment of bovine IMI caused by strains of *S. aureus* that are positive or negative for β-lactamase.

The purpose of this study was to determine the effect of β-lactamase production by *S. aureus* strains isolated from bovine IMI on the activity of selected antimicrobial agents.

MATERIALS AND METHODS

Bacteria

A total of 107 *S. aureus* strains was used in the study. All strains had been isolated from cases of
TABLE 1. Summary of MIC data for strains of *Staphylococcus aureus* that were isolated from bovine IMI and that were positive or negative for β-lactamase.

<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th>no.</th>
<th>P</th>
<th>AMP</th>
<th>OX</th>
<th>CF</th>
<th>XNL</th>
<th>P + N</th>
<th>E</th>
<th>PIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>70</td>
<td>≤0.06</td>
<td>0.13</td>
<td>0.25</td>
<td>0.25</td>
<td>1.0</td>
<td>≤0.06</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>1.0</td>
<td>≤0.06</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>≤0.06–32</td>
<td>≤0.06–4.0</td>
<td>≤0.06–64.0</td>
<td>≤0.06–8.0</td>
<td>0.25–1.0</td>
<td>≤0.06–32.0</td>
<td>0.25–64.0</td>
<td>0.13–64.0</td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
<td>0.5</td>
<td>1.0</td>
<td>0.25</td>
<td>0.25</td>
<td>1.0</td>
<td>≤0.06</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td></td>
<td>16.0</td>
<td>4.0</td>
<td>1.0</td>
<td>0.5</td>
<td>1.0</td>
<td>≤0.06</td>
<td>64.0</td>
<td>64.0</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>≤0.06–32.0</td>
<td>0.13–8.0</td>
<td>0.13–64.0</td>
<td>0.13–32.0</td>
<td>0.5–2.0</td>
<td>≤0.06–32.0</td>
<td>0.13–64.0</td>
<td>0.13–64.0</td>
</tr>
</tbody>
</table>

1β-Lactamase production determined using a chromogenic cephalosporin disk method.
2P = Penicillin, AMP = ampicillin, OX = oxacillin, CF = cephalothin, XNL = ceftiofur, P + N = penicillin plus novobiocin, E = erythromycin, and PIR = pirlimycin.
3MIC<sub>50</sub> = Value at which 50% of isolates are at or below MIC; MIC<sub>90</sub> = value at which 90% of isolates are at or below MIC.

bovine mastitis in the United States, Canada, and Europe. All isolates were stored at −70°C in trypticase soy broth containing 10% glycerol. Prior to testing, all isolates were serially cultured twice on trypticase soy agar containing 5% sheep blood and incubated for 24 h at 35°C under aerobic conditions.

### β-Lactamase Production

All isolates were tested for β-lactamase production using a chromogenic cephalosporin disk method (Cefinase<sup>™</sup>; Becton-Dickinson Microbiology Systems, Cockeysville, MD). Briefly, a Cefinase<sup>™</sup> disk for each test organism was dispensed into a sterile petri dish and moistened with one drop of sterile water. Colony material from a 24-h culture of the test organism was removed using a sterile bacteriological loop, applied to the surface of a Cefinase<sup>™</sup> disk, and observed for up to 1 h. Development of a red color was considered to indicate a positive response. *Staphylococcus aureus* ATCC 29213 was included as the positive control, as recommended by the manufacturer.

### MIC Determinations

The MIC were determined using a commercially prepared microdilution panel containing the following antimicrobial agents: penicillin, ampicillin, oxacillin, cephalin, cephalzin, penicillin plus novobiocin, erythromycin, and pirlimycin. This method adhered to currently recommended standards (8). The dilution range tested for all the compounds was 0.06 to 64.0 μg/ml, except for penicillin plus novobiocin, which was tested at 0.06 and 0.12 μg/ml, respectively, to 64.0 and 128.0 μg/ml, respectively. In addition to the test organisms, the following quality control strains also were tested: *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

### RESULTS AND DISCUSSION

Of the 107 *S. aureus* strains that were tested, 70 were negative for β-lactamase production, and 37 were positive for β-lactamase production. The MIC for the *S. aureus* strains tested in this study that were either positive or negative for β-lactamase production are summarized in Table 1. The MIC values for the quality control organisms were within recommended ranges for all the antimicrobial agents tested (8).

In the present study, five compounds (penicillin, ampicillin, oxacillin, cephalin, and ceftiofur) representing the β-lactam class of antimicrobial agents were tested. Penicillin is the oldest representative of this class of compounds and was dramatically affected by β-lactamase production (Table 1). For example, the MIC<sub>90</sub> (value at which 90% of isolates were at or below MIC) for penicillin for the *S. aureus* strains that were negative for β-lactamase was 0.25 μg/ml compared with 16.0 μg/ml for the strains that were positive for β-lactamase. The activity of ampicillin, a semi-synthetic penicillin specifically modified to be more resistant to β-lactamases, was not as greatly affected as penicillin; the MIC<sub>90</sub> for the strains that were negative and positive for β-lactamase were 0.5 and 4.0 μg/ml, respectively. However, the MIC<sub>90</sub> values for penicillin and ampicillin were well above the 0.25 and 0.5 μg/ml levels, respectively, that are used to categorize staphylococci as being resistant to these compounds (8).

Oxacillin is an anti-staphylococcal penicillin that is resistant to β-lactamase and that belongs to the same class as nafcillin, methicillin, and cloxacillin (8). Ox-
acillin is the recommended agent used to detect staphylococci that are resistant to methicillin because oxacillin more reliably detects methicillin-resistance than do nafcillin and cloxacinil; oxacillin is also more stable under normal storage conditions than is methicillin (8). Oxacillin demonstrated increased stability to β-lactamase; the MIC$_{90}$ for strains that were negative and positive for β-lactamase were below the 2.0-µg/ml level that is used to categorize isolates as being susceptible to oxacillin (8). However, 5 strains, 2 that were negative for β-lactamase and 3 that were positive for β-lactamase, were resistant to oxacillin. The MIC values were ≥64.0 µg/ml. Current recommendations (8) are that methicillin-resistant _S. aureus_ (MRSA) strains should be reported as being resistant to all β-lactam antimicrobial agents, including cephalosporins, because of poor treatment efficacy.

The cephalosporins are generally categorized into generations based on activity against Gram-positive and Gram-negative bacteria (10, 12). The first generation cephalosporins demonstrate good to excellent activity against Gram-positive bacteria, but activity against the Gram-negative bacteria is dependent on the strain (10, 12). Second generation cephalosporins remain active against Gram-positive bacteria but are more active against Gram-negative bacteria (10, 12). Third generation cephalosporins demonstrate good to moderate Gram-positive activity but have enhanced activity against the Gram-negative bacilli, including _Pseudomonas_ spp. (10). In the present study, cephapirin was included as a representative of the first generation cephalosporins, and ceftiofur was included to represent third generation cephalosporins. Both compounds demonstrated good activity against _S. aureus_ strains that were both positive and negative for β-lactamase. The MIC$_{90}$ values were 0.5 and 1.0 µg/ml for cephapirin and ceftiofur, respectively. However, only 2 of the 5 MRSA strains tested were susceptible to cephapirin (MIC ≤ 8.0 µ/ml) but all 5 strains were resistant to ceftiofur. Based on current National Committee for Clinical Laboratory Standards (8) recommendations, all five MRSA strains should be reported as resistant to both cephapirin and ceftiofur. Moreover, ceftiofur is not currently approved for treatment of bovine mastitis and should not be reported for mastitis pathogens.

Penicillin in combination with the coumeramycin DNA gyrase inhibitor, novobiocin, is currently approved for treatment of bovine IMI. Thornsberry et al. (11) examined the time-kill kinetics of penicillin plus novobiocin against _S. aureus_ and determined that the combination exhibited enhanced bactericidal activity against the test strain. In the present study, penicillin plus novobiocin demonstrated excellent activity against the strains of _S. aureus_ that were negative for β-lactamase; the MIC$_{90}$ was ≤0.06 µg/ml. Against the _S. aureus_ strains that were positive for β-lactamase, the MIC$_{90}$ for the combination was 0.25 µg/ml. This value is in agreement with previous MIC data reported for novobiocin alone (11). Thus, the combination is active against staphylococci that are positive for β-lactamase because novobiocin is unaffected by β-lactamases. Indeed, Thornsberry et al. (11) demonstrated that the combination was active against a _S. aureus_ strain that demonstrated an induced β-lactamase when grown in the presence of penicillin alone. The combination of penicillin plus novobiocin was not active against the MRSA strains tested in this study; all strains had MIC values ≥8.0 µg/ml.

The macrolide and lincosaminide antimicrobial agents act by inhibiting RNA-dependent bacterial protein synthesis (1, 4, 12). Because macrolide and lincosaminide antimicrobial agents have common targets in the bacterial cell, organisms that are resistant to one class are often resistant to the other class (1, 4, 12). Erythromycin was used in the current study to represent the macrolides; pirlimycin represented the lincosaminides. Both compounds demonstrated good activity against the _S. aureus_ strains that were negative for β-lactamase with MIC$_{90}$ values of 0.5 µg/ml. The MIC$_{90}$ for both erythromycin and pirlimycin for the strains that were positive for β-lactamase were >64.0 µg/ml. However, the MIC data for the individual strains indicated that 8 strains (including the 5 MRSA strains) were also macrolide-lincosaminide resistant. Recalculation of the MIC$_{90}$ without these strains yielded values for both

<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th>no.</th>
<th>E</th>
<th>PIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>65</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC$_{50}$</td>
<td></td>
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<td>0.25</td>
</tr>
<tr>
<td>MIC$_{90}$</td>
<td></td>
<td>0.25–0.5</td>
<td>0.13–0.5</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC$_{50}$</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC$_{90}$</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>0.13–1.0</td>
<td>0.25–0.5</td>
</tr>
</tbody>
</table>

1β-Lactamase production determined using a chromogenic cephalosporin disk method.

2MIC$_{50}$ = Value at which 50% of isolates are at or below MIC; MIC$_{90}$ = value at which 90% of isolates are at or below MIC.
erythromycin and pirlimycin that were equivalent for both strains that were positive or negative for β-lactamase (Table 2). These data indicate that production of β-lactamase does not affect the activity of either erythromycin or pirlimycin. Brook et al. (2) considered that the intrinsic resistance of clindamycin, another lincosaminide antimicrobial agent, to β-lactamases accounted for the ability of this compound to eliminate organisms that produce β-lactamase, such as S. aureus, Prevotella spp., and Fusobacterium spp.

CONCLUSIONS

Both penicillin plus novobiocin and pirlimycin were active against strains of S. aureus that were positive or negative for β-lactamase. Additionally, these data emphasize the need for mastitis bacteriology laboratories to identify MRSA accurately because these strains are resistant to all compounds currently approved for treatment of bovine mastitis.

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REFERENCES


