Clinical isolates of *Staphylococcus aureus* from 13 hospitals in Japan were divided into 66 strains of methicillin-sensitive *Staphylococcus aureus* (MSSA; MIC $\leq 3.13 \mu g/ml$: inoculum size $10^6$ and $10^8$ CFU/ml) and 53 strains of methicillin-resistant *Staphylococcus aureus* (MRSA; $\geq 12.5 \mu g/ml$: $10^6$ CFU/ml). The MICs of five $\beta$-lactam antibiotics against the MSSA and MRSA strains were determined with inoculum sizes of $10^6$ and $10^8$ CFU/ml.

In the MSSA strains, the MICs of cefaclor, and cefazolin, in that order, shifted towards the resistant side with an inoculum size of $10^8$ CFU/ml as compared with $10^6$ CFU/ml. But those of methicillin and cefmetazole were unchanged.

In the MRSA strains, the MICs of all the $\beta$-lactam antibiotics tested shifted towards the resistant side according to the increase in inoculum size.

Substrate profiles in $\beta$-lactamase from *S. aureus* were determined. Ampicillin, cefaclor, and cefazolin, in that order, were hydrolyzed. The results reflected the effect of the inoculum size on the MICs against the MSSA strains.

**Key words**: *S. aureus*, Inoculum size, $\beta$-lactamase, MSSA, MRSA

**INTRODUCTION**

Since 1980, MRSA strains have been frequently isolated from clinical specimens. UBUKATA, UTSUI and HARTMAN have independently reported that the resistance of MRSA against $\beta$-lactam antibiotics was due to the production of penicillin-binding protein 2' (PBP 2'). Before that, however, $\beta$-lactamase had been considered the main mechanism of resistance against $\beta$-lactam antibiotics in *S. aureus*. Most of the recent clinical isolates of *S. aureus*, irrespective of MRSA and MSSA, have produced $\beta$-lactamase when tested with nitrocephin. We therefore think it important to distinguish between $\beta$-lactamase and PBP 2' as the cause of the resistance. In this paper we elucidate the contribution of $\beta$-lactamase to the resistance of *S. aureus* against $\beta$-lactam antibiotics.
Table 1. Influence of culture temperature on the preculture viable cell count

<table>
<thead>
<tr>
<th>Strain</th>
<th>Preculture</th>
<th>Viable cell count ($\times 10^9$ CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30°C, 20 h</td>
</tr>
<tr>
<td>S. aureus 209 P</td>
<td>1.3</td>
<td>0.98</td>
</tr>
<tr>
<td>MS353</td>
<td>1.3</td>
<td>0.48</td>
</tr>
<tr>
<td>M-12</td>
<td>1.1</td>
<td>0.95</td>
</tr>
<tr>
<td>BI-1</td>
<td>1.4</td>
<td>2.3</td>
</tr>
<tr>
<td>BI-6</td>
<td>0.91</td>
<td>0.65</td>
</tr>
<tr>
<td>BI-9</td>
<td>1.2</td>
<td>0.58</td>
</tr>
<tr>
<td>BI-110</td>
<td>1.2</td>
<td>0.42</td>
</tr>
<tr>
<td>BI-186</td>
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<tr>
<td>BI-354</td>
<td>0.62</td>
<td>0.38</td>
</tr>
<tr>
<td>BI-355</td>
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<tr>
<td>BI-359</td>
<td>0.73</td>
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<tr>
<td>BI-364</td>
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<tr>
<td>BI-369</td>
<td>0.69</td>
<td>0.81</td>
</tr>
<tr>
<td>BI-373</td>
<td>2.5</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Medium: Sensitivity Test Broth (Nissui)

RESULTS

Influence of culture temperature on the preculture viable cell count. In a previous report Laverdiere et al. showed the effect of inoculum size on the MICs of $\beta$-lactam antibiotics against S. aureus. In determining the MIC, it is therefore important to prepare the inoculum size exactly.

Viable cell counts of the precultures which were incubated at 30°C or 37°C for 20 h for 14 S. aureus strains selected at random are shown in Table 1. In the preculture at 30°C the viable cell counts of all strains tested were about $1 \times 10^9$ CFU/ml. On the other hand, at 37°C those of several strains decreased to about $1 \times 10^8$ CFU/ml. The preculture condition for the MIC determination was therefore set up at 30°C for 20 h.

MICs against MSSA. The MICs of five $\beta$-lactam antibiotics, namely, methicillin, ampicillin, cefazolin, cefaclor, and cefmetazole, against 66 MSSA strains were determined. The MIC distributions at 10⁶ and 10⁸ CFU/ml are shown in Figs. 1 and 2.

At 10⁶ CFU/ml, as compared with 10⁸ CFU/ml, the curves of the MIC distributions of ampicillin, cefaclor, and cefazolin, in that order, were gently-sloping and shifted towards the resistant side. On the other hand, those of methicillin and cefmetazole were unchanged by the inoculum size.

MICs against MRSA. The MICs of the same five $\beta$-lactam antibiotics against 53 MRSA strains were determined. The MIC distributions at 10⁶ and 10⁸ CFU/ml are shown in Figs. 3 and 4. In MRSA the MIC distributions of all five $\beta$-lactam antibiotics were higher than those in MSSA.
antibiotics were influenced by the inoculum size and shifted towards the resistant side.

**Stability to β-lactamases.** The stability of β-lactam antibiotics to a typical β-lactamase produced by *S. aureus* (plasmid pI 258-mediated β-lactamase) and β-lactamases from one clinical MSSA strain and two clinical MRSA strains were determined. The relative rate of hydrolysis is shown in Table 2.

These enzymes had the same substrate profile. Ampicillin was the most unstable, and subsequently cefaclor, cephaloridine, cefazolin, in that order, were hydrolysed, though methicillin and cefmetazole were not.

**DISCUSSION**

There are known to be two resistance mechanisms to β-lactam antibiotics in *S. aureus*. As to β-lactamase, our experiments on MSSA showed that the more unstable the β-lactam antibiotic to β-lactamase, the more the MIC was influenced by the inoculum size. We therefore assume that β-lactamase is the main resistance factor in MSSA.

In MRSA, two factors, both PBP 2' and β-lactamase, may be involved in the resistance. Methicillin and cefmetazole were little hydrolyzed by β-lactamase, but their MICs against the MRSA strains were changed to some extent by the inoculum size. In MRSA, the MICs of all β-lactam antibiotics were influenced greatly by the inoculum size. UBUKUTA has suggested the reason for this is the production of inducible PBP 2' [1, 8]. We think it is also important that highly-methicillin-resistant isolates (>400 µg/ml) can be selected easily on methicillin-containing agar plates from methicillin-intermediate-resistant strains (50 µg/ml) [9].

In this study we determined the stability of β-lactam antibiotics against β-lactamases originating from MSSA and MRSA. No difference was found in the substrate profiles of both enzymes, whose production was inducible. By comparing the affinity to and the inducibility of PBP 2' of β-lactam antibiotics under the same conditions, their antibacterial activity against MRSA strains can be
explained more clearly.

References

黄色ブドウ球菌に対する各種β-ラクタム剤の抗菌力
—接種菌量の影響とβ-ラクタマーゼ安定性について

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臨床材料より分離した黄色ブドウ球菌119株を、methicillin感受性株66株（MSSA; MIC≤3.13μg/ml：接種菌量1×10⁸並びに10⁹CFU/ml）、methicillin耐性株53株（MRSA; MIC≥12.5μg/ml:10⁹CFU/ml）の二群に分け、それぞれについて各種β-ラクタム剤のMICを接種菌量1×10⁸、10⁹CFU/mlの二点を取って測定した。

MSSAについては1×10⁸に比して10⁸接種でampicillin>cefaclor>cefazolinの順でMICの耐性側への移行がみられたが、cefmetazole、methicillinは接種菌量によるMICの変動、すなわち耐性化がみられなかった。

MRSAについては1×10⁸に比して10⁸接種ですべてのβ-ラクタム剤でMICの耐性側への移行がみられた。

各種薬剤の黄色ブドウ球菌由来のβ-ラクタマーゼに対する相対加水分解速度を測定したが、ampicillin>cefaclor>cefazolinの順に分解され、MSSAにおける接種菌量の影響を反映する結果が得られた。