The opportunistic human pathogen *Enterococcus faecium* overproduces the low-affinity PBP5. In clinical strains, mutations in PBP5 further reduce its acylation rate by β-lactams. Previous studies have reported that ceftaroline had poor inhibitory activity against β-lactam-resistant *E. faecium* strains. In this study, we show that ceftaroline exhibits killing activity against our laboratory-derived ampicillin-resistant *E. faecium* mutant that overproduces a wild-type PBP5 and that ceftaroline inactivates PBP5 much faster than benzylpenicillin and faster than ceftobiprole.
Ceftaroline (Fig. 2 and Table 1) revealed that at twice the MIC values, all high-molecular-mass PBPs were inhibited by the antibiotic (Fig. 2) and that the low-molecular-mass PBP6, a D, D-carboxypeptidase (16), was not affected. This inhibition pattern is completely different from that obtained with benzylpenicillin, for which PBP5 is the most resistant PBP (17). The IC₅₀ for ceftaroline on PBP5 were 1.40 ± 0.09 mg/liter and 0.7 mg/liter when determined with membrane preparations and the purified sPBP5, respectively. The kinetic parameters governing the acylation of PBP5 by ceftaroline were determined by using the sPBP5 (a soluble PBP5 from which the N-terminal membrane anchoring peptide was removed), which was overproduced and purified as previously described, except that the molecular sieve step was eliminated (18). The kinetics model used has been described previously (17). On the basis of the data shown in Fig. 3, the second-order rate constant k₃₂/K for ceftaroline was 950 ± 70 M⁻¹ s⁻¹, i.e., 50 to 100 times higher than the value reported for benzylpenicillin (15 to 24 M⁻¹ s⁻¹), which indicates that ceftaroline inactivates sPBP5 much faster than benzylpenicillin (5) and faster than ceftobiprole, another anti-MRSA cephalosporin (k₃₂/K = 110 ± 10 M⁻¹ s⁻¹ [13]). While the inhibitory activity of ceftaroline for E. faecium PBP5 is significant, the k₃₂/K rate constant for sPBP5 is 15 to 25 times lower than those determined for MRSA PBP2a (23,600 ± 2,000 M⁻¹ s⁻¹ [unpublished data]) produced and purified in our laboratory (19) and for the penicillin-resistant Streptococcus pneumoniae (23,600 ± 2,000 M⁻¹ s⁻¹ [unpublished data]).

TABLE 1 Inhibition of PBPs from E. faecium D63 and D63r strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antibiotic</th>
<th>IC₅₀&lt;sup&gt;a&lt;/sup&gt; (mg/liter) for PBP 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>MIC (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D63r</td>
<td>Benzylpenicillin</td>
<td>1.6 ± 0.4</td>
<td>0.06 ± 0.01</td>
<td>0.6 ± 0.3</td>
<td>8 ± 5</td>
<td>55 ± 15</td>
<td>2 ± 1</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Cefepine</td>
<td>1.7 ± 0.6</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;50</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
<td>0.9 ± 0.6</td>
<td>0.5 ± 0.3</td>
<td>0.04 ± 0.01</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;50</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td>Ceftobiprole</td>
<td>0.7 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>1 ± 0.2</td>
<td>&gt;16</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Ceftaroline</td>
<td>0.63 ± 0.09</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.97 ± 0.03</td>
<td>1.38 ± 0.1</td>
<td>&gt;50</td>
<td>2</td>
</tr>
<tr>
<td>D63</td>
<td>Benzylpenicillin</td>
<td>0.9 ± 0.7</td>
<td>0.1 ± 0.07</td>
<td>1.3 ± 0.6</td>
<td>10 ± 8</td>
<td>75 ± 25</td>
<td>1.5 ± 1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Cefobiprole</td>
<td>0.6 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>1.5 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>&gt;16</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Ceftaroline</td>
<td>0.64 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.212 ± 0.03</td>
<td>0.53 ± 0.03</td>
<td>&gt;50</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Concentration of β-lactam antibiotic that reduced binding of fluorescent ampicillin by 50% compared to a control containing no drug. IC₅₀ for D63r PBP5 against antibiotics other than ceftaroline were previously published (17).
toccus pneumoniae PBP2x (12,600 M$^{-1}$ s$^{-1}$) (20). The sPBP5-ceftaroline adduct was very stable. Indeed, no free enzyme could be detected after a 4-hour incubation of the isolated acyl-enzyme at 37°C.

We have shown that ceftaroline inactivates the low-affinity E. faecium PBP5 more efficiently than benzylpenicillin. It exhibits bacterial killing against our laboratory-derived ampicillin-resistant E. faecium mutant that overproduces the wild-type PBP5. Such a profile differs from those of most E. faecium clinical isolates, which were reported as resistant to all β-lactams, including ceftaroline (6). Like other cephalosporins, ceftaroline is not indicated for treatment of enterococcal infections. It is likely that most E. faecium clinical isolates produce mutant PBP5 forms with reduced affinity. This suggests that simple overexpression of wild-type PBP5 is sufficient to moderately increase the MIC for ceftaroline in the presence of amino acid substitutions in the protein that are necessary to confer high-level resistance. Among the β-lactams tested to date, ceftaroline has the highest affinity for wild-type PBP5, which makes it a potentially useful tool for PBP5 studies.

ACKNOWLEDGMENTS
We acknowledge the support of the Belgian Federal Government (IUAP program P7/44 iPROS). This study was supported by Forest Laboratories, Inc. Scientific Therapeutics Information, Inc., provided editorial coordination, which was funded by Forest Research Institute, Inc.

Cefaroline powder was supplied by Forest Laboratories, Inc. Forest Laboratories, Inc., had no involvement in the design, collection, analysis, interpretation of data, and decision to present these results.

REFERENCES