Reduced In Vitro Activity of Ceftaroline by Etest among Clonal Complex 239 Methicillin-Resistant Staphylococcus aureus Clinical Strains from Australia

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A total of 421 methicillin-resistant Staphylococcus aureus (MRSA) clinical isolates were tested for ceftaroline susceptibility by Etest (bioMérieux). A multidrug resistant phenotype was found in 40.9%, and clonal complex 239 (CC239) was found in 33.5%. Ceftaroline nonsusceptibility (MIC, >1.0 μg/ml) was 16.9% overall. Nonsusceptibility was significantly higher in CC239 (41.1%, 58/141) and in isolates with a multidrug resistant phenotype (35.5%, 61/172) compared with comparators (P < 0.0001). Nonsusceptibility of common multidrug resistant MRSA clones limits the empirical use of ceftaroline for these infections.

Ceftaroline, an oxyimino-cephalosporin active against methicillin-resistant Staphylococcus aureus (MRSA) due to enhanced affinity for penicillin binding protein (PBP) 2a, was approved for use in Australia in 2013 for the treatment of complicated skin and soft tissue infections (SSTIs) and community-acquired bacterial pneumonia (CABP), following approvals for the same indications in the United States in 2010 and Europe in 2012. S. aureus MIC breakpoints have been set by the Clinical and Laboratory Standards Institute (CLSI) (1) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2). Both report a susceptibility MIC of ≤1.0 μg/ml, although they differ in the resistance designation (CLSI, resistant MIC of >4.0 μg/ml; EUCAST, resistant MIC of >1.0 μg/ml). Resistance appears to be associated with decreased PBP2a binding affinity (3–7) and heteroresistance (8). Reports have largely demonstrated minimal resistance, although geographical variation has been noted (4, 7, 9–33) (Table 1). More recently, in hospital-associated MRSA (HA-MRSA) isolates from China, ceftaroline nonsusceptibility was 33.5% (84/251); most (95.2%) belonged to clonal complex (CC) 8, and sequence type (ST) 239-III was the majority (9). Similarly, ceftaroline nonsusceptible MRSA isolates from Eastern Australia were all ST239-III clones (10). A German study also demonstrated increased ceftaroline MIC50 and MIC90 values for isolates of clonal lineages ST228 and ST239 (7).

Nonduplicate, consecutive clinical MRSA isolates were tested from patients treated at the Alfred Hospital, a tertiary-care metropolitan hospital in Melbourne, Australia, over two time periods: July to December 2010 and August to November 2013. Ceftaroline was not in use clinically before 2014. Isolates from 2010 and 2013 were identified using Vitek 2 and Vitek MS (bioMérieux), respectively. Susceptibility testing was performed using the Vitek 2 Gram-positive susceptibility card (AST-P612), applying EUCAST breakpoints. The multidrug resistant phenotype was defined as resistance to ≥3 non-β-lactam antibiotics, including ciprofloxacin, erythromycin/clindamycin, gentamicin, co-trimoxazole, and tetracycline. All isolates were assessed for ceftaroline and vancomycin susceptibility by Etest (bioMérieux). Isolates from 2010 that had been frozen were thawed and subcultured twice before testing. Isolates from 2013 were collected and tested in real time. Ceftaroline Etest were set up on Mueller–Hinton agar (Becton Dickinson BBL 211438), incubated, and read per manufacturer’s guidelines. Ceftaroline nonsusceptibility was defined as a MIC of >1.0 μg/ml. Typing was performed by a high-resolution melting-based method, giving inferred CCs (34).

A total of 421 nonduplicate MRSA isolates were identified for testing. Of the total, 270 isolates were from 2010, and 151 isolates were from 2013. SSTIs accounted for the majority (70.3%) of specimens. Strains with a multidrug-resistant phenotype made up 40.9% of the isolates, although this proportion declined over the two time periods, from 47.0% (127/270) in 2010 to 29.8% (24/151) in 2013. CC239, the dominant HA-MRSA strain found in Australia, and often multidrug resistant (11), was the most commonly identified clonal complex, accounting for 33.5% of all isolates. This proportion also decreased between the two time periods, from 41.5% (112/270) in 2010 to 19.2% (29/151) in 2013.

In isolates with a multidrug-resistant phenotype (84.9%, 146/172) and in CC239 isolates (83.7%, 118/141) compared with those that had a non-multidrug-resistant phenotype (32.9%, 82/249) or another CC type (39.3%, 110/280), ceftaroline nonsusceptibility was significantly higher in CC239 (41.1%, 58/141) and in isolates with a multidrug resistant phenotype (35.5%, 61/172) compared with comparators (P < 0.0001).
The majority of ceftaroline-nonsusceptible isolates had a multidrug-resistant phenotype (85.9%, 61/71), were typed as CC239 (81.7%, 58/71) and had a vancomycin MIC of 1.0 g/ml (76.1%, 54/71). All ceftaroline-nonsusceptible isolates had MICs that were either 1.5 g/ml or 2.0 g/ml, representing the upper tail of a unimodal distribution, and were characterized as resistant by EUCAST criteria and intermediate by CLSI (Fig. 1). A two-sample test of proportions demonstrated that ceftaroline nonsusceptibility was significantly higher in CC239 MRSA isolates than in non-CC239 isolates (58/141 [41.1%] versus 13/280 [4.6%]; P < 0.0001). Similarly, ceftaroline nonsusceptibility in multidrug-resistant MRSA isolates was significantly higher than in non-multidrug-resistant isolates (61/172 [35.5%] versus 10/249 [4.0%]; P < 0.0001).

MIC gradient strip testing and disc diffusion remain the most common ways for clinical laboratories to test ceftaroline susceptibility in the absence of automated methods and with the impracticalities of BMD (35). Ceftaroline gradient strip tests have been reported to underestimate (7), overestimate (12), and demonstrate reasonably good MIC correlation with BMD (10). Livermore et al. reported that discrimination between MICs of 1.0 and 2.0 g/ml by Etest, compared with agar dilution, was poor (36). Despite this, and despite differences in clinical breakpoints set by CLSI (1) and EUCAST (2), an Etest ceftaroline MIC of 1.0 g/ml remains an important and conservative breakpoint for the laboratory to report susceptibility.

The pharmacokinetic and pharmacodynamic index that best correlates with ceftaroline efficacy is the percentage of time during the dosing interval that free-drug concentrations remain above the MIC of the infecting organism (fT>MIC). In a Staphylococcus aureus neutropenic murine thigh model, the mean ± standard deviation was 66.5 ± 10.2% when using an Etest MIC of 1.0 g/ml and 65.5 ± 10.0% when using a gradient strip (Abbott et al. 1998). This is consistent with a previous study (Livermore et al. 2000) that reported a mean ± standard deviation of 65.5 ± 9.4% and 66.5 ± 9.7% using a gradient strip and Etest, respectively, at an MIC of 1.0 g/ml.

### Table 1: Published reports of ceftaroline susceptibility in MRSA isolates

<table>
<thead>
<tr>
<th>Time period</th>
<th>Region</th>
<th>No. isolates</th>
<th>Source</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>% susceptible&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
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<tr>
<td>1997–2008</td>
<td>Australia</td>
<td>103</td>
<td>Blood</td>
<td>0.5</td>
<td>1.0</td>
<td>100</td>
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<td>2008</td>
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<td>2,254</td>
<td>SSTI&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.0</td>
<td>94.8</td>
<td>13</td>
</tr>
<tr>
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<td>2.0</td>
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<td></td>
</tr>
<tr>
<td>2008–2009</td>
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<td>215</td>
<td>RESP&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>0.5</td>
<td>100</td>
<td>18</td>
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<tr>
<td></td>
<td></td>
<td>151 HA-MRSA&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>100</td>
<td></td>
</tr>
<tr>
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<td>RESP</td>
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<td>1.0</td>
<td>96.2</td>
<td>19</td>
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<td>Europe</td>
<td>331</td>
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<td>SSTI and RESP</td>
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<td>Various</td>
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<td>1.0</td>
<td>96.8</td>
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<tr>
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<td>98.9</td>
<td>27</td>
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<td>2011</td>
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<td>2.0</td>
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<td>Various</td>
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<td>1.0</td>
<td>98.2&lt;sup&gt;i&lt;/sup&gt;</td>
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<tr>
<td>2011</td>
<td>China</td>
<td>251 HA-MRSA</td>
<td>SSTI</td>
<td>1.0</td>
<td>2.0</td>
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<td>30</td>
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<tr>
<td>2012</td>
<td>Global</td>
<td>4,324</td>
<td>Various</td>
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<td>Germany</td>
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<td>Various</td>
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<td>63.9&lt;sup&gt;i&lt;/sup&gt;</td>
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<td>2012–2013</td>
<td>U.K.</td>
<td>531</td>
<td>SSTI</td>
<td>1.0</td>
<td>1.0</td>
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<td>SSTI</td>
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<td>1.0</td>
<td>97.0</td>
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<tr>
<td>2013</td>
<td>Australia</td>
<td>100</td>
<td>Various</td>
<td>0.5</td>
<td>2.0</td>
<td>85.0&lt;sup&gt;i&lt;/sup&gt;</td>
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<td>2013</td>
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<td>SSTI</td>
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<td>1.0</td>
<td>98.8&lt;sup&gt;i&lt;/sup&gt;</td>
<td>32</td>
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<td>2014</td>
<td>India</td>
<td>50</td>
<td>Various</td>
<td>0.5</td>
<td>1.0</td>
<td>96.0&lt;sup&gt;i&lt;/sup&gt;</td>
<td>33</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolates were tested for susceptibility to ceftaroline by reference broth microdilution methods, as described by CLSI M07-A9, unless otherwise indicated.

<sup>b</sup> Susceptibility testing by MIC evaluator (MICE) strips (Oxoid).

<sup>c</sup> SSTI, skin and soft tissue infection.

<sup>d</sup> RESP, respiratory.

<sup>e</sup> CA-MRSA, community-associated MRSA.

<sup>f</sup> HA-MRSA, health care-associated MRSA.

<sup>g</sup> Study drug was ceftaroline-avibactam.

<sup>h</sup> Includes isolates from Chile, of which 83.9% had a ceftaroline MIC of 2.0 μg/ml.

<sup>i</sup> Susceptibility testing by Etest (bioMérieux).

<sup>j</sup> Broth microdilution methods, as described by Etest (bioMérieux).

<sup>k</sup> Broth microdilution methods, as described by BSAC (British Society for Antimicrobial Chemotherapy).
deviation $\%T_{>\text{MIC}}$ required for bacterial stasis, 1-log$_{10}$ kill, and 2-log$_{10}$ kill was 26 ± 8, 33 ± 9, and 45 ± 13, respectively. Less killing was observed with more widely spaced dosing intervals (12 h and 24 h) (37). Suggestions that ceftaroline susceptibility test interpretive criteria could be as high as a MIC of ≤2.0 μg/ml (4, 38) are based on achieving an $\%T_{>\text{MIC}}$ target of 26%, reflecting bacterial stasis, and would be considered the minimum value required when uncomplicated infections in patients with an intact immune system are being treated (39). At a MIC of 2.0 μg/ml, standard dosing of ceftaroline with 600 mg twice daily demonstrated lower target attainments, aiming for 1- or 2-log$_{10}$ kill (74.5% and 28.8%, respectively, in simulated patients with normal renal function, applying $\%T_{>\text{MIC}}$ of 36% and 51% for a 1- and 2-log$_{10}$ kill, respectively) (38). Monte Carlo simulation with

FIG 1 Ceftaroline MIC distributions in relation to antibiotic susceptibility phenotype and clonal complex (CC). Dark shaded area represents ceftaroline nonsusceptible MIC range. CC22, clonal complex 22 strain; CC239, clonal complex 239 strain; mMRSA, multidrug-resistant MRSA phenotype; nmMRSA, non-multidrug-resistant MRSA phenotype.
ceftaroline administered 600 mg every 8 hours as a 2-h infusion in patients with normal renal function demonstrated a higher probability of treatment success (from 72% in CAPB and 79% in SSTI to ≥99% in both), and the every-8-hour schedule may represent a better option than standard dosing, especially when targeting MRSA (40). In practice, off-label dosing has been commonly reported for serious infections (e.g., bacteremia, endocarditis, MRSA pneumonia) with good clinical outcomes, although such dosing risks higher toxicity rates (41).

Cefaroline resistance among MRSA isolates from Melbourne, Australia, especially the isolates with a multidrug-resistant phenotype and the CC239 strain, is significant and would preclude its empirical use prior to dedicated susceptibility testing. Empirical usage in other settings should be determined at an individual institutional level. Cefaroline nonsusceptibility has been identified prior to the clinical use of the drug and, therefore, does not represent the emergence of resistance. Despite the limitations of this study, which might be biased toward hospital-specific clones, or which might represent the underlying genetic background to nonsusceptibility and the dynamic relationship that is seen between the pathogen and the drug. In regions where CC239 contributes to a large proportion of MRSA, caution should be exercised in using ceftaroline for suspected or known MRSA infections. Although the association of CC239 and the multidrug-resistant phenotype are inherently linked, the clinical appreciation at the time of culture result, that a multidrug-resistant MRSA isolate has a higher background rate of ceftaroline nonsusceptibility, is an important one. Further studies are required on the clinical efficacy and safety of using more intensive ceftaroline dosing regimens in such settings.

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