Ceftaroline fosamil salvage therapy: an option for reduced-vancomycin-susceptible MRSA bacteraemia

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Objectives: To examine the activity of ceftaroline against reduced-vancomycin-susceptible MRSA isolates.

Methods: One-hundred and three MRSA blood culture isolates (predominantly ST239-MRSA-III), with varying vancomycin phenotypes, had their ceftaroline MICs determined by broth microdilution and MIC Evaluator strip (Oxoid-Thermo Fisher). Statistical analyses were performed that examined relationships with vancomycin and daptomycin MICs. Mutations in mecA were also examined.

Results: All 103 isolates (including 60 heteroresistant vancomycin-intermediate Staphylococcus aureus/vancomycin-intermediate S. aureus) were susceptible to ceftaroline, with one isolate displaying heteroresistance that may be related to a mecA mutation. Higher ceftaroline MICs were associated with vancomycin-susceptible S. aureus isolates.

Conclusions: This study highlights that ceftaroline fosamil is an option for salvage therapy based on in vitro activity.

Keywords: broth microdilution, MICE strips, ‘see-saw’ effect

Introduction

Despite improvements in the clinical management of patients, bacteraemia caused by MRSA is associated with high mortality rates.1 Due to the acquisition of the mecA gene, as part of the staphylococcal chromosome cassette (SCC) mec element, MRSA produces a low-affinity penicillin-binding protein (PBP2A) that confers resistance to most β-lactam antibiotics.2 While the glycopeptide vancomycin is the mainstay of antibiotic treatment for MRSA bacteraemia,3 the effectiveness of vancomycin has been challenged in recent times (at least in part) due to the emergence of reduced-vancomycin-susceptible strains: heteroresistant vancomycin-intermediate S. aureus (hVISA) and vancomycin-intermediate S. aureus (VISA).4 The optimal therapy for infections caused by such strains remains unclear as in vitro cross-resistance to daptomycin with clinical failure has been noted.5 Although alternative agents such as linezolid have in vitro activity, documented success has been limited to case reports.6

Ceftaroline, which is the active metabolite of ceftaroline fosamil, is the first cephalosporin with activity against MRSA due to its high affinity for PBP2A.7 Although ceftaroline fosamil has been registered by the EMA and the FDA for the treatment of complicated skin and soft tissue infections (cSSTIs) and community-acquired pneumonia, it has only received an MRSA-specific indication in the treatment of cSSTIs in both regions; this is not surprising as the pneumonia trials were not designed to assess the treatment of MRSA pneumonia. In any case, its utility in MRSA bacteraemia as initial or salvage therapy is of great interest based on data showing improved clinical outcomes with β-lactam therapy compared with vancomycin, albeit in the management of MSSA.8 However, it is currently unclear whether ceftaroline remains active in salvage therapy, particularly with respect to isolates selected for by vancomycin failure. In this in vitro study, the ceftaroline susceptibility levels of a fully characterized set of MRSA bacteraemia isolates (including vancomycin phenotypes established through population analysis profiling (PAP)) were determined in order to establish the utility of ceftaroline fosamil in salvage therapy.

Methods

Isolates and definitions

One-hundred and three non-duplicate MRSA bacteraemia isolates (99 of which are ST239 MRSA III) from 64 patient episodes, which were collected over a 12 year period (1997–2008) at Liverpool hospital, Sydney, Australia,
were included in this study; isolate characteristics are shown in Table 1. Subsequent blood culture isolates (from a single patient) that were obtained more than 7 days (average 11 days) after the initial bacteraemia isolate (despite appropriate therapy) were classified as persistent; note that, in all cases, patients were receiving vancomycin monotherapy. Where a new MRSA bacteraemia episode occurred more than 30 days after the initial episode, isolates were classified as recurrent.

Isolate Sa0365, which was determined to be ceftaroline fosamil heteroresistant (see ‘Results and discussion’), was obtained from a 25-year-old female post-gastrectomy complicated by a deep surgical site infection. The patient was treated with vancomycin monotherapy and surgical debridement, and made a full recovery.

Antibiotic MIC and resistance phenotype determination
Ceftaroline MICs were determined by broth microdilution (BMD) using CAMHB (Oxoid-Thermo Fisher, Hampshire, UK), as described by the CLSI. In addition, ceftaroline MICs were also determined by MIC Evaluator (MICE) strips (Oxoid) using Mueller–Hinton agar (Oxoid), according to the manufacturer's instructions. All isolates had previously undergone hVISA/VISA characterization by PAP and vancomycin MIC testing, the latter via both BMD (with the addition of a 1.5 mg/L step) and Etest. Daptomycin MICs by Etest had also previously been determined.

S. aureus ATCC 25923 and 29213 were utilized as controls and were within acceptable ranges.

To confirm a ceftaroline-heteroresistant S. aureus phenotype, PAP was performed in triplicate, as previously described. In short, ceftaroline (Forest Laboratories, New York, USA) was added to brain heart infusion agar (BHIA; Becton Dickinson, Franklin Lakes, USA) at the following concentrations (mg/L): 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0. Bacterial test suspensions with inocula of 10⁷ and 10⁴ cfu/mL were spread onto BHIA plates containing all of the above ceftaroline concentrations and only those between 0 and 1.0 mg/L, respectively (NB: the lower limit of detection was 10² cfu/mL). To determine the stability of the heteroresistant phenotype, daily BMD MICs were determined for colonies obtained on the BHIA plate with the highest ceftaroline concentration after subculture on antibiotic-free BHIA.

Statistical analyses
Following assessment of distribution, descriptive analysis was performed for all antimicrobials tested. Correlation between variables was determined via Spearman's test; a P<0.05 was considered significant. All calculations were computed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL, USA).

Results and discussion
To determine the activity of ceftaroline against a fully characterized MRSA bacteraemia isolate collection, with varying vancomycin resistance phenotypes, ceftaroline MICs were determined by BMD and MICE strip. Although agreement (within ±1 dilution) between the methodologies was high at 99% (102/103), correlation between methods was significantly different (Spearman’s r 0.467; P<0.01) secondary to ceftaroline strip results being 1 dilution higher than BMD results in 34% (35/103) of isolates. Cumulative susceptibility rates (Table 2) showed that all isolates were susceptible to ceftaroline by both BMD and MICE strip. Overall, the ceftaroline MIC50 and MIC90 were 0.5 and 1 mg/L, respectively, by BMD, while the MIC50 and MIC90 were both 1 mg/L by MICE strip (Table 1). These results are similar to a previous Australian study that reported a BMD MIC50 and an MIC90 of 0.75 and 1 mg/L, respectively, for the dominant hospital-acquired MRSA clones, including ST239-MRSA-III. An inverse relationship between β-lactam and glycopeptide MICs or a ‘see-saw’ effect has previously been documented for

Table 1. Characteristics of MRSA isolates

<table>
<thead>
<tr>
<th>Isolate characteristic</th>
<th>BMD/Etest or MICE strip MIC (mg/L)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ceftaroline</td>
</tr>
<tr>
<td></td>
<td>MIC50</td>
</tr>
<tr>
<td>All (n=103)</td>
<td>0.5/1</td>
</tr>
<tr>
<td>Isolates grouped by blood culture definition</td>
<td></td>
</tr>
<tr>
<td>initial (n=63)</td>
<td>0.5/1</td>
</tr>
<tr>
<td>persistent (n=26)</td>
<td>1/1</td>
</tr>
<tr>
<td>recurrent (n=14)</td>
<td>0.5/1</td>
</tr>
<tr>
<td>Isolates grouped by vancomycin PAP result</td>
<td></td>
</tr>
<tr>
<td>VSSA (n=43)</td>
<td>1/1</td>
</tr>
<tr>
<td>hVISA (n=54)</td>
<td>0.5/1</td>
</tr>
<tr>
<td>VISA (n=6)</td>
<td>0.5/0.5</td>
</tr>
</tbody>
</table>

Table 2. MIC frequency distribution of ceftaroline for 103 MRSA study isolates based on testing method

<table>
<thead>
<tr>
<th>Method</th>
<th>range</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.25–1</td>
<td>2  (1.9)</td>
<td>54 (54.4)</td>
<td>47 (100)</td>
</tr>
<tr>
<td>MICE strip</td>
<td>0.25–2</td>
<td>1 (1)</td>
<td>22 (22.3)</td>
<td>80 (100)</td>
</tr>
</tbody>
</table>

See the ‘Methods’ section for isolate details.
BMD was not performed for daptomycin.
Importantly, an overall ‘see-saw’ effect was also observed in this study, as ceftaroline MICs were inversely correlated to vancomycin MICs, irrespective of the MIC test method used (Spearman’s $r = -0.444$; $P < 0.01$). This is best illustrated in Table 1, where hVISA isolates had an overall lower ceftaroline MIC50 and a significantly reduced mean ceftaroline MIC, irrespective of the method used, when compared with vancomycin-susceptible S. aureus (VSSA) isolates (0.6 versus 0.87 mg/L; $P < 0.01$ for BMD). Similarly, a decrease in the mean ceftaroline MIC by BMD of the persistent/recurrent isolates, compared with their respective initial isolates, was also observed (0.71 versus 0.79 mg/L; $P = 0.2$). Although this decrease was not significant, secondary to the limited number of isolates in the persistent/recurrent group, this data still supports an in vivo ‘see-saw’ effect, as seen previously with other $\beta$-lactam antibiotics. However, no correlation between daptomycin and ceftaroline MICs was observed, despite a direct correlation between daptomycin and vancomycin Etest MICs (Spearman’s $r = 0.260$; $P < 0.01$).

It is interesting to note that one VSSA isolate (Sa0365) displayed potential ceftaroline heteroresistance based on initial MICE strip testing. Sa0365 ceftaroline MICE strip results showed an MIC of 1 mg/L based on the manufacturer’s interpretive criteria; however, a potentially heterogeneous subpopulation of microcolonies was observed growing towards (but not fully reaching) the 2 mg/L increment on the strip. Further MICE strip testing performed on a subculture of these microcolonies confirmed a ceftaroline MIC of 2 mg/L. Subsequently, ceftaroline PAP of Sa0365 showed that it was able to grow at a concentration of 2.5 mg/L (Figure 1). Five colonies that grew at a ceftaroline concentration of 2.5 mg/L during PAP testing were, on a daily basis for a period of 2 days, subcultured on antibiotic-free media and underwent ceftaroline MIC testing by BMD. After the first day, only one of the colonies maintained an elevated ceftaroline MIC (2 mg/L), while the other colonies reverted to the background MIC of 1 mg/L. Following another overnight subculture in antibiotic-free media, all ceftaroline MICs had reverted to 1 mg/L, thus indicating that the heteroresistant phenotype was unstable.

Based on the colony counts from the PAP, the frequency at which heteroresistance occurred was 1 in every $1.3 \times 10^{15}$ cells and this is similar to results obtained previously for a range of ceftaroline-heteroresistant clinical MRSA strains of different genetic backgrounds (ST239 not included). Of note, this frequency is low and, given differences in inoculum sizes, ceftaroline heteroresistance is more likely to be undetected using BMD compared with MICE strip testing (1.5 $\times 10^{6}$ versus $1.5 \times 10^{8}$ cfu/mL, respectively). Furthermore, it is unlikely that automated susceptibility testing will detect these isolates, similar to detection issues that occur with hVISA. Of note, this isolate demonstrated low vancomycin MICs with a non-hVISA phenotype based on vancomycin PAP. Although this heteroresistant ceftaroline phenotype was unstable and the clinical consequences remain unclear, it is speculated that treatment failure is likely based on previous infections involving heteroresistance phenotypes (e.g. hVISA). Therefore, additional study is needed, in order to define isolates that require further testing; we would suggest (at a minimum) that persistent isolates close to breakpoint ‘failing’ ceftaroline therapy should be tested. Unfortunately, PAP remains the definitive test but, unlike the situation with hVISA, MICE strip testing may indicate the presence of ceftaroline heteroresistance.

A recent study of different MRSA STs and SCCmec types showed that two mutations in mecA, which encodes PBP2A, were

![Figure 1. Ceftaroline PAP of S. aureus ATCC 25923 and Sa0365. Also shown for Sa0365 are its vancomycin PAP result and BMD/Etest MICs of vancomycin and daptomycin (T. Barbagiannakos, S. J. van Hal and J. Mercer, unpublished data).](image-url)
associated with decreased ceftaroline susceptibility in ST228-MRSA-I isolates.\textsuperscript{16} A nucleotide change (C to T) at positions 715 and 1339 in mecA resulted in a glutamate to lysine change at codons 239 and 447, respectively.\textsuperscript{16} Isolates with the Glu447Lys mutation, which is located within the penicillin-binding domain (PBD) of PBP2A, had a ceftaroline MIC of 8 mg/L, while those with the Glu239Lys mutation (located outside of the PBD) had MICs of 2 mg/L.\textsuperscript{16} In our study, isolate Sa0365 contained the Glu239Lys mutation (data not shown), but despite displaying heteroresistance, it had a ceftaroline MIC of only 1 mg/L by BMD and MICE strip. While further studies are required, these results (in combination) suggest that the Glu239Lys mutation requires additional genetic changes in order to confer elevated ceftaroline MICs and thus could play a role in ceftaroline heteroresistance; note that such changes may be ST specific. In support of this, an intrinsic ST-specific difference in ceftaroline susceptibilities was observed in a recent survey of common hospital-acquired MRSA STs in Australia, which noted that a larger proportion of ST239-MRSA-III isolates (40.8%, n = 211) had ceftaroline MICs $\geq$1 in comparison with ST22-MRSA-IV isolates (23.1%, n = 212).\textsuperscript{13}

Conclusions

Ceftaroline resistance in \textit{S. aureus} is a rare phenomenon, as only 0.04% of strains tested worldwide have been reported to have MICs $\geq$2 mg/L.\textsuperscript{16} In our study, all 103 bacteraemia isolates tested were susceptible to ceftaroline and had MICs $\leq$1 mg/L; however, one isolate had a ceftaroline heteroresistant phenotype, which was unstable and may be associated with a PBP2A mutation (Glu239Lys). Further investigations are needed to identify potentially ‘resistant’ isolates and the associated genetic factors that drive such resistance.

Although the current mainstay of therapy for MRSA bacteraemia remains vancomycin,\textsuperscript{3} the optimal treatment following vancomycin failure is unknown, especially as there are concerns regarding daptomycin cross-resistance, particularly in association with hVISA/VISA isolates. Based on our data (and that of previous studies), ceftaroline not only remains active but also displays enhanced activity following vancomycin failure (secondary to a ‘see-saw’ effect).\textsuperscript{17–20} This study represents the largest subset of hVISA/VISA isolates (60/103) examined (to date) for ceftaroline susceptibility and demonstrates that ceftaroline cross-resistance did not occur in the context of vancomycin exposure. As such, these in \textit{vivo} results (along with a growing body of clinical evidence)\textsuperscript{21–23} support the use of ceftaroline fosamid as salvage therapy for the treatment of MRSA bloodstream infections.

References


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Transparency declarations

None to declare.


