Characterization of methicillin-resistant Staphylococcus aureus displaying increased MICs of ceftaroline

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Received 8 November 2011; returned 23 January 2012; revised 7 February 2012; accepted 8 February 2012

Objectives: To characterize the mechanisms responsible for elevated MICs of ceftaroline for methicillin-resistant Staphylococcus aureus (MRSA).

Methods: During the 2008 Assessing Worldwide Antimicrobial Resistance Evaluation (‘AWARE’) surveillance programme, four S. aureus collected from separate patients in Athens, Greece, demonstrated ceftaroline MICs of 4 mg/L. These isolates were clonally related and one strain (13101) was selected for further characterization. Two strains (4981 and 4977) displaying ceftaroline MICs of 1 and 2 mg/L, respectively, were included for comparison. All strains originated from the same hospital. Penicillin-binding protein (PBP) affinities for ceftaroline and comparators were determined. Strains were typed by single-locus typing (i.e. spa typing), multilocus sequence typing (‘MLST’) and by multiple-locus variable-number tandem repeat fingerprinting (MLVF). The presence of Pantone–Valentine leucocidin and the staphylococcal cassette chromosome mec types was assessed. We also performed nucleotide sequencing of the mecA encoding PBP2a promoter and ribosomal binding site (rbs) regions and mecR1.

Results: Ceftaroline demonstrated the highest PBP2a affinity with strain 4981 (ST5-MRSA-II) (IC₅₀ 0.06 mg/L; MIC 1 mg/L). Strains 4977 and 13101 (both ST239-MRSA-III) showed indistinguishable MLVF profiles. Ceftaroline PBP2a binding affinity in strains 4977 (IC₅₀ 0.25 mg/L; MIC 2 mg/L) and 13101 (IC₅₀ 1 mg/L; MIC 4 mg/L) was 4- and 16-fold lower than 4981, respectively. Strain 4981 contains a wild-type PBP2a, while strains 4977 and 13101 have N146K and E150K alterations in the non-penicillin-binding domain. Additionally, 13101 has one substitution (H351N) in the transpeptidase domain. Alterations in the mecR1, mecA promoter or rbs regions were not observed.

Conclusions: Increased ceftaroline MICs were associated with decreased PBP2a binding affinity and reflected alterations in PBP2a.

Keywords: anti-MRSA agents, resistance mechanisms, penicillin-binding protein 2a mutations, MRSA lineages, ST239

Introduction

Ceftaroline fosamil, the pro-drug of the active metabolite ceftaroline, is a new, broad-spectrum cephalosporin approved in 2010 by the US FDA for the treatment of community-acquired bacterial pneumonia (‘CABP’) and acute bacterial skin and skin structure infections (‘ABSSSIs’). Ceftaroline is active against Gram-positive pathogens, including methicillin-resistant Staphylococcus aureus (MRSA). Activity against MRSA is a consequence of high binding affinity to penicillin-binding protein (PBP) 2a, the additional PBP protein present in MRSA that confers resistance to other β-lactam antibiotics. Most β-lactam antimicrobial agents have a high affinity for PBP1, 2 and 3, which are essential for staphylococcal cell growth. In addition, ceftaroline has activity against many common wild-type Gram-negative pathogens [i.e. non-extended-spectrum β-lactamase (‘non-ESBL’) or AmpC-producing isolates].

As part of the 2008 Assessing Worldwide Antimicrobial Resistance Evaluation (‘AWARE’) programme, the activities of ceftaroline and comparators were evaluated against consecutive and non-duplicate clinically relevant S. aureus (6665 strains) from the USA, 12 European countries and Israel. Ceftaroline exhibited...
potent activity against USA methicillin-susceptible \textit{S. aureus} (MSSA; MIC<sub>50/90</sub> 0.25/0.25 mg/L) and MRSA (MIC<sub>50/90</sub> 1/1 mg/L), and European strains (MSSA MIC<sub>50/90</sub> 0.25/0.5 mg/L and MRSA MIC<sub>50/90</sub> 1/2 mg/L).\textsuperscript{1} Four \textit{S. aureus} strains (0.06\% of all \textit{S. aureus}) collected from unique bacteremic patients at a single site in Athens, Greece, demonstrated reproducible ceftaroline MICs of 4 mg/L.\textsuperscript{1} The current study was designed to identify the mechanisms responsible for the decreased susceptibility to ceftaroline in these MRSA clinical isolates.

Materials and methods

The four Greek MRSA strains showing decreased susceptibility to ceftaroline (with MICs of 4 mg/L) were clonally related (two subtypes; similarity coefficient >84\%) according to PFGE typing results; thus one representative strain (13101) was selected and included in this study.\textsuperscript{1} In addition, one strain (4981) displaying the modal ceftaroline MIC value (1 mg/L) found among European MRSA isolates;\textsuperscript{1} and another MRSA (4977) showing an MIC result of 2 mg/L were included for comparison purposes. All three selected strains originated from the same Greek medical centre. Two representative strains of international MRSA clones having similar multilocus sequence typing (MLST) and staphylococcal cassette chromosome mec type results to the evaluated \textit{S. aureus} isolates were also included for control purposes, as follows: \textit{S. aureus} N315 (New York/ Japan clone; ST5-MRSA-II; GenBank accession number D86934; ceftaroline MIC 1 mg/L) and \textit{S. aureus} BAA-39 (Hungarian clone; ST239-MRSA-III; GenBank accession number NZ_AEEK01000041; ceftaroline MIC 1 mg/L).

The three selected clinical strains (13101, 4981 and 4977) were typed by single-locus typing (i.e. spa typing), MLST and by multiple-locus variable-number tandem repeat fingerprinting (MLVF; formerly MLVA), as previously described.\textsuperscript{5–7} In addition, the presence of Pantone–Valentine leucocidin (PVL) genes (\textit{lukF-PV} and \textit{lukS-PV}) and the SCCmec type results to the evaluated \textit{S. aureus} isolates were also included for control purposes, as follows: \textit{S. aureus} N315 (New York/ Japan clone; ST5-MRSA-II; GenBank accession number D86934; ceftaroline MIC 1 mg/L) and \textit{S. aureus} BAA-39 (Hungarian clone; ST239-MRSA-III; GenBank accession number NZ_AEEK01000041; ceftaroline MIC 1 mg/L).

Results

Antimicrobial susceptibility profiles for the strains included in this study are shown in Table 1, while molecular characterization results and IC<sub>50</sub> for ceftaroline and comparators are displayed in Table 2. A correlation between the ceftaroline binding affinity for PBP2a and ceftaroline MIC was observed. Ceftaroline demonstrated the highest PBP2a affinity for the \textit{S. aureus} control strain 4981 (ST5-MRSA-II) (IC<sub>50</sub> 0.06 mg/L), which had a ceftaroline MIC result (1 mg/L) at the modal value for European MRSA clinical isolates. The mecA gene sequence analysis of 4981 (Figure 1) predicted an amino acid sequence identical to that of the \textit{S. aureus} N315 control (ST5-MRSA-II; ceftaroline MIC 1 mg/L).

\begin{table}[h]
\centering
\caption{MICs of ceftaroline and tested comparator agents for \textit{S. aureus} included in this evaluation}
\begin{tabular}{lccc}
\hline
\textbf{Antimicrobial agent} & \textbf{4981} & \textbf{4977} & \textbf{13101} \\
\hline
Ceftaroline & 1 & 2 & 4 \\
Oxacillin & >2 & >2 & >2 \\
Ceftriaxone & >32 & >32 & >32 \\
Cefepime & >16 & >16 & >16 \\
Imipenem & 4 & >8 & >8 \\
Piperacillin/tazobactam & >64 & >64 & >64 \\
Tigecycline & 0.12 & 0.25 & 0.25 \\
Erythromycin & >2 & >2 & <0.25 \\
Clindamycin & >2 & >2 & <0.25 \\
Levofoxacin & >4 & >4 & >4 \\
Linezolid & 2 & 1 & 2 \\
Vancomycin & 1 & 1 & 1 \\
Daptomycin & 0.25 & 0.25 & 0.25 \\
Trimethoprim/sulfamethoxazole & <0.5 & 0.25 & 0.25 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Molecular typing results and PBP binding affinities of \textit{β}-lactams for selected \textit{S. aureus}}
\begin{tabular}{lcccccc}
\hline
\textbf{Strain/drug} & \textbf{MIC (mg/L)} & \textbf{IC<sub>50</sub>} (mg/L) & \textbf{PBP1} & \textbf{PBP2a} & \textbf{PBP2} & \textbf{PBP3} & \textbf{PBP4} \\
\hline
4981 (unique MLVF, ST5, t002, SCCmec type II and PVL-negative)\textsuperscript{9} & & & & & & & \\
Ceftaroline & 1 & 1 & 0.06 & 0.25 & 0.25 & 1 \\
Ceftriaxone & >32 & 4 & 8 & 0.25 & 1 & 4 \\
Cefotaxime & >32 & 0.06 & 64 & 0.25 & 0.25 & 1 \\
4977 (MLVF ‘A’, ST239, t037, SCCmec type III and PVL-negative)\textsuperscript{9} & & & & & & & \\
Ceftaroline & 2 & 0.25 & 0.25 & 0.25 & 0.25 & 1 \\
Ceftriaxone & >32 & 0.12 & 4 & 0.25 & 0.12 & 0.12 \\
Cefotaxime & >32 & 0.12 & 2 & 0.12 & 0.12 & 0.12 \\
13101 (MLVF ‘A’, ST239, t037, SCCmec type III and PVL-negative)\textsuperscript{9} & & & & & & & \\
Ceftaroline & 4 & 0.25 & 1 & 0.25 & 0.25 & 0.25 \\
Ceftriaxone & >32 & 0.06 & 32 & 0.25 & 0.06 & 1 \\
Cefotaxime & >32 & 4 & >128 & 0.5 & 0.03 & 16 \\
\hline
\end{tabular}
\end{table}

Ceftaroline exhibited a reduced (4-fold) binding affinity for PBP2a in \textit{S. aureus} 4977 (ST239-MRSA-III) (IC<sub>50</sub> 0.25 mg/L; MIC 2 mg/L) when compared with that observed for isolate 4981 (IC<sub>50</sub> 0.06 mg/L). Strain 4977 had two amino acid substitutions (N146K and E150K) in the non-penicillin-binding domain (nPBD) of PBP2a when compared with that of BAA-39 (ST239-MRSA-III control). Finally, the lowest ceftaroline binding affinity for the PBP2a protein was found for strain 13101 (ST239-MRSA-III).
Table 2. Binding affinities of ceftaroline and comparators for PBP1, PBP2, PBP3 and PBP4 show greater differences depending on the agent and isolate tested.

Discussion

The ceftaroline PBP2a affinity results for all MRSA strains described here were similar to those previously reported (IC₅₀ ranged from 0.01–1 mg/L). In the series of MRSA isolates in the present study showing different ceftaroline MICs, there was an inverse correlation between PBP2a binding affinity and ceftaroline MIC, which is expected because the anti-MRSA activity of ceftaroline reflects binding and inhibition of PBP2a. Isolate 4981, showing a ceftaroline MIC of 1 mg/L, has a wild-type PBP2a amino acid sequence. MRSA strains 4977 and 13101, with increased ceftaroline MICs, demonstrated two mutations (N₁₄₆K and E₁₅₀K) in the nPBD.

No nucleotide modification was observed in the mecR₁ promoter or ribosomal binding site regions in any of the strains.

Figure 1. Partial amino acid alignment of PBP2a from S. aureus 4981 (ST5-MRSA-II; ceftaroline MIC 1 mg/L), 4977 (ST239-MRSA-III; ceftaroline MIC 2 mg/L) and 13101 (ST239-MRSA-III; ceftaroline MIC 4 mg/L) compared with those of S. aureus N315 (New York/Japan clone; ST5-MRSA-II; GenBank accession number D86934; ceftaroline MIC 1 mg/L) and S. aureus BAA-39 (Hungarian clone; ST239-MRSA-III; GenBank accession number NZ_000000041; ceftaroline MIC 1 mg/L). Comparison of amino acid sequences was performed within strains showing similar genetic backgrounds (i.e. MLST and SCCmec typing results). Differences in amino acids are highlighted. Boxed amino acids represent differences between ST5-MRSA-II and ST239-MRSA-III strains.

The affinities of ceftaroline for PBP2a were tested for comparison with the following antibiotics: amoxicillin (IC₅₀ 0.9 mg/L), cefazolin (IC₅₀ 0.7 mg/L), ceftriaxone (IC₅₀ 64 mg/L), cefotaxime (IC₅₀ 8 mg/L) and ceftazidime (IC₅₀ 0.1 mg/L). The affinities of ceftaroline for PBP2a of MRSA 13101 were 32- and >128-fold higher than those of ceftriaxone and cefotaxime (IC₅₀ 0.06 mg/L) in strain 4977, ceftaroline (IC₅₀ 0.25 mg/L) exhibited affinity for PBP2a 8- and 16-fold higher than cefotaxime (IC₅₀ 2 mg/L) and ceftriaxone (IC₅₀ 4 mg/L), respectively. The affinity of ceftaroline for PBP2a of MRSA 13101 was 32- and >128-fold higher than those of ceftriaxone (IC₅₀ 32 mg/L) and cefotaxime (IC₅₀ >128 mg/L), respectively.

In Table 2, the binding affinities of ceftaroline and comparators for PBP1, PBP2, PBP3 and PBP4 are also presented. The IC₅₀ for PBP2 and PBP3 are relatively low (ranging from 0.03 to 1 mg/L) for all three drugs for each tested strain. The IC₅₀ for PBP1 and PBP4 show greater differences depending on the agent and isolate tested.

Discussion

The ceftaroline PBP2a affinity results for all MRSA strains described here were similar to those previously reported (IC₅₀ ranged from 0.01–1 mg/L). In the series of MRSA isolates in the present study showing different ceftaroline MICs, there was an inverse correlation between PBP2a binding affinity and ceftaroline MIC, which is expected because the anti-MRSA activity of ceftaroline reflects binding and inhibition of PBP2a. Isolate 4981, showing a ceftaroline MIC of 1 mg/L, has a wild-type PBP2a amino acid sequence. MRSA strains 4977 and 13101, with increased ceftaroline MICs, demonstrated two mutations (N₁₄₆K and E₁₅₀K) in the nPBD. This region is far from the transpeptidase active site and within a domain not involved in β-lactam binding. However, alterations in this region, including E₁₅₀K, have been indirectly implicated in decreased susceptibility to β-lactam agents, perhaps due to modified protein–protein interactions.

The S. aureus 13101 isolate, displaying the highest ceftaroline MIC result (4 mg/L), had a third PBP2a modification (H₃₅₁N) in the transpeptidase domain when compared with the wild-type sequence from strain BAA-39. Previously characterized mutants of MRSA with elevated MICs of β-lactams (including other
anti-MRSA agents) had amino acid substitutions in the penicillin-binding domain of PBP2a.\textsuperscript{11,12} The contribution of the H\textsubscript{351}N substitution to the reduced activity of ceftaroline against strain 13101 is not certain, since other clonally related strains (all also carrying N\textsubscript{146}K and E\textsubscript{150}K) with MIC results of 4 mg/L were found not to contain H\textsubscript{351}N (data not shown). However, it is important to emphasize that \( \beta \)-lactam resistance in \textit{S. aureus} may also be influenced by other unknown factors within a strain’s genetic background.\textsuperscript{15,13} Certainly not all amino acid substitutions in PBP2a are associated with decreased susceptibility to ceftaroline, since amino acid differences at positions 204 and 246 were observed among control strains exhibiting similar ceftaroline MICs. Therefore the effect of the H\textsubscript{351}N substitution on ceftaroline MICs needs further investigation.

In summary, isolates of MRSA with ceftaroline MIC results at \( \geq 4 \) mg/L are not commonly found. A set of isolates recovered from a single medical centre in Greece with ceftaroline MICs ranging from 1 to 4 mg/L were characterized. Overall, increased ceftaroline MIC results correlated with decreased binding affinity for PBP2a.\textsuperscript{9} Alterations have been identified in PBP2a, which appear to have contributed to the reduction in binding affinity and the increase in MIC, albeit further experiments are needed. Moreover, ceftaroline showed good affinity to PBP2 and PBP3 (\( IC_{50} \) 0.25 and 0.25–0.5 mg/L, respectively) among all tested strains.

### Acknowledgements

Preliminary findings of this work have been presented previously at the Forty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009 (Poster C2-138) and at the Fiftieth Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, MA, 2010 (Poster C1-1445).

### Funding

This study was supported by Forest Laboratories, Inc. The editorial assistance was funded by Forest Research Institute, Inc.

### Transparency declarations

JMI Laboratories has received research/education grants in the last 2 years from API, Anacor, Astellas, AstraZeneca, Bayer, Cubera, Cephalon, Cubist, Daiichi, Enanta, Forest, GlaxoSmithKline, Johnson & Johnson (Ortho McNeil), Merck, Novartis, Optimer, Ordway, Pfizer, Shionogi, The Medicines Company, Theravance and TREK Diagnostics. D. B. is an employee of Cubera, Inc., and wholly owned subsidiary of Forest Laboratories, Inc.; D. B. owns stocks and options in the company. All other authors: none to declare.

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

### Scientific Therapeutics Information, Inc., provided editorial assistance.

### References