Structural Insights into the Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) Activity of Ceftobiprole*

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Background: Ceftobiprole is a β -lactam recently developed to treat methicillin-resistant *Staphylococcus aureus* (MRSA) by inhibiting its antibiotic resistance determinant PBP2a.

Results: The PBP2a-ceftobiprole complex reveals an extensive binding interface with two distinct inhibitor conformations.

Conclusion: Ceftobiprole inhibits PBP2a via increased stabilization of the Michaelis complex followed by acylation.

Significance: We report the first structure of a resistant PBP inhibited by a competent anti-MRSA β -lactam.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant strain of *S. aureus* afflicting hospitals and communities worldwide. Of greatest concern is its development of resistance to current last-line-of-defense antibiotics; new therapeutics are urgently needed to combat this pathogen. Ceftobiprole is a recently developed, latest generation cephalosporin and has been the first to show activity against MRSA by inhibiting essential peptidoglycan transpeptidases, including the β -lactam resistance determinant PBP2a, from MRSA. Here we present the structure of the complex of ceftobiprole bound to PBP2a. This structure provides the first look at the molecular details of an effective β -lactam-resistant PBP interaction, leading to new insights into the mechanism of ceftobiprole efficacy against MRSA.

Methicillin-resistant *Staphylococcus aureus* (MRSA)⁶ is a strain of *S. aureus* that has developed resistance to the β -lactam antibiotics, which include penicillins (e.g. methicillin, oxacillin, and dicloxacillin) and cephalosporins (1–3). Over the past dec-

ade, there has been a striking increase in the prevalence of hospital-acquired MRSA infections as well as an emergent epidemic of community-acquired MRSA infections (4). Most disconcerting is the development of resistance in *S. aureus* to current last-resort antibiotics including vancomycin (5) and linezolid (6). Ceftobiprole (see Fig. 1*a*) is among a new generation of cephalosporin β -lactams that combines activity against MRSA with broad spectrum activity against Gram-negative bacteria and other Gram-positive bacteria (7, 8). Ceftobiprole was the first β -lactam shown to possess anti-MRSA activity (and also the first antibiotic with potency against both MRSA and *Pseudomonas aeruginosa*); in addition to inhibiting the methicillin-susceptible PBPs of *S. aureus* (2), it remains able to target both *Enterococcus faecalis* and penicillin-resistant *Streptococcus pneumoniae*. Thus, ceftobiprole has a broad spectrum of activity encompassing several prominent pathogens. To elucidate the molecular details of the action of ceftobiprole against its target for inhibition, PBP2a, we present here the structure of PBP2a in complex with ceftobiprole.

The bacterial cell wall peptidoglycan is a complex structure composed of glycan strands cross-linked through peptide side chains. Transpeptidases belong to a group of enzymes known historically as penicillin-binding proteins (PBPs), which catalyze the formation of specific cross-links between the peptide side chains of neighboring glycan strands, a process essential to cell wall integrity (9, 10). The mechanism of β -lactam inhibition of this transpeptidation step in bacterial cell wall biosynthesis is well documented: β -lactams function specifically as substrate analogs of the D-Ala-D-Ala peptidoglycan side chain termini upon which a PBP acts, forming a long-lived inhibitory covalent acyl-enzyme complex with the nucleophilic serine within the enzyme active site. The region of the active site that normally accommodates the deacylating acceptor moiety of an adjacent peptidoglycan strand or a potential hydrolyzing water molecule remains occupied by the fused ring system of the β -lactam; hence, the antibiotic-bound PBP cannot undergo its usual subsequent deacylation. With the enzyme now inactivated, the consequent loss of cell wall cross-linking ultimately leads to cell lysis and death (11–13). This can be described by Reaction 1.

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The atomic coordinates and structure factors (code 4DKI) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (<http://www.rcsb.org/>).

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⁶ The abbreviations used are: MRSA, methicillin-resistant *Staphylococcus aureus*; MME, monomethyl ether; PBP, penicillin-binding protein; PDB, Protein Data Bank; r.m.s.d., root mean square deviation.

Here, the β -lactam initially binds a PBP noncovalently (with dissociation constant K_d) to form the Michaelis complex. The PBP serine nucleophile then attacks the β -lactam peptide bond to create the covalent acyl-enzyme complex (at the rate indicated by the constant k_2). Nucleophilic attack by water would regenerate the enzyme; however, β -lactam antibiotics are hydrolyzed extremely slowly by PBPs (reflected by low k_3 values (14, 15)) so in effect the acyl-PBP is now irreversibly inhibited. PBP2a is believed to resist inactivation via a very slow acylation step (its k_2 is 3 orders of magnitude lower than β -lactam sensitive PBPs (16)). Meanwhile, PBP2a exhibits β -lactam binding affinity (K_d) and poor deacylation efficiency (k_3) values comparable with sensitive PBPs (17, 18). Thus, a β -lactam with improved binding affinity (decreased K_d) would feature increased inhibition resulting from greater overall acylation efficiency (k_2/K_d); in other words, the low probability that the Michaelis complex will yield an acylation event would be compensated for by increasing the frequency with which the Michaelis complex forms.

MRSA evades inhibition by β -lactams through acquisition of an exogenous, β -lactam-resistant PBP, PBP2a (also referred to as PBP2'), encoded by the *mecA* gene (19–23). Crystal structures of both the methicillin-susceptible PBP2 (24) and methicillin-resistant PBP2a (16) have provided the molecular details describing the mechanism of β -lactam resistance achieved by PBP2a. Most significantly, they show a structural rearrangement around the active site serine (Ser-403) which is located in a narrow, extended cleft in the resistant PBP2a (see Fig. 2). This rearrangement, although still allowing for initial binding of the antibiotic, causes displacement and misalignment of the β -lactam with respect to the serine nucleophile. Therefore, a productive β -lactam arrangement in the PBP2a active site requires a significant conformational change to occur, presumably making initiation of the acylation reaction less frequent (decreased k_2). It is postulated that any anti-MRSA β -lactam would owe its effectiveness to increased hydrophobic interaction with PBP2a, increasing the frequency of formation of the Michaelis complex and compensating for the energetic cost of rearrangement (16).

To test these existing hypotheses about anti-MRSA β -lactam drug design, we obtained the first structure of PBP2a in complex with ceftobiprole (deposited in the Protein Data Bank (PDB) with accession code 4DKI). Ceftobiprole is a pyrrolidinone cephalosporin that has an oxymino aminothiazolyl substituent (R1 group) linked to the 7-amino group of the cephalosporin nucleus, conferring stability to hydrolysis by many β -lactamases. A vinylpyrrolidinone moiety (R2 group) located at position 3 facilitates interaction with the narrow groove of the PBP2a active site and favors the acylation of PBP2a (Fig. 1a).

TABLE 1
Data collection and refinement statistics

Crystal parameters ^a	
Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions: $a \times b \times c$, Å	80.8 × 103.5 × 186.5
Resolution, Å	2.9 (3.06–2.9)
Wavelength, Å	1.11588
No. reflections	106,806
No. unique reflections	32,591
Average redundancy	3.3 (2.8)
Completeness, %	91.9 (63.5)
$I/\sigma I$	15.9 (3.3)
R_{sym} ^b	0.07 (0.248)
Refinement statistics ^c	
Atoms in the crystallographic asymmetric unit	1275 observable amino acid residues, 2 ceftobiprole molecules, 39 H ₂ O, 7 Cd ²⁺ , 4 Cl [−] , 4 bicarbonate ions.
$R_{\text{work}}/R_{\text{free}}$	0.173/0.237
r.m.s.d. bond lengths, Å	0.010
r.m.s.d. bond angles, degrees	1.31

^a Statistics for highest resolution shell are given in parentheses.

^b $R_{\text{sym}} = \sum (|I_{\text{hkl}}| - \langle I \rangle) / \sum I_{\text{hkl}}$.

^c 5% of reflections were excluded from refinement and used to calculate R_{free} .

EXPERIMENTAL PROCEDURES

Protein Expression, Purification, and Crystallization—*S. aureus* PBP2a was prepared as described previously (16). Briefly, a construct expressing residues 23–668 (with the mutation Y23M) produced soluble recombinant protein in *Escherichia coli*, lacking the N-terminal transmembrane sequence. PBP2a was purified to near homogeneity through sequential Q-Sepharose, CM-Sepharose, hydroxyapatite, and Sephacryl-100 chromatography steps. The protein was concentrated to 20 mg/ml in a final buffer of 5 mM NaHCO₃, pH 8.0, 150 mM NaCl. Crystals of the orthorhombic form of PBP2a were grown using 1 ml of protein crystal stock mixed with 1 ml of reservoir solution (100 mM Hepes, pH 7.0, 0.88 M NaCl, 20% (v/v) PEG 500 MME, and 16 mM CdCl₂) in a typical sitting drop vapor-diffusion experiment.

Derivatization, Data Collection, and Structure Determination—Ceftobiprole (Basilea Pharmaceutica) was solubilized in dimethyl sulfoxide (with 0.2% v/v TFA, agitated for 20 min at room temperature) to give a stock solution of 20 mM. This was diluted 1:200 into PBP2a cryoprotection solution (100 mM Hepes, pH 7.0, 1 M NaCl, 28% (v/v) PEG 550 MME, and 16 mM CdCl₂). This derivatization solution was added directly to the crystallization drop (in three 1- μ l increments, over a time course of 35 min) and left overnight to promote acylation. Acylated PBP2a crystals were plunge-cooled to 100 K and screened for diffraction quality. Data were collected at Beamline 8.3.1 of the Advanced Light Source (Berkeley, CA), using x-rays of 1.116 Å wavelength. Full statistics are reported in Table 1.

Data Processing and Refinement—Data were processed using MOSFLM, scaled with SCALA, and data file manipulations performed using the CCP4 suite of programs (25). Due to the isomorphous nature of the crystals with those previously reported (16), the coordinates of the PBP2a nitrocefin acyl-enzyme (PDB code 1MWS) were used to generate initial phases. The possibility of model bias arising from the inclusion of the nitrocefin adduct in the initial phase estimates was negated through deletion of the nitrocefin adduct from the model and the usage of the Prime and Switch procedure in RESOLVE (26) to provide maps for the modeling of the ceftobiprole moiety.

FIGURE 1. Chemical structures of MRSA-active and -inactive cephalosporins along with electron density maps and models of ceftobiprole covalently bound to Ser-403 at the PBP2a active site. *a*, general chemical structure of the cephalosporin antibiotics, indicating the positions of variable R1 and R2 groups and chemical structures of ceftobiprole, nitrocefin, and methicillin. *b*, final model and $2F_o - F_c$ map contoured at 1σ . The ceftobiprole R1 group is shown in both of its proposed conformations A and B, with all atoms equivalent up to C7[C2] (indicated by an arrow in panel *a*). *c*, results of test refinement with ceftobiprole in the A conformation, with $2F_o - F_c$ (blue, 1σ) and $F_o - F_c$ (green, 3σ) maps shown. The $F_o - F_c$ difference density is continuous with the modeled and unmodeled $2F_o - F_c$ electron density, suggesting either multiple conformations or chemical modification of the terminal heterocycle of R1. With no evidence for the latter, and precedent for multiple R1 orientations among other transpeptidase structures, the modeling of two conformations was chosen. *d*, as in *c*, with ceftobiprole modeled and test refined in the B conformation. Ceftobiprole is colored by CMYK convention (C, yellow; N, blue; O, red; S, green), and σ represents the root mean square electron density in the crystallographic unit cell.

Initial geometric restraints for ceftobiprole were generated using the PRODRG server (27). For the initial refinement stages, CNS was used (28), with later stages using TLS in REFMAC (29). Due to the moderate resolution of the data, refinement utilized NCS restraints for residues 27–402 and 404–668, with the acyl-Ser-403 excluded. Final refinement and map improvement were completed using TLS in BUSTER-TNT (30). PRODRG-generated ceftobiprole bond length and angle restraints used in the final refinement were manually adjusted using recently published small molecule bond lengths and angles (31); bond lengths and angles for its aminothiadiazolyl moiety were derived from the crystal structure of the analogous five-membered ring of methyl (Z)-(5-amino-1,2,4-thiadiazol-3-yl)-((fluoromethoxy)imino) acetate found in the Cambridge Structural Database (32). The final model has an $R_{\text{work}}/R_{\text{free}}$ of 0.173/0.237 and is of good stereochemical quality (Table 1). Although the difference between R_{work} and R_{free} may seem

somewhat large (0.064), it seems to us reasonable given the resolution of the data; it is also consistent with previously reported ligand-bound structures of PBP2a (PDB code, $R_{\text{work}}/R_{\text{free}}$ difference as follows: 1MWU, 0.061; 1MWT, 0.062; 1MWR, 0.054). Figures were generated using PyMOL (33) and UCSF Chimera (34).

Differential Scanning Calorimetry—Thermal stability of PBP2a (isolated as above) under various conditions was investigated by differential scanning calorimetry using the VP-DSC microcalorimeter (MicroCal GE Healthcare). Solutions of 10 μM PBP2a in 20 mM Tris, adjusted to pH 7.5 with H_2SO_4 , 1 M NaCl with and without β -lactam were used as samples. All solutions were thoroughly degassed before use, and the reference cell in each experiment was filled with an aliquot of buffer against which the protein solution had previously been dialyzed overnight. All samples were scanned from 30 to 70 $^\circ\text{C}$, at a rate of 1 $^\circ\text{C}/\text{min}$. Data were base-line-corrected, smoothed using a

FIGURE 2. Structural comparisons demonstrating PBP2a structural rearrangements upon binding of ceftobiprole and the importance of serine nucleophile exposure. *a*, PBP2a apo structure (orange) compared with PBP2a in complex with ceftobiprole (enzyme in green, ceftobiprole atomic bonds in black, and electron density contoured at 1σ in gray). Secondary structure is shown as transparent ribbons, with backbone and side chain atoms overlaid in solid color. Upon binding, structural rearrangements must take place to accommodate ceftobiprole. These include a shift (toward the left in the figure) of the β -sheet, most notably at strand $\beta 3$, as well as a clockwise twist (relative to the direction of $\beta 3$) of the residue backbone and side chain atoms. The arrow diagram illustrates these rearrangements, and the inset depicts the whole PBP2a molecule with the active site indicated by the inhibitor (black). PBP2a domains are shown as follows: N-terminal extension (NTE), non-penicillin-binding domain (NPB), and transpeptidase domain (TP). *b*, methicillin-susceptible endogenous PBP2 (blue) superimposed on the resistant PBP2a (orange). The O γ atom of the PBP2 serine nucleophile (Ser-398) is 2.0 Å distant from that of PBP2a Ser-403 and so is far more exposed at the opening of the active site cleft, presumably contributing to its greater availability for acylation *in vivo*.

Savitsky-Golay 9-point smoothing algorithm, and analyzed using Origin Scientific plotting software.

RESULTS

PBP2a-Ceftobiprole Crystal Structure—The enzyme-inhibitor complex captured in our x-ray structure shows clear electron density connecting the nucleophile Ser-403 with ceftobiprole, indicating the presence of an acyl-enzyme species. This is consistent with the reaction mechanism typical for β -lactams. Importantly, this observation indicates that ceftobiprole

FIGURE 3. Differential scanning calorimetry traces for PBP2a and acyl-enzyme complexes formed with β -lactams. *A* (blue), heat capacity change in the presence of 1 mM imipenem. *B* (magenta), heat capacity change in the presence of 100 μ M benzylpenicillin. *C* (green), heat capacity change observed for native protein. *D* (red), heat capacity change in the presence of 10 μ M ceftobiprole. Each β -lactam was also present in the reference cell, and traces in the absence of protein showed only a steady decline over the temperature range studied (data not shown).

behaves largely like a traditional β -lactam and does not derive its activity from an entirely novel mechanism.

Despite the moderate resolution of the diffraction data, it is clear that in monomer A of PBP2a the R1 substituent in the acyl-enzyme complex occupies two positions, denoted conformation A, in which the R1 group is oriented toward the R2 group and residue Thr-600 of PBP2a, and conformation B, in which the R1 group is oriented away from the R2 group and toward Ser-403 (Fig. 1, *b–d*).

Comparison of the enzyme-inhibitor complex structure with the previously published apo structure of PBP2a (PDB code 1VQQ) allows insight into the conformational changes required to accommodate ceftobiprole binding (Fig. 2*a*) (16). Upon acylation of PBP2a with ceftobiprole, a twisting of the central β -sheet, most notably at strand $\beta 3$, alters the position and orientation of Ser-598, Gly-599, and Thr-600 to avoid steric clashes with the reacted β -lactam. These backbone (and side chain C_β) atoms twist, with a backbone-plus- C_β r.m.s.d. ~ 1.2 Å. The neighboring Ala-601 and Glu-602 residues reposition themselves in response to the R1 substituent (all-atom r.m.s.d. ~ 1.5 Å), as do residues Arg-612 and Gln-613 (all-atom r.m.s.d. ~ 2.9 Å; for comparison, 625 C_α pairs of PBP2a upon acylation vary with r.m.s.d. ~ 0.5 Å). The conformational change also involves movement of the N terminus of helix $\alpha 2$ such that Ser-403 becomes more exposed and therefore better positioned to react (backbone-plus- C_β , residues 403 and 404 have a

TABLE 2

Thermal stability determined for PBP2a upon acylation

Compound	T_m	ΔT_m	Peak height	Peak width at half-height	Total heat capacity
	°C	°C	mcal/°C	°C	mcal
None	45.8		6.00	3.75	26.25
Ceftobiprole	47.5	+1.7	3.04	6.88	23.32
Cephalothin	45.3	−0.5	3.83	4.03	24.95
Nitrocefin	45.2	−0.6	3.95	3.83	23.12
Piperacillin	43.4	−2.4	4.54	3.80	20.95
Benzylpenicillin	41.3	−4.5	4.48	3.75	19.31
Imipenem	36.5	−9.3	3.63	4.17	18.70

r.m.s.d. ~ 0.9 Å). This is an important feature because serine nucleophile accessibility appears to be a major factor in determining whether acylation will occur as illustrated by the placement of Ser-403 deeper within the PBP2a active site cleft than the equivalent nucleophile in the methicillin-susceptible PBP2 (PDB code 2OLU; Fig. 2B; 16, 24). Apart from such reorganization events, the opposing side of the active site cleft evidently responds to acylation largely via rigid-body movement of regions 421–475 (r.m.s.d. of 220 backbone-plus- C_β atoms ~ 0.9 Å) and 501–526 (r.m.s.d. of 100 backbone-plus- C_β r.m.s.d. ~ 1.1 Å).

Differential Scanning Calorimetry—In addition to our x-ray structure, thermal stability of PBP2a apo enzyme and six different acyl-enzyme complexes were compared using differential scanning calorimetry (Fig. 3). Of six β -lactams tested, only ceftobiprole was observed to induce an increase in the thermal stability of the complex formed with PBP2a (Table 2). The apo enzyme had a thermal transition (T_m) at 45.8 °C which was increased to 47.5 °C with ceftobiprole. The cephalosporins cephalothin and nitrocefin each reproducibly caused a small (0.5–0.6 °C) shift to a lower temperature while also causing a decrease in the peak height and inducing a broadening of the transition. Piperacillin and benzylpenicillin produced larger shifts (2–4 °C) toward lower T_m , and imipenem produced a profound decrease in the thermal stability (T_m) of the protein, lowering the T_m by >9 °C.

DISCUSSION

The ceftobiprole-PBP2a structure represents the first transpeptidase complex with a β -lactam containing an oxyimino aminothiadiazolyl R1 group captured in both A and B conformations in the same structure (each conformation has been observed individually in studies involving the similar R1 group of cefotaxime, but with different enzymes (35–37)). In general, it is observed that the R1 substituents tend toward conformation A in low molecular weight transpeptidases (monofunctional PBPs) and conformation B in the high molecular weight enzymes (multimodular PBPs).

We propose that movement in the outermost part of the active site region allows ceftobiprole to bind to the narrow cleft via hydrophobic interaction with the extended R2 group. Electron density is clear for the Tyr-446 side chain encompassed within this region and the ceftobiprole R2 group, whereas enzyme acylation has accompanied a disordering of the Met-641 region. Met-641, Thr-600, and Tyr-446 sandwich the rings of the R2 substituent (forming favorable pi-pi stacking interactions); however, no hydrogen bonding occurs involving the CO or NH groups of the ceftobiprole R2 moiety. The hydrophobic

nature of the R2 group was initially proposed to allow a non-specific mode of interaction with PBP2a, which would allow for the β -lactam to be repositioned to accommodate any rearrangements near the Ser-403 nucleophile during the transition from the Michaelis complex to the acyl-PBP intermediate. The planarity and hydrophobicity of R2 indeed seem essential to allow the β -lactam extended access to the narrow active site groove; these properties mediate a close fit with Tyr-446, Met-641, and Thr-600 and correspond well with the features of the pentaglycine cross-bridge, characteristic for *S. aureus*, which links together peptide side chains from neighboring glycan strands. Additionally, the clustering of anti-MRSA β -lactam structures around cephalosporins (and some carbapenems but not generally penams) supports the importance of this planar interaction (16). An increased number of van der Waals contacts with the R2 substituent of cephalosporins, which is not seen with penicillin nor with methicillin, is proposed to increase the binding affinity and result in the increased overall acylation rate observed for cephalosporins such as nitrocefin over that of penicillin G (18). The binding event is also associated with a rearrangement of the elements on the opposing side of the active site cleft, most notably at strand β_3 and helix α_2 . Because Thr-600 is located in strand β_3 , contacts R2, and also forms part of the oxyanion hole, it appears to play a pivotal role in linking acylation to substrate binding in the course of β_3 strand rearrangements. The role of the β -lactam in facilitating changes at helix α_2 is less obvious, although movement of Ser-403 into the acylation position seems only possible via avoidance of steric clashes with helix α_2 residue Lys-406 and strand β_3 upon formation of the Michaelis complex. Ring opening of the lactam forces the R1 and R2 groups into close proximity, an interaction well tolerated in the A conformation of ceftobiprole, but strikingly detrimental in bulkier compounds like methicillin, consistent with MIC₉₀ values of ≤ 4 mg/liter for ceftobiprole compared with 1600 mg/liter for methicillin (7, 39). Without information on the Michaelis complex with an unreacted β -lactam, it is difficult to identify roles for the R1 and R2 groups in directly facilitating these conformational changes, because only the end point of the acylation reaction is observed.

Ceftobiprole binding raises the thermal transition of PBP2a by ~ 2 °C. Such increased thermal stability upon ligand binding is usually associated with the formation of additional interactions between the protein and ligand, which act to stabilize the complex. In contrast, the large decrease in thermal stability of PBP2a induced by imipenem, piperacillin, and benzylpenicillin (and to a lesser extent cephalothin and nitrocefin) suggests that the acyl-enzyme complexes formed with these β -lactams lose

stabilizing interactions during complex formation that are not compensated for by additional interactions with the inhibitor.

Changes in thermal stability of the acyl-enzyme complexes may influence kinetic measurements. For example, at 37 °C (a temperature often chosen for investigation of these kinetics (18)), the native protein is at the beginning of the unfolding transition and can be expected to be unstable under these conditions, whereas the imipenem complex (T_m 36.5 °C) would be at its thermal transition midpoint. Even at 25 °C, one would expect significant denaturation to occur if the measurements extend over hours (40). This suggests two possible modes of PBP2a inhibition: destabilization (e.g. by imipenem) or irreversible inhibition by stabilization of the Michaelis complex followed by acylation (e.g. by ceftobiprole).

The effectiveness of ceftobiprole is attributed to a combination of increased rate of acylation, higher intrinsic affinity, and a lower deacylation rate, all leading to greater inhibition of the active site of the transpeptidase (8). The differences between the resistant and susceptible PBPs in *S. aureus* result from altered positioning of the serine nucleophile, yielding large differences in acylation rates (41, 42). Ceftobiprole effectively acylates and inhibits PBP2a and displays potent inhibition against other PBPs (e.g. PBP2x in penicillin-resistant *S. pneumoniae* and PBP3 in *E. coli*) (43–45). Importantly, the potential for the development of resistance against ceftobiprole appears to be low, and it has been shown to be stable against hydrolysis by penicillinases produced by *S. aureus* as well as class A and C β -lactamases produced by certain Gram-negative bacteria (7, 38, 46). In the presence of β -lactams, PBP2a can function as the sole PBP in cell wall biosynthesis. Therefore, it represents an excellent target for specific inhibitors against MRSA. The novel, broad spectrum antibiotic activity of ceftobiprole gives it great promise both in empiric therapy and in the design of other β -lactams to combat the growing resistance of several prominent pathogens.

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