Activities of Ceftobiprole and Other β-Lactams against *Streptococcus pneumoniae* Clinical Isolates from the United States with Defined Substitutions in Penicillin-Binding Proteins PBP 1a, PBP 2b, and PBP 2x

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The activities of ceftobiprole and other β-lactams were examined with 30 *Streptococcus pneumoniae* isolates containing multiple *pbp1a*, *pbp2b*, and *pbp2c* mutations. The highest ceftobiprole MIC was 1 μg/ml, while the comparator MICs were 16 to 64 μg/ml. Fifty percent inhibitory concentrations for penicillin-binding protein 2x were 0.5 μg/ml (ceftobiprole) and 4 μg/ml (ceftriaxone) in a penicillin- and ceftriaxone-resistant isolate.

Community-acquired pneumonia (CAP) is diagnosed in 4,000,000 patients per year in the United States. *Streptococcus pneumoniae* is the leading bacterial cause of CAP (20 to 60% of cases) (3, 18). *Haemophilus influenzae*, *Staphylococcus aureus*, and atypical pathogens are also causative agents (3 to 10%) (3, 18).

Combination therapy with an extended-spectrum cephalosporin and a macrolide is one of the CAP treatments recommended for adults in hospital settings (3, 18). Against *S. pneumoniae* and *H. influenzae*, the extended-spectrum cephalosporins are some of the most active β-lactam agents (19). However, against *S. aureus*, primarily methicillin-resistant strains, these cephalosporins are considered clinically ineffective (12, 13).

Ceftobiprole, a new parenteral cephalosporin in phase 3 clinical trials, exhibits a broad spectrum of activities against many clinically important gram-negative and gram-positive pathogens, including the common CAP pathogens and many clinically important gram-negative and gram-positive pathogens, including the common CAP pathogens. In clinical trials, exhibits a broad spectrum of activities against many clinically important gram-negative and gram-positive pathogens, including the common CAP pathogens (19). However, against *S. aureus*, primarily methicillin-resistant strains, these cephalosporins are considered clinically ineffective (12, 13).

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Ceftobiprole is differentiated from other parenteral cephalosporins due to its low MICs for *S. aureus*, including methicillin-resistant strains, and for cefotaxime- or ceftriaxone-resistant *S. pneumoniae* (12–14).

β-Lactam resistance in *S. pneumoniae* is caused by mutations in the penicillin-binding domains of one or more of its six penicillin-binding proteins (PBPs) resulting from point mutations or mosaic genes (9–11, 15). Altered PBP 1a, PBP 2x, and PBP 2b are the most important PBPs for β-lactam resistance among clinical isolates (1, 2, 9, 22).

In this study, the activities of ceftobiprole against 30 recent clinical *S. pneumoniae* isolates from six U.S. states with varied penicillin susceptibilities and defined mutations in the penicillin-binding domains of *pbp1a*, *pbp2b*, and *pbp2c* were examined.

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All MICs except for ceftobiprole MICs were determined with panels manufactured by Trek Diagnostic Systems (Cleveland, OH), using CLSI methods (5, 6). Ceftobiprole-containing minimum was prepared fresh for each assay.

Approximately 1-kb fragments of *pbp1a*, *pbp2x*, and *pbp2b* encompassing the penicillin-binding domain of each gene were PCR amplified as described by Nichol et al. (17) and sequenced by ACGT (Wheeling, IL). Competition assays were performed with a protocol based on the work of Sifaoui et al. (20), using Bocill-FIL (In vitrogen, Carlsbad, CA). PBPs were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and visualized using a Lumimager (Roche Diagnostics, Indianapolis, IN).

Seven of the 30 isolates were penicillin susceptible, with all β-lactam MICs being ≤0.03 μg/ml. These isolates had no mutations in the penicillin-binding motifs of *pbp1a*, *pbp2b*, and *pbp2c* (Table 1).

For the eight penicillin-intermediate isolates, penicillin MICs were 0.12 μg/ml for three and 1 μg/ml for five isolates. The former group had a T-to-A substitution in the PBP 2b SSNT motif (genotype 1), with corresponding β-lactam MICs of ≤0.25 μg/ml (Table 1). In addition to the previous PBP 2b substitution, a penicillin MIC of 1 μg/ml was associated with a T-to-A change in the STMK motif and an L-to-V change in the LKSGT motif of PBP 2x (genotype 2). Although these isolates were resistant to cefuroxime, cefotaxime and ceftaxone-resistant MICs were ≤1 μg/ml, and the ceftobiprole MICs were ≤0.25 μg/ml (Table 1).

The remaining 15 isolates were penicillin resistant. Seven isolates with genotype 3 had PBP 1a substitutions of T to A in the STMK motif and P to T in the SRNVP motif associated with penicillin MICs of 2 to 4 μg/ml, in addition to the PBP 2x and PBP 2b changes previously mentioned (Table 1). These seven isolates were not susceptible to cefuroxime, and some were not susceptible to ceftriaxone, cefotaxime, and amoxicillin-clavulanic acid; the ceftobiprole MICs for these isolates...
were \( \leq 0.5 \) \( \mu \text{g/ml} \) (Table 1). Isolates with genotype 4 had an additional substitution in PBP 2b of A to G in the KTGTA motif and a T-to-S change in the STMK motif of PBP 1a, as opposed to the T-to-A change seen in genotype 3 (Table 1). These changes corresponded with an increased penicillin MIC (8 \( \mu \text{g/ml} \)), resistance to ceftriaxone and amoxicillin-clavulanic acid, and an intermediate interpretation for ceftobiprole, while cefotaxime retained MICs of \( \leq 0.25 \) \( \mu \text{g/ml} \) (Table 1). For six isolates resistant to all the \( \beta \)-lactams (MICs, 4 to 64 \( \mu \text{g/ml} \)), cefotaxime MICs were \( \leq 1 \) \( \mu \text{g/ml} \) (Table 1). These isolates (genotype 5) had the same PBP substitutions as genotype 4 isolates, with the addition of an M-to-F change in the STMK motif of PBP 2x (Table 1).

Against the penicillin- and ceftriaxone-resistant isolate 8819, ceftobiprole had a higher affinity for PBP 2x than did ceftriaxone, with 50% inhibitory concentrations (IC\(_{50}\)) of 0.5 \( \mu \text{g/ml} \) and 4 \( \mu \text{g/ml} \) for ceftobiprole and ceftriaxone, respectively (Fig. 1). Both drugs had low affinities (IC\(_{50}\) of \( > 4 \) \( \mu \text{g/ml} \)) for PBP 2b (Fig. 1), as expected, since cephalosporins do not have PBP 2b as a primary target (8).

In this study, the PBP 1a, 2b, and 2x substitutions found in and adjacent to the penicillin-binding motifs, SXXK, SXXN, and KT/SG, were similar to substitutions reported by others (1, 7, 16, 17, 21). Recent reports identified additional PBP 2x substitutions important for the development of high-level \( \beta \)-lactam resistance in clinical isolates, with I371T, R384G, and M400T mutations having the greatest impact on resistance (4, 23). The I371T and R384G substitutions were found in all

![Ceftobiprole binding](http://aac.asm.org/)

**FIG. 1.** Binding of ceftobiprole and ceftriaxone to PBPs 2x, 2a, and 2b from *S. pneumoniae* isolate 8819 (penicillin resistant, ceftriaxone resistant).
isolates with genotypes 2 to 5. These substitutions impede β-lactam binding by causing a conformational change in a loop that is adjacent to the active-site cavity (4). The M400T substitution was found only in isolates with genotype 5. This substitution appears to be important for a low acylation efficiency and is found in association with the well-characterized PBP 2x substitution M339F (4). In our experiments, cefobiprole was able to bind to this mutated PBP 2x protein (IC50 0.5 μg/ml) and was the only β-lactam tested that had low MICs (0.5 to 1 μg/ml) for isolates with this PBP 2x substitution.

Previous reports by Kosowska et al. (14), who used agar dilution assays, and Hebeisen et al. (12) identified cefobiprole MICs as high as 2 to 4 μg/ml for penicillin-resistant S. pneumoniae strains. However, like Jones et al. (13), we did not identify any cefobiprole MICs of >1 μg/ml in microdilution broth assays. It will be important in the future to determine whether additional PBP mutations are associated with high cefobiprole MICs or whether there were MIC differences due to different testing methodologies.

In summary, six PBP genotypes were found among the 30 pneumococcal isolates, with cefobiprole having the lowest MICs for the organisms. The highest cefobiprole MIC for our collection was 1 μg/ml for isolates that were resistant to the other β-lactams (MICs of 16 to 64 μg/ml). β-Lactam MIC increases correlated closely with increases in the numbers of PBP 1a, 2x, and 2b substitutions. These results explain why cefobiprole retains activity against S. pneumoniae isolates resistant to extended-spectrum cephalosporins and penicillins.

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REFERENCES