Comparative Study of the Susceptibilities of Major Epidemic Clones of Methicillin-Resistant Staphylococcus aureus to Oxacillin and to the New Broad-Spectrum Cephalosporin Ceftobiprole

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Multidrug-resistant strains of Staphylococcus aureus continue to increase in frequency worldwide, both in hospitals and in the community, raising serious problems for the chemotherapy of staphylococcal disease. Ceftobiprole (BPR; BAL9141), the active constituent of the prodrug ceftobiprole medocaril (BAL5788), is a new cephalosporin which was already shown to have powerful activity against a number of bacterial pathogens, including S. aureus. In an effort to test possible limits to the antibacterial spectrum and efficacy of BPR, we examined the susceptibilities of the relatively few pandemic methicillin-resistant S. aureus (MRSA) clones that are responsible for the great majority of cases of staphylococcal disease worldwide. We also included in the tests the highly oxacillin-resistant subpopulations that are present with low frequencies in the cultures of these clones. Such subpopulations may represent a natural reservoir from which MRSA strains with decreased susceptibility to BPR may emerge in the future. We also tested the efficacy of BPR against MRSA strains with reduced susceptibility to vancomycin and against MRSA strains carrying the enterococcal vancomycin resistance gene complex. BPR was shown to be uniformly effective against all these resistant MRSA strains, and the mechanism of superb antimicrobial activity correlated with the strikingly increased affinity of the cephalosporin against penicillin-binding protein 2A, the protein product of the antibiotic resistance determinant mecA.

Since the introduction of antibiotics in the 1940s, Staphylococcus aureus, a frequent cause of potentially life-threatening hospital-borne infections, has become increasingly resistant to most chemotherapeutic agents. In 1960, the first clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA) appeared in the clinical environment and multidrug-resistant derivatives of MRSA clones began to spread worldwide, initially in the hospital environment but more recently in the community as well (11, 22). To treat MRSA infections, new antibiotics were developed, and beginning in the mid-1990s, the glycopeptide antibiotic vancomycin became the therapy of first choice for MRSA infections (3). Recently, MRSA isolates with reduced susceptibilities to vancomycin (vancomycin-intermediate S. aureus [VISA]) began to appear in several countries (4, 20, 25, 27) and MRSA strains that had acquired the enterococcal vanA gene complex (vancomycin-resistant S. aureus [VRSA]) were also recovered from clinical specimens. These VRSA strains also carried the β-lactam resistance gene mecA and were therefore resistant to both glycopeptide and β-lactam antibiotics (4, 24).

Ceftobiprole (BPR), the active component of the water-soluble prodrug ceftobiprole medocaril (BAL5788) (19), is a new cephalosporin that was shown to have powerful activity against many gram-positive pathogens (2, 15).

The primary purpose of this study was to determine the potency of this novel drug against highly oxacillin-resistant MRSA strains that exist at low frequencies in cultures of the most widely spread pandemic clones of MRSA (10). Under selective pressure, such subpopulations of highly β-lactam-resistant cells could become the source of lineages with increased BPR MICs. We also included in this study two MRSA strains that carried the enterococcal vancomycin resistance gene (VRSA strains) and two MRSA strains with reduced susceptibility to vancomycin (VISA strains).

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are described in Table 1. Mu50 was the first known MRSA isolate with reduced susceptibility to vancomycin (VISA) and was recovered from clinical specimens in Japan in 1996 (20). Another, more recent VISA isolate, isolate JH9, was recovered in the United States in 2000 from a bacteremic patient who underwent extensive chemotherapy with vancomycin during a 2-month period (25). VRS1 (HIP11714) is a highly VRSA clinical isolate that carries the enterococcal vanA gene complex and was obtained from the NARRSA strain collection. Strain COLVA is a highly vancomycin-resistant transconjugant generated by introducing a plasmid-associated copy of Tn1546 from donor isolate VRS1 into MRSA strain COL (24).

S. aureus strains were grown in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) with aeration at 37°C or on tryptic soy agar (TSA; Difco Laboratories) at 37°C. Bacterial growth was monitored by measurement of the optical density at 620 nm (OD620) with a spectrophotometer (model 2800; UNICO, Dayton, NJ). VRSA isolates COLVA and VRS1 were grown overnight in 5 ml of TSB supplemented with 10 μg/ml of vancomycin in order to induce the expression of the vanA resistance gene. Overnight cultures of COLVA and VRS1 were then centrifuged at 3,700 rpm at 4°C for 10 min, and the resulting pellets were washed twice with 5 ml of TSB to remove the vancomycin and were resuspended in 5 ml of TSB before they were spread on TSA plates for determination of their population analysis profiles (PAPs).

Antibiotics. BPR (provided by Johnson & Johnson Pharmaceutical Research and Development, L.L.C., Raritan, NJ) was prepared by the addition of 99 μl of dimethyl sulfoxide and 10 μl of glacial acetic acid to 1.5 mg of powder, and the mixture was then diluted with 891 μl of distilled water. Vancomycin and oxacillin were purchased from Sigma, St. Louis, MO.

Cloning of the mecA gene, excluding the sequence encoding the membrane anchor. The mecA gene, which encodes the extracellular domain of penicillin-binding protein (PBP) 2A, was amplified by PCR from plasmid pSTSW-2C.
Table 1. S. aureus strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolation</th>
<th>Molecular typing</th>
<th>Oxacillin resistance phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2125</td>
<td>Denmark</td>
<td>I</td>
<td>Hetero</td>
</tr>
<tr>
<td>HPV107</td>
<td>Portugal</td>
<td>IA</td>
<td>Hetero</td>
</tr>
<tr>
<td>BK2464</td>
<td>United States</td>
<td>II</td>
<td>Homo</td>
</tr>
<tr>
<td>HDE288</td>
<td>Portugal</td>
<td>VI</td>
<td>Hetero</td>
</tr>
<tr>
<td>BK2529</td>
<td>United States</td>
<td>IVd</td>
<td>Hetero</td>
</tr>
<tr>
<td>HAR24</td>
<td>United Kingdom</td>
<td>II</td>
<td>Hetero</td>
</tr>
<tr>
<td>HAR22</td>
<td>United Kingdom</td>
<td>VN</td>
<td>Hetero</td>
</tr>
<tr>
<td>USA300</td>
<td>United States</td>
<td>IVa</td>
<td>Hetero</td>
</tr>
<tr>
<td>COL</td>
<td>United Kingdom</td>
<td>I</td>
<td>Hetero</td>
</tr>
<tr>
<td>HU254</td>
<td>Brazil</td>
<td>II</td>
<td>Homo</td>
</tr>
<tr>
<td>VRS1</td>
<td>United States</td>
<td>II</td>
<td>Homo</td>
</tr>
<tr>
<td>COLVA</td>
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<td>I</td>
<td>Hetero</td>
</tr>
<tr>
<td>Mu50</td>
<td>Japan</td>
<td>II</td>
<td>Homo</td>
</tr>
<tr>
<td>JH</td>
<td>United States</td>
<td>II</td>
<td>Hetero</td>
</tr>
</tbody>
</table>

Abbreviations: MLST, multilocus sequence typing; ST, sequence type; SCCmec, staphylococcal chromosomal cassette mec; NA, not available; CA, community associated; Homo, homogeneous; Hetero, heterogeneous.

Recent studies in several laboratories have already documented the powerful antimicrobial activity of the newly developed β-lactam antibiotic BPR against several species of bacteria (13). While the greatly improved MIC of BPR is welcome news, experience with previously developed antimicrobial agents shows that the appearance of BPR-resistant news, experience with previously developed antimicrobial teria (13). While the greatly improved MIC of BPR is welcome

described here, we examined the activity of BPR against such

TABLE 2. Susceptibilities to oxacillin and BPR of major epidemic MRSA clones and their oxacillin- and BPR-resistant subpopulations

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml) for cell majority</th>
<th>Subpopulation growing on (µg/ml):</th>
<th>MIC (µg/ml) for OXA-resistant subpopulation</th>
<th>BPR MIC (µg/ml) for BPR-resistant subpopulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OXA*</td>
<td>BPR</td>
<td>OXA</td>
<td>BPR</td>
</tr>
<tr>
<td>E2125</td>
<td>6</td>
<td>0.8</td>
<td>400</td>
<td>6</td>
</tr>
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<td>HPV107</td>
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<td>0.8</td>
<td>400</td>
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</tr>
<tr>
<td>BK2464</td>
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<td>1.5</td>
<td>400</td>
<td>1.5</td>
</tr>
<tr>
<td>HDE288</td>
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<td>0.8</td>
<td>400</td>
<td>3</td>
</tr>
<tr>
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<td>1.5</td>
<td>1.5</td>
<td>400</td>
<td>3</td>
</tr>
<tr>
<td>HAR24</td>
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<td>1.5</td>
<td>800</td>
<td>3</td>
</tr>
<tr>
<td>HAR22</td>
<td>100</td>
<td>0.8</td>
<td>400</td>
<td>1.5</td>
</tr>
<tr>
<td>USA300</td>
<td>0.8</td>
<td>1.5</td>
<td>800</td>
<td>1.5</td>
</tr>
<tr>
<td>COL</td>
<td>&gt;800</td>
<td>3</td>
<td>&gt;800</td>
<td>3</td>
</tr>
<tr>
<td>HU25</td>
<td>400</td>
<td>3</td>
<td>800</td>
<td>3</td>
</tr>
</tbody>
</table>

*OXA, oxacillin.

b ND, not determined.

the data to GraFit (version 4.9) software (Erithacus Software Ltd., United Kingdom).

Conformational change of PBP 2A by BPR and oxacillin. PBP 2A (1 µM) was dissolved in 20 mM sodium phosphate buffer, pH 7.2, containing 30 mM NaCl in order to determine changes induced in the circular dichroic (CD) spectra by the addition of 20 µM of BPR or oxacillin. The CD spectra were recorded in an AVIV-62 CD spectrometer (1-cm path length; AVIV Biomedical, Inc.). PBP 2A was incubated in the absence and in the presence of β-lactam antibiotics for 30 min at 25°C before the CD spectra were recorded. The effect of each β-lactam by itself on the CD spectra, in the absence of PBP 2A, was subtracted (16, 17). BPR dissolved in 10% acetonitrile and 1% phosphoric acid solution at 1 mM was used as a stock solution for the CD experiments.

Peptidoglycan purification and analysis by high-pressure liquid chromatography (HPLC). Peptidoglycan was prepared from S. aureus cells by methods described previously (8).

RESULTS AND DISCUSSION

Recent studies in several laboratories have already documented the powerful antimicrobial activity of the newly developed β-lactam antibiotic BPR against several species of bacteria (13). While the greatly improved MIC of BPR is welcome news, experience with previously developed antimicrobial agents shows that the appearance of BPR-resistant S. aureus strains in the clinical environment is only a matter of time. It was also documented that strains resistant to a new antimicrobial agent often appeared from bacterial lineages that have already become resistant to previously extensively used antimicrobials, thus generating multidrug-resistant strains (12). Thus, newly emerging MRSA isolates were often also resistant to penicillin and other previously used therapeutic agents, such as streptomycin, tetracycline, and erythromycin (6). This also seems to be the case with the most recently emerging VISA and VRSA strains, which invariably carry the mechanism of methicillin resistance, reflecting the primary therapeutic use of these agents against infections caused by multidrug-resistant MRSA strains. In view of this background of the history of antimicrobial resistance, we performed experiments to identify a potential source of resistant strains that may emerge upon the introduction of BPR into clinical use.

A most likely source for such BPR-resistant strains appears to be the highly oxacillin-resistant subpopulations that are present at various low frequencies in cultures of most MRSA lineages with a heterogeneous phenotype (10). In the studies described here, we examined the activity of BPR against such highly oxacillin-resistant subpopulations of staphylococci which are present in the relatively few major pandemic MRSA clones that are responsible for most MRSA disease worldwide both in hospitals and in the community (1).

Efficacy of BPR against major epidemic MRSA clones. Table 1 shows the geographic origin, date of isolation, clonal type, and genetic backgrounds (sequence type, staphylococcal chromosomal cassette mec type, spa type), as well as the oxacillin resistance phenotypes of several pandemic MRSA lineages. The MICs for oxacillin and BPR exhibited by both the majority and the minority populations of bacteria present in the cultures of the heteroresistant strains are shown in Table 2.

The PAPs of these bacterial strains were determined for both oxacillin and BPR (Fig. 1). The most interesting features of these PAPs are the sharply contrasting shapes of the population analysis curves obtained with the two antibiotics when the same bacterial strains are compared. In contrast to the oxacillin-heteroresistant phenotypes of most MRSA clones, the shapes of the population analysis curves obtained with BPR indicates a virtual absence or a greatly reduced frequency of the highly resistant subpopulations (9). Indeed, the distribution of BPR MICs against the majority of cells and the resistant minority population appears to be in the same range of 0.8 to 6 µg/ml (Table 2).

In order to examine this issue more closely, we picked single colonies of bacteria that grew on the agar plates containing 100 µg/ml or 200 µg/ml of oxacillin (Fig. 1). After establishing the stability of high-level resistance in these colonies through serial passages, we used them as inocula for liquid cultures, which were then reexamined for their profiles of susceptibility to oxacillin and BPR. The stability of the resistant phenotype in most of these subpopulations has been demonstrated before (29). Figure 1 shows that these original “minority” subpopulations produced virtually homogeneous oxacillin-resistant cultures of MRSA with high MICs (Table 2). We also examined the BPR susceptibilities of these highly oxacillin-resistant cultures. In striking contrast to the nearly homogeneous and high oxacillin MICs, the MIC for BPR remained uniformly low for each of the strains tested (Fig. 1 and Table 2). In fact, the spread of BPR MICs against these highly oxacillin-resistant subpopulations was within the same narrow range of 0.8 to 1.5 µg/ml already demonstrated against both the majority and the
FIG. 1. Oxacillin and BPR susceptibility profiles of major epidemic MRSA clones and their homogeneous oxacillin-resistant subpopulations. Aliquots of an overnight culture were plated after serial dilution on TSA containing increasing concentrations of oxacillin (circles) or BPR (squares). The numbers of CFU were counted after incubation for 48 h at 37°C. The antibiotic susceptibility profiles (closed symbols and solid lines) were first determined for E2125 (A), HPV107 (B), BK2464 (C), HDE288 (D), BK2529 (E), HAR24 (F), HAR22 (G), USA300 (H), COL (I), and HU25 (J). Colonies were then picked from the subpopulations that were able to grow in the presence of high concentrations of oxacillin (indicated by the arrowheads with asterisks), and the oxacillin and BPR susceptibility profiles were further determined (open symbols and dashed lines). Colonies were also picked from the subpopulations that were able to grow in the presence of elevated concentrations of BPR (arrowheads), and the BPR susceptibility profiles of the bacteria were determined (see Table 2).
The selection of highly oxacillin-resistant subpopulations from individual MRSA strains did not increase the BPR MICs. We also picked single colonies of bacteria that grew on agar plates containing 1.5 or 3 μg/ml of BPR (Fig. 1) and determined their susceptibilities to BPR. The BPR MICs for the BPR-resistant subpopulations remained unchanged (Table 2).

Activity of BPR against MRSA strains with decreased susceptibility to vancomycin (VISA). We tested the BPR susceptibilities of two clinical VISA isolates with somewhat different mechanistic features. Strain Mu50 represents the first VISA isolate recovered from clinical specimens in Japan in 1996 (20). The second VISA strain, strain JH9, was recovered from a bacteremic patient in the United States in 2000 (25). While both of these VISA strains have vancomycin MICs within a similar range (6 to 12 μg/ml), they differed in their autolytic phenotypes (18, 28). Both Mu50 and JH9 are single-locus variants of NY/JP clone ST5 (sequence type 5) and show homogeneous (Mu50) and heterogeneous (JH9) resistance to oxacillin. While the oxacillin MICs for the majority of cells for Mu50 and JH9 were >800 and 3 μg/ml respectively, the corresponding MICs for BPR were 3 and 0.8 μg/ml (Fig. 2A and B).

Activity of BPR against highly VRSA strains carrying the enterococcal Tn1546 vancomycin resistance gene complex. Clinical VRSA isolate VRS1 showed a high level of homogeneous resistance to both oxacillin and vancomycin (MICs, >800 μg/ml) (Fig. 2C). A second VRSA strain, named COLVA, was generated in the laboratory by introducing a plasmid carrying the Tn1546 transposon from clinical strain VRS1 into highly oxacillin-resistant MRSA strain COL (24). Both VRS1 (Fig. 2C) and COLVA (Fig. 2D) shared a very low MIC for BPR of 1.5 μg/ml. This is in contrast to the uniformly high MICs of over 800 μg/ml for both oxacillin and vancomycin.

Earlier studies (24) have demonstrated that oxacillin at sub-MICs can effectively lower the vancomycin MIC of strain COLVA and convert the homogeneous vancomycin-resistant phenotype of this strain to a heterogeneous one. We tested the synergistic effect of BPR on the vancomycin resistance phenotype of VRSA strains VRS1 and COLVA. Addition of BPR at a concentration of 1 μg/ml to the vancomycin-containing agar plates produced cultures with the heterogeneous vancomycin susceptibility phenotype in both strain VRS1 (Fig. 3A) and strain COLVA (Fig. 3B), similar to what was already shown for oxacillin in the case of strain COLVA (24), and resulted in a drastic reduction of the vancomycin MIC (Fig. 4). The big difference between the two synergists was in the absolute concentrations of the β-lactam compounds necessary to substantially reduce the level of vancomycin resistance in the majority of cells. While oxacillin concentrations as high as 50 μg/ml
were necessary to reduce the level of vancomycin resistance (and produce the heterogeneous phenotype) (24), a drastic reduction of the vancomycin MIC of VRS1 and COLVA was achieved with as little as 1 μg/ml of BPR (Fig. 4).

Similar to the case of heterogeneous oxacillin-resistant strains, we selected from the synergy experiments highly vancomycin-resistant subpopulations (Fig. 3A and B) of VRS1 and COLVA, named VRS1* and COLVA*, respectively, that produce cultures composed of these more highly vancomycin-resistant subpopulations. We tested the susceptibility to BPR of VRS1* and COLVA*. Figure 3C shows that such highly vancomycin-resistant subpopulations (VRS1* and COLVA*) retained, unaltered, extremely low BPR MICs (1.5 μg/ml).

Comparison of bactericidal and bacteriolytic activities of BPR and oxacillin. The susceptibility of MRSA strain COL to the killing and lytic effects of oxacillin and BPR were compared by exposing exponentially growing cultures of the bacteria at mid-log phase of growth to concentrations of the two antibiotics equivalent to 10× the MICs. The cultures were incubated with the two antibiotics under aerobic conditions, and at different time intervals the number of surviving bacteria was determined. Figure 5 shows that oxacillin and BPR, used at equivalent concentrations in terms of multiples of their MICs, had indistinguishable bactericidal activities. After 24 h of incubation with 10× the MICs of the antibiotics, both oxacillin and BPR reduced the initial viable titer of the bacteria from 10^8 to about 10^3 CFU/ml.

Bacterial cells surviving the 24-h exposure to 10× the MIC of BPR were diluted to remove residual antibiotics and used to generate fresh cultures, which were again exposed to the same 10× MIC of BPR. Such a second exposure to BPR did not enrich the culture in bacterial cells that would have become less susceptible to the bactericidal activity of the antibiotic (Fig. 5).

In parallel experiments of exactly the same design used to generate the results shown in Fig. 5, the bacteriolytic activities of BPR and oxacillin were compared by monitoring the decrease in OD_{620} against time. No differences in the lytic potentials of BPR and oxacillin could be detected (data not shown).

Mechanism of action of BPR. The remarkable antimicrobial activity of BPR against MRSA strains, including the highly oxacillin-resistant subpopulations produced by each one of the widely spread epidemic MRSA clones, raises the possibility that BPR may have a novel, as yet not fully characterized property not shared by other β-lactam antibiotics, and such a hypothetical mechanism may be the basis of BPR's superior antimicrobial power.

In order to test this, we performed several assays (assays 1 through 4) to probe the mode of action of BPR in *S. aureus*.

(i) Assay 1: titration of affinity of BPR for PBPs of MRSA strain COL. Membrane preparations from a PBP 2 insertional mutant of MRSA strain COL (mutant RU130) were used to determine the relative affinities of oxacillin and BPR against the PBPs of the bacteria by an in vitro competition assay with radioactive benzylpenicillin. Membrane preparations were pre-incubated with increasing concentrations of the various antibiotics, after which the preparations received a single common concentration of the radioactive penicillin reagent, as described in Materials and Methods. The rationale for using the PBP 2 insertional mutant was to allow the clear detection of PBP 2A free of PBP 2 in the penicillin-binding assay since these two PBPs have virtually identical migrations in SDS-
polyacrylamide gels. Mutant RU130, in which Tn551 was inserted into the transpeptidase domain of the protein, was shown to produce a truncated PBP 2 of faster mobility, thus enabling one to evaluate the affinity of PBP 2A free from interference (23).

Figure 6 shows the results of the PBP competition assays. While a concentration of oxacillin as high as 2,500 g/ml was necessary to fully saturate PBP 2A, a BPR concentration as low as 6.0 g/ml was sufficient to achieve this. The penicillin-binding assay with BPR also showed that, in parallel with its high affinity for PBP 2A, this β-lactam was also able to saturate PBPs 1, 3, and 4 as well within an extremely low range of concentrations.

(ii) Assay 2: IC_{50}s of BPR and oxacillin for purified PBP 2A. The Bocillin FL-binding assay with purified PBP 2A was carried out to confirm the penicillin-binding assay with membrane preparations described above. PBP 2A was incubated with different concentrations of BPR or oxacillin prior to addition of a constant concentration of Bocillin FL. As shown in Fig. 7, 66% of PBP 2A was inhibited by BPR at 10 μM, whereas only 5% of PBP 2A was inhibited by oxacillin used at the same 10 μM concentration. These data indicate that BPR has very high binding affinity for the purified PBP 2A protein compared to oxacillin. This is consistent with the result of the affinity titration assay with membrane preparations.

The IC_{50} of these antibiotics for purified PBP 2A were 3.2 ± 0.4 μM (1.7 ± 0.2 μg/ml) for BPR and 710 ± 80 μM (320 ± 35 μg/ml) for oxacillin, indicating about a 220-fold higher efficiency of BPR than of oxacillin for the inhibition of PBP 2A. This IC_{50} of oxacillin for PBP 2A (320 ± 35 μg/ml) isolated from MRSA strain COL is similar to those (both 300 μg/ml) obtained for PBP 2A isolated from the MRSA strains 67-O and 27R (5). The IC_{50} of BPR for PBP 2A (1.7 ± 0.2 μg/ml) is also comparable to those (~0.5 μg/ml and 0.9 μg/ml, respectively) reported for PBP 2A purified from MRSA strains P8-Hom (15) and OC 3726 (7). These IC_{50}s are remarkably low compared to the values obtained for other β-lactam antibiotics. The new cephalosporin BPR appears to exhibit the highest affinity of binding to PBP 2A among the β-lactam antibiotics studied so far (5, 7, 15).

(iii) Assay 3: conformational change of PBP 2A by BPR and oxacillin. The conformational change of PBP 2A by oxacillin was demonstrated by Fuda et al. (17). The conformational change of PBP 2A by BPR was investigated to elucidate if the binding of BPR to PBP 2A would occur in a way similar to that for oxacillin. Figure 8 shows the conformational change of PBP 2A either by BPR or by oxacillin. As mentioned before, since the binding affinity of oxacillin for PBP 2A is very low, a large conformational change of PBP 2A did not occur when the protein was exposed to 20 μM of oxacillin. However, the same concentration of BPR caused dramatic α-helical relaxation at 208 nm and 222 nm, as shown in Fig. 8. This result indicates not
and without sub-MICs (0.4 g/ml) of BPR (A) or in the presence of a subinhibitory concentration (0.4 g/ml) of BPR (B). Following digestion with mutanolysin, muropeptides were separated by HPLC and detected by measurement of the absorbance at 206 nm.

The exact enzymatic mechanism by which bacterial transpeptidases and transglycosylases (PBPs) recognize their cross-linked oligomers has remained unclear, despite extensive efforts (21). This was particularly the case for PBP 2A, the active site of which appears to be accessible only to β-lactam inhibitors capable of inducing an initial allosteric type interaction which “opens up” the structure of this protein, as Fuda and colleagues recently demonstrated (16). The appearance of a cephalosporin such as BPR with powerful activity against MRSA strains should be encouraging for medicinal chemists, since it suggests that modification of the β-lactam core structure may still be a rewarding avenue for producing weapons effective against the sophisticated mechanism of resistance that has evolved in methicillin-resistant staphylococci (21).

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