ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1993, p. 2045–2053
0066-4804/93/102045-09$02.00/0

Vol. 37, No. 10

MINIREVIEW

Penicillin-Binding Proteins and Bacterial Resistance to β-Lactams

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INTRODUCTION

β-Lactam antibiotics are potent, broad-spectrum, bactericidal agents of low toxicity to eukaryotes and widespread clinical use. They encompass an enormous number of mostly semisynthetic compounds which can be conveniently divided into the bicyclic penicillins (penams, penems, carbapenems, oxapenams) and cephalosporins (cephems, cephamycins, oxacephems, carbacephems) and the monocyclic monobactams (39, 109, 110). "Natural" susceptibility to β-lactams varies widely among bacterial species. For example, *Neisseria gonorrhoeae* is susceptible to penicillin G at 0.05 μg/ml, and thus, a strain for which the MIC was 0.5 μg/ml would be considered resistant, whereas a *Pseudomonas aeruginosa* strain for which the MIC was the same would be considered susceptible (157). Susceptibility to β-lactam antibiotics reflects the combined effects of binding to targets (penicillin-binding proteins [PBPs]), stability to β-lactamases, and, in gram-negative bacteria, outer-membrane permeability. Similarly, resistance reflects a change in any of the three components. Bacterial resistance to β-lactams has generally increased in the past 50 years, reflecting the extensive usage of this class of compounds.

This minireview focuses on β-lactam resistance mediated by altered PBPs in clinically important bacteria. Traditionally, the major mechanism of β-lactam resistance has involved β-lactamases, particularly plasmid-mediated β-lactamases (78, 121). In gram-negative bacteria, β-lactamases are periplasmic and act in combination with altered outer membrane permeability (112, 171). In gram-positive bacteria, they are exocellular, although they are probably associated with the cell wall through electrostatic interactions (138, 140). This topic will not be further discussed here, because there are several excellent reviews on β-lactamases (2, 4, 14, 15, 78, 121, 139), the interplay between β-lactamases and outer membrane permeability (111–113), and β-lactamase inhibitors (16, 22, 23, 28, 87, 128). There are also several recent reviews on the biochemistry and genetics of PBPs and penicillin-sensitive enzymes (42, 43, 47, 54, 56, 149, 161, 172), topics important for the understanding of PBP-mediated resistance but discussed only briefly in this minireview. For a proper perspective, PBP-associated resistance should be viewed in the broad context of antibacterial resistance (13, 77, 106).

The targets for β-lactam antibiotics are cell wall-synthesizing enzymes (penicillin-sensitive enzymes), which are commonly detected by their ability to bind covalently radiolabeled penicillin (hence the name PBPs) (8, 54, 172). Key to their detection is the stability of the penicillin-enzyme complex, which permits analysis by conventional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by fluorography (147). PBPs are present in almost all bacteria, but they vary from species to species in number, size, amount, and affinity for β-lactam antibiotics, usually following taxonomic lines (50). They are localized nonrandomly on the outer face of the cytoplasmic membrane (79) and are anchored through short hydrophobic carboxy- or amino-terminal sequences; in gram-negative bacteria, they are pseudoperiplasmic (119). The major enzymatic activities associated with PBPs are peptidoglycan transpeptidase, which is believed to be essential, and DD-carboxypeptidase, which is believed to be dispensable. To date, only PBPs with DD-carboxypeptidase activity have been examined in any great detail. Some PBPs, particularly those of low molecular weight, also have weak β-lactamase activity which, although of no major physiological significance, may nevertheless interfere with PBP binding studies in intact bacteria (93).

A given organism contains four to eight PBPs with molecular sizes of 35 to 120 kDa; by convention, these are numbered in the order of decreasing molecular size. Once the number of PBPs for an organism is established, additional PBPs are numbered as derivatives of established ones. This is done to avoid renumbering PBPs and thereby causing confusion with the older literature. For example, when the 91-kDa PBP 1 of *Escherichia coli* was resolved into two components, they became PBPs 1a and 1b (152), while a novel 78-kDa PBP found in methicillin-resistant *Staphylococcus aureus* (MRSA) became PBP 2a (66, 134) or PBP 2′ (167, 169). Essential PBPs usually have a high molecular mass (60 to 120 kDa), have peptidoglycan transpeptidase activity, and control such fundamental processes as cell growth and division. In a given organism, there are two to four essential PBPs and, thus, potentially multiple β-lactam targets. Their inhibition can lead to cell lysis, death, or growth arrest. Binding of β-lactam antibiotics to PBPs is usually measured indirectly, as decreased binding of commercially available radiolabeled penicillin G (50, 89, 152).

Altered PBPs associated with β-lactam resistance are more commonly found in gram-positive than in gram-negative bacteria (Table 1). In laboratory strains, high-level, PBP-mediated resistance occurs in several steps rather than as a single-step mutation (148). Possibly, the steric similarity of β-lactams to the natural substrate makes difficult decreased affinity for the former with full retention of catalytic activity toward the latter by the target enzymes. By inference, clinical resistance may require the introduction of multiple amino acid substitutions in the target enzyme(s), in effect, resculpturing the active site (68, 69). Nevertheless, once present in a strain, resistance may rapidly spread along with the resistant strain (clonal spread).

TABLE 1. PBP-associated resistance in clinically important bacteria[a]

Organism	Resistant to:	PBP (alteration)	Reference
S. aureus	Methicillin, most β-lactams	2a[b] (decreased binding)	66, 134, 167, 169
	Cephalexin[c]	3 (decreased binding)	52, 90
S. epidermidis	Methicillin, most β-lactams	2a[b] (decreased binding)	125, 137, 155
S. pneumoniae	Ampicillin	1a[d] (decreased binding), 2x (decreased binding), 2a[d] (decreased binding), 2b[d] (decreased binding)	21, 35, 36, 61, 64, 92, 180
E. faecalis	Most β-lactams[e]	1, 3 (decreased binding)	26, 51
	Most β-lactams	3 (overproduced)	174
E. faecium	Most β-lactams[e]	1, 2 (decreased binding)	175
E. coli	Mecillinam[c]	2 (overproduced)	148
	Cephalexin[c]	3 (decreased binding)	68, 69
P. aeruginosa	Piperacillin	3 (decreased binding)	57
	Cefsulodin[c]	3 (decreased binding)	58
H. influenzae	Ampicillin[c]	4 (decreased binding)	102
	β-Lactams	3, 4, 5, (decreased binding)	20, 101, 102, 142
A. calcoaceticus	β-Lactams	1, 3 (decreased binding)	116
N. gonorrhoeae	β-Lactams	1, 2 (decreased binding)	33
	β-Lactams	2[d] (decreased binding)	10, 38, 150
N. meningitidis	β-Lactams	2[d] (decreased binding)	153
B. fragilis	Cefoxitin[c]	1 (decreased binding)	173
	Cefoxitin	1, 2 (decreased binding)	124

[a] Clinical isolates, unless indicated otherwise.
[b] Novel PBP.
[c] Laboratory strain.
[d] Hybrid PBP (segments imported).
[e] Organism naturally resistant.

GRAM-POSITIVE BACTERIA

Perhaps the most common example of PBP-mediated clinical resistance is MRSA, a major pathogen of increasing importance (12, 17, 41). MRSA isolates show high-level resistance to all β-lactams. They may be homogeneously or heterogeneously resistant; in the latter case, cells with increased levels of resistance may be present at low frequency (typically, 10^{-4} to 10^{-7}) in the form of one or more subpopulations. A reduction in the growth temperature (from 37 to 30°C) or an increase in the osmolality of the growth medium can increase the proportion of the resistant cells modestly (e.g., to 10^{-3}), dramatically (e.g., to 10^{-1}) (99), or not at all (67). MRSA strains first appeared in 1961 (80), but became a serious threat in the 1980s. Since then, MRSA strains have caused hospital outbreaks of colonization and infection throughout the world. The coexistence of multiple antibiotic resistance, particularly quinolone resistance, in MRSA is an alarming recent development (130, 144). Multiply resistant strains of MRSA have so far remained susceptible to vancomycin, currently the single reserve antibiotic for the treatment of MRSA infections. Nonetheless, the emergence of vancomycin resistance in enterococci (25), and more worryingly, its recent transfer to *S. aureus* in the laboratory (114), is a cause for concern.

Normally, *S. aureus* has five PBPs with molecular sizes of 87 (PBP 1), 80 (PBP 2), 75 (PBP 3), 70 (PBP 3′), and 41 (PBP 4) kDa (51, 133, 154). Of these, PBPs 1, 2, and 3 are essential and have high affinities for β-lactam antibiotics (51, 132). PBP 1 may be the primary peptidoglycan transpeptidase (5), PBP 2 (recently resolved into two components [19]) is a transpeptidase functioning in nongrowing cells (5), PBP 3 is a septation-associated transpeptidase (49), and PBP 4 is a DD-carboxypeptidase and transpeptidase involved in secondary cross-linking of the peptidoglycan (177). Interestingly, the transglycosylase activities are separate from those of the penicillin-sensitive transpeptidases in *S. aureus* (118), suggesting an organization of the peptidoglycan synthetic machinery different from that in *E. coli*.

In MRSA, an additional 78-kDa PBP, 2a (66, 134) or 2′ (167, 169), is invariably found (55, 67) and has provided new insights into the mechanism of resistance. PBP 2a has a low affinity for β-lactams and may catalyze a penicillin-insensitive transpeptidation (31, 32). It is encoded by a chromosomal gene (*mecA*), which is located on a 30-kb DNA segment (*mec* determinant) of unknown source (98). Transformation of methicillin-susceptible *S. aureus* with a *mecA*-containing plasmid converts it to MRSA (160a, 165). However, cellular levels of PBP 2a do not correlate with levels of methicillin resistance (105). The expression of PBP 2a is affected by growth conditions such as pH and temperature, the presence of a penicillinase plasmid (72), and a regulatory region (*mecR*) on the *mec* determinant (71, 160). The presence of NaCl in the growth medium also affects the phenotypic expression of methicillin resistance, but through inhi-

bition of autolysis (18, 94). An insertion element, IS*431*, on *mec* may be responsible for deletion of *mecA* and reversion to the susceptibility phenotype on subcloning (170). The DNA region between *mecA* and IS*431* varies in length among strains, the variability being due to a 40-bp repeat (135).

In addition to *mecA*, another chromosomal gene, *femA* (factor essential for methicillin resistance) has been implicated in methicillin resistance (6). It encodes a 48-kDa protein which does not affect expression of PBP 2a but affects the glycine content of peptidoglycan, cell wall turnover, and susceptibility to β-lactams (59, 60, 95). Additional chromosomal factors that contribute to methicillin resistance have recently been mapped (7).

PBP 2a has been proposed to have evolved from the fusion of a regulatory region for β-lactamase and a PBP structural gene of unknown origin since it is induced by β-lactams (145). There is some evidence for differential induction of PBP 2a by different β-lactams, and claims have been made that some β-lactams have activity against MRSA (9, 73, 97, 122). However, to date no β-lactam has been proven to be clinically useful against MRSA, although new β-lactams that bind to PBP 2a and that are active against MRSA may emerge in the future. In this context, the recent cloning of PBP 2a and the expression of a truncated (22 amino acids deleted from the N terminus), water-soluble form in *E. coli* are hopeful developments (176).

Fosfomycin, a structural analog of phosphoenolpyruvate which inhibits the condensation of PEP and UDP-GlcNAc to form UDP-MurNAc (81), decreases PBP 2a expression and acts synergistically with β-lactams against MRSA (168). It also increases PBP 2 expression and weakly antagonizes β-lactams against methicillin-susceptible *S. aureus* (107). The clinical implications of these findings are unclear.

Methicillin resistance associated with the overproduction of PBP 4 is relatively rare, although it has been reported in a laboratory strain (48) and in clinical isolates (162). In the former case, the organism remained susceptible to cefoxitin, which binds to PBP 4.

Moderate (~10-fold) resistance specifically to cephalexin, cephradine, or cefaclor has been reported in clinical isolates (90) and has been found to be associated with decreased binding to PBP 3 (52).

Staphylococcus epidermidis, the most common coagulase-negative *Staphylococcus* spp. (103), has a PBP pattern and a methicillin resistance mode similar to those of *S. aureus* (50, 82). The organism is commonly found in prosthetic devices and is thus a nosocomial pathogen of increasing importance. In both *S. epidermidis* and *Staphylococcus haemolyticus*, another coagulase-negative *Staphylococcus* spp., PBP 2a (*mecA* gene) is homologous to the PBP 2a of *S. aureus* (125, 136, 137, 155, 166). Polymerase chain reaction has recently been used for the genotypic identification of methicillin-resistant, coagulase-negative staphylococci (129, 164).

Streptococcus pneumoniae, the most common cause of pneumonia, has six PBPs with molecular sizes of 43 to 100 kDa. PBPs 1a/1b (100 kDa) and 2a/2x/2b (95 to 78 kDa) are potential lethal targets for β-lactam antibiotics (64), while PBP 3 (43 kDa) is a DD-carboxypeptidase (63). β-Lactam resistance in clinical isolates is associated exclusively with extensive PBP alterations, including changes in electrophoretic mobilities on SDS-PAGE. In the resistant strains, β-lactam binding to four high-molecular-mass PBPs (PBPs 1a, 2x, 2a, and 2b) is decreased (62, 64, 65, 120, 180). Three of the PBP genes for resistant strains, those for PBPs 1a (100 kDa), 2x (82 kDa), and 2b (78 kDa), are mosaics, which are believed to have arisen by homologous interspecies gene transfer (21, 35, 36, 61, 92). Alarmingly, altered PBP 1a and PBP 2x are also implicated in pneumococcal resistance to expanded-spectrum cephalosporins and have been shown to be transferable into susceptible strains in a single step (104). Interestingly, peptidoglycan structure is altered in resistant strains, implying changes in the substrate specificities of penicillin-sensitive enzymes (44, 45). PBP 2x has recently been cloned in *E. coli*, and a truncated, soluble, but enzymatically active form has been purified (91).

Viridans group streptococci are also increasingly resistant to β-lactam antibiotics; their resistance is associated with altered PBPs, particularly PBP 2b (78 kDa). This PBP is identical to the PBP 2b of *S. pneumoniae*, suggesting lateral transfer of the altered PBP 2b gene from *S. pneumoniae* (37). In addition, transformation experiments have shown that β-lactam resistance can be transferred from *S. pneumoniae* to viridans group streptococci (126, 127).

Enterococcus faecalis and *Enterococcus faecium* are naturally resistant to most β-lactams (70). The two organisms have similar PBP patterns: five PBPs, of which 1 (105 kDa) and 3 (79 kDa) are associated with resistance (26, 51, 174). PBP 3 (PBP 5 in the numbering system of Fontana et al. [40]) has a low affinity and acylation rate for β-lactams and is overproduced in resistant strains (40, 175). Recent evidence suggests that additional mechanisms may be involved in high-level clinical resistance to β-lactams (86).

GRAM-NEGATIVE BACTERIA

Clinical resistance to β-lactams in gram-negative bacteria is not commonly associated with alterered PBPs (150). This is probably due to the effectiveness of β-lactamases, coupled with reduced outer membrane permeability, in producing resistance. For example, there are no reports of PBP-mediated clinical resistance in *E. coli*, although there are such reports of laboratory resistance. Since this organism is the best understood one, its PBPs merit a more detailed discussion.

There are seven PBPs in *E. coli*, of which 1 (90 kDa), 2 (66 kDa), and 3 (60 kDa) are essential and are involved, respectively, in elongation, shape, and septation (146). All three are bifunctional enzymes with transpeptidase and transglycosylase activities (74–76, 88, 108, 156a) that probably reside in the amino- and carboxy-terminal halves of the protein, respectively (151). β-Lactam antibiotics that bind to PBP 1 (cephaloridine) cause lysis, while those that bind to PBP 2 (amdinocillin [mecillinam]) produce giant spherical cells and those that bind to PBP 3 (aztreonam, carumonam) result in filamentation (30, 47, 146). PBP 1 has been resolved into two genetically distinct components, 1a and 1b, with similar biochemical and physiological functions but different affinities toward β-lactam antibiotics (152). PBP 1a is sensitive to most β-lactam antibiotics, while PBP 1b is relatively resistant (30). PBP 1b has been further resolved into three components, α, β, and γ, in order of decreasing molecular size, with similar enzymatic activities and encoded by a single gene (83, 156). PBP 2 is highly sensitive to amdinocillin (mecillinam), some penicillins, and, to a lesser extent, thienamycin and clavulanic acid. PBP 3 is sensitive to most β-lactam antibiotics, being generally less affected by structural changes on the β-lactam nucleus than is PBP 1b or 2 (47). It is the target of most β-lactamase-stable β-lactam antibiotics such as ceftazidime and the monobactams carumonam and aztreonam. Laboratory mutants resistant to mecillinam (148) and cephalexin (68, 69) have reduced

affinities for of PBPs 2 and 3, respectively. Interestingly, the latter mutants still undergo septation, suggesting that it is possible for them to preserve their function while decreasing their affinities for β-lactams. However, the cell shape of PBP 3 (N361S) mutants is affected: the polar caps are pointed instead of hemispherical, suggesting an impaired ability of PBP 3 to process the normal substrate (159). PBP-mediated resistance has not been found in clinical isolates so far, despite the widespread use of PBP 3-specific β-lactams such as aztreonam and ceftazidime.

The low-molecular-mass PBPs 4 (49 kDa), 5 (42 kDa), and 6 (40 kDa) do not appear to be essential, because binding of β-lactam antibiotics does not affect growth. Nonetheless, mutants lacking PBPs 5 and 6 are hypersusceptible to β-lactams (11, 158), possibly reflecting the loss of the weak β-lactamase activity associated with the two PBPs. This β-lactamase activity may be physiologically significant, since it is coupled with the outer membrane permeability barrier. Two additional low-molecular-mass PBPs, 7 (32 kDa) and 8 (29 kDa), found in some preparations (141, 147) and suggested to be functionally distinct (163) may simply be degradation products of PBPs 5 and 6 (47).

Enterobacter, *Klebsiella*, and *Salmonella* spp. have PBP profiles very similar to that of *E. coli* (29, 143), while *Proteus* and *Serratia* PBPs are somewhat different, although they are still correlatable with *E. coli* PBPs (29, 50, 117). So far, PBP-mediated clinical resistance has not been found in these organisms.

The PBP pattern of *P. aeruginosa* is also correlatable with that of *E. coli* (115), and binding of β-lactam antibiotics generally results in morphological changes similar to those observed in *E. coli* (29). Interestingly, fosfomycin decreases PBP 3 expression and antagonizes β-lactams, although the latter effect may be secondary to β-lactamase induction (131). Non-β-lactamase-mediated resistance to β-lactams in clinical isolates and laboratory mutants has been reported and is associated with reduction in PBP 3 binding (57, 58).

In *Haemophilus influenzae*, eight PBPs with molecular sizes of 27 to 90 kDa have been detected (96). PBP 2 (80 kDa) and PBP 3 (75 kDa) correspond, on the basis of their sensitivities to β-lactam antibiotics, to PBPs 1a and 2 of *E. coli* (96), while PBPs 4 (68 kDa) and 5 (64 kDa) have transpeptidase activity in permeabilized cells (100). *Haemophilus* spp. (and *Neisseria* spp.) have relatively permeable outer membranes, as evidenced by their susceptibilities to the hydrophobic penicillin G and erythromycin (24). Non-β-lactamase-mediated resistance to β-lactams in clinical isolates is associated with decreased binding to PBPs 3 (75 kDa), 4 (68 kDa), and 5 (64 kDa) (PBP numbering and molecular sizes are those of Makover et al. [96]) (20, 101, 102, 142).

In *N. gonorrhoeae*, three major PBPs with molecular sizes of 90 (PBP 1), 63 (PBP 2) and 48 (PBP 3) kDa have been detected (PBP numbering and molecular sizes are those of Dougherty et al. [33]) (3, 33). PBP 2 is the primary target for most β-lactam antibiotics, while PBP 1 is generally less sensitive (34). Non-β-lactamase-mediated resistance to β-lactams in clinical isolates is associated with decreased binding to PBPs 1 and 2 (33). Comparison of the sequences of the PBP 2 (*penA*) genes from susceptible and resistant strains revealed multiple amino acid substitutions and insertions within the transpeptidase domain but close similarity among resistant strains (150). Specifically, the *penA* gene is identical to its susceptible counterpart in the first two-thirds of the gene sequence but is extensively modified in the remainder. The insertion of an additional residue (Asp-345A) was a universal feature among resistant strains and was primarily responsible for resistance (10). It has been proposed that the altered *penA* genes are hybrids, the resistant portion being introduced from a closely related species and subsequently spread clonally (38, 150). The distribution of this hybrid PBP and the threat it poses are not yet clear.

In the related organism *Neisseria meningitidis*, a hybrid structural gene for PBP 2 has also been implicated in β-lactam resistance (179). It has been suggested that the *penA* gene was introduced into both *N. gonnorhoeae* and *N. meningitidis* by transformation from the commensal organism *Neisseria flavescens* (153).

In *Acinetobacter calcoaceticus*, an emerging pathogen, six PBPs have been detected (50, 84, 116), of which PBPs 1 (94 kDa), 2 (92 kDa), and 5 (59 kDa) are implicated in resistance to β-lactam antibiotics. The PBP pattern of *Acinetobacter baumannii* is markedly different (46), and in laboratory mutants resistant to imipenem, binding to all PBPs was reduced, possibly reflecting β-lactamase interference. The clinical implications of these laboraratory findings are not yet clear.

In the anaerobe *Bacteroides fragilis*, a major pathogen naturally resistant to most β-lactams by virtue of its β-lactamases and outer membrane permeability (27), four PBPs have been detected. Of these, PBP 1 (100 kDa) may be the major peptidoglycan transpeptidase and PBP 2 (86 kDa) may be involved in septation (53). PBP 2 is the target for most β-lactam antibiotics (53, 123). Cefoxitin resistance in laboratory mutants has been associated with reduced binding to PBP 1 (173), while in clinical isolates cefoxitin resistance has been associated with reduced binding to both PBPs 1 and 2 (124). Resistance to cephalothin and other cephalosporins is associated with reduced binding to PBP 3 (65 kDa) (178). Other *Bacteroides* spp. have PBP patterns similar to that of *B. fragilis* (123), but their resistance to β-lactams has not yet been examined.

CONCLUDING REMARKS

In the past 50 years, much progress has been made in the chemistry and biology of β-lactam antibiotics, perhaps the most successful therapeutic agents. Guided largely by serendipity, there is now some understanding of how substituents on the β-lactam nucleus affect antibacterial activity. There is also some understanding of the mechanisms by which β-lactams affect bacteria and the types of defences bacteria put up to defeat or circumvent β-lactams. At the molecular level, there is some understanding of the structure of the β-lactam targets and their intimate relationship with β-lactamases. Both are β-lactam-interacting enzymes related structurally, mechanistically, and evolutionarily (54, 56, 85). In some cases, as with PBP 2a and the β-lactamase of *S. aureus*, they even share control elements for their expression (145). Their competition for the same β-lactam may lead to cell death or survival, which has been elegantly quantitated in *E. coli* as the "target accessibility index" (112, 171).

The studies summarized in this minireview lead to several conclusions, which could serve as a flexible framework for new data. PBP-mediated resistance poses a serious threat to the effectiveness of β-lactam antibiotics against some of the most common human pathogens (staphylococci, pneumococci, gonococci). In these organisms, β-lactamases are either absent (*S. pneumoniae*) or ineffective toward currently used β-lactams (*S. aureus*, *N. gonorrhoeae*). Thus, under the selection pressure of β-lactams and aided by a high transformation efficiency, portions of homologous chromo-

somal PBP genes have been introduced from related species. Resistance has subsequently spread worldwide, aided by the homogenization of the world population, along with the organisms.

PBP-mediated resistance has propelled continued research and drug development, both academic and industrial. Yet, overcoming PBP-mediated resistance remains a challenge. Perhaps the most immediate need is to find inhibitors of PBP 2a of MRSA. The recent progress toward a substrate-based spectrophotometric assay for high-molecular-mass PBPs (1) and a water-soluble form of PBP 2a suitable for inhibitor screening (176) is a promising development in that direction. A greater challenge will be to find inhibitors of the altered PBPs of *S. pneumoniae*. Like MRSA, this organism tends to be multiresistant, but unlike MRSA, there is considerable variability in the resistant PBPs which may complicate drug design. In *Neisseria* spp., PBP-mediated resistance has not yet been coupled with resistance to other antibiotics, and thus, the need to overcome β-lactam resistance is not urgent. Nevertheless, resistance trends point to multiresistant organisms in the future.

The immense versatility of bacteria has made antibiotic discovery a Sisyphian task. Under the selective pressure of β-lactams, bacteria have produced a vast array of β-lactamases. More recently, they have remodeled the active sites of vulnerable enzymes and preserved function, defying the close similarity of β-lactams to the natural substrate. They have evolved altered, although still functional, peptidoglycan. Inhibitors of the emerging resistant PBPs are likely to be narrow-spectrum antibiotics and may, too, face the ultimate challenge: an altered target.

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