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REVIEW

β-Lactam Resistance in ESKAPE Pathogens Mediated Through Modifications in Penicillin-Binding Proteins: An Overview

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ABSTRACT

Bacteria acquire β-lactam resistance through a multitude of mechanisms among which production of β-lactamases (enzymes that hydrolyze β-lactams) is the most common, especially in Gram-negatives. Structural changes in the high-molecular-weight, essential penicillin-binding proteins (PBPs) are widespread in Gram-positives and increasingly reported in Gram-negatives. PBP-mediated resistance is largely achieved by accumulation of mutation(s) resulting in reduced binding affinities of β-lactams. Herein, we discuss PBP-mediated resistance among ESKAPE pathogens that cause diverse hospital- and community-acquired infections globally.

Keywords: PBP; ESKAPE; Resistance; β-Lactam; Antibiotic; Peptidoglycan

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Key Summary Points

Penicillin-binding proteins (PBP)-mediated resistance is the key mechanism of β-lactam resistance in both Gram-negative and Gram-positive pathogens.

This review describes the types of PBPs and the effect of mutations that lead to antibiotic binding specificity/affinity on β-lactam resistance in ESKAPE pathogens. Further, it highlights the importance of multiple PBP-binding antibiotics in the treatment of infectious diseases.

Few β-lactamase inhibitors could potentially bind to PBP2 and exhibit antimicrobial activity.

INTRODUCTION

β-Lactam antibiotics are widely used for the treatment of common as well as serious bacterial infections. These antibiotics exert their antibacterial action through inhibition of penicillin-binding proteins (PBPs), the enzymes involved in bacterial cell wall synthesis. Bacteria acquire resistance to β-lactams by different mechanisms; however, production of β-

lactamases (enzymes that hydrolyze β-lactams) and modifications in PBPs are prominently reported. β-Lactamase-mediated resistance is the primary mechanism in Gram-negative bacteria, whereas PBP modifications are common among Gram-positive bacteria, with the exception of penicillinase in *Staphylococcus aureus*. However, this is not a rule anymore, with mutations in PBPs being reported for β-lactam resistance in Gram-negative pathogens. Several reviews have documented in detail the role of β-lactamases in β-lactam resistance [1, 2]. Here, we describe PBPs and their modifications that impact β-lactam resistance in ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens, which are known to cause diverse hospital- and community-acquired infections globally.

This article is based on previously conducted studies and does not contain any new studies with human participants or animal performed by any of the authors.

PEPTIDOGLYCAN AND PBPS

Bacteria require synthesis of peptidoglycan, an important polymer framework, for constructing the cell wall. The cell wall is essential for bacterial survival since it helps bacteria to maintain shape and sustain internal osmotic pressure, homeostasis of cellular functioning, and stress management. During cell wall biosynthesis, sugar moieties of the peptidoglycan are connected to each other (transglycosylation) to form linear chains that are then interlinked (transpeptidation) using short peptides attached to sugars. PBPs are integral membrane-bound enzymes (with catalytic active site domain in periplasmic space) that catalyze these important reactions. Transpeptidation reaction sites are the target of most β-lactams. They mimic D-alanine-D-alanine (D-ala-D-ala) dipeptide, recognized as normal substrate by PBPs, and, hence, are involved in stable covalent bond formation, leading to PBP inhibition. This substrate-mimicking-based block in peptidoglycan synthesis by β-lactams gradually weakens the bacterial cell wall, resulting in cell rupture [3] (Fig. 1A).

All species of bacteria contain several PBPs that perform diverse functions in peptidoglycan synthesis during cell wall formation. After binding to invading antibiotics, they could be neutralized or display resistance. Table 1 presents a summary of different PBPs, their molecular significance, and their association with β-lactams. PBPs are broadly categorized into two types on the basis of their molecular mass: low-molecular-mass (class C PBPs; cPBPs; carboxypeptidases/endopeptidases) and high-molecular-mass PBPs. High-mass PBPs are further classified into class A and B PBPs. Class A enzymes (aPBPs) can perform transglycosylation as well transpeptidation (dual function), whereas class B enzymes (bPBPs) are monofunctional (transpeptidases). Active site binding or interaction affinity by which β-lactams target PBPs is similar among PBPs of diverse bacterial species with differences in sequences of other coding region and overall architectures only, but there are distinct ways in which bacteria modify their PBPs to resist β-lactams [4] (Fig. 1B).

PBPS MODIFICATIONS AND β-LACTAM RESISTANCE IN GRAM-POSITIVE BACTERIA

Enterococcus spp.

Members of the *Enterococcus* genus (denote "E" in ESKAPE) represent notable pathogens causing serious hospital-acquired infections. *E. faecalis* and *E. faecium* are the two most prevalent disease-causing enterococci. Bacteria belonging to the *Enterococcus* genus are intrinsically resistant to cephalosporin β-lactams. *E. faecium*, which is considered to be more pathogenic than *E. faecalis*, express a low-affinity class B PBP (PBP5) to achieve β-lactam resistance. In *E. faecium*, PBP5 exists in two forms that signify their extent of β-lactam resistance. The first is PBP5 susceptible [ampicillin minimum inhibitory concentration (MIC) $\leq$ 2 mg/L], and the other is PBP5 resistant (ampicillin MIC $\geq$ 16 mg/L). This classification

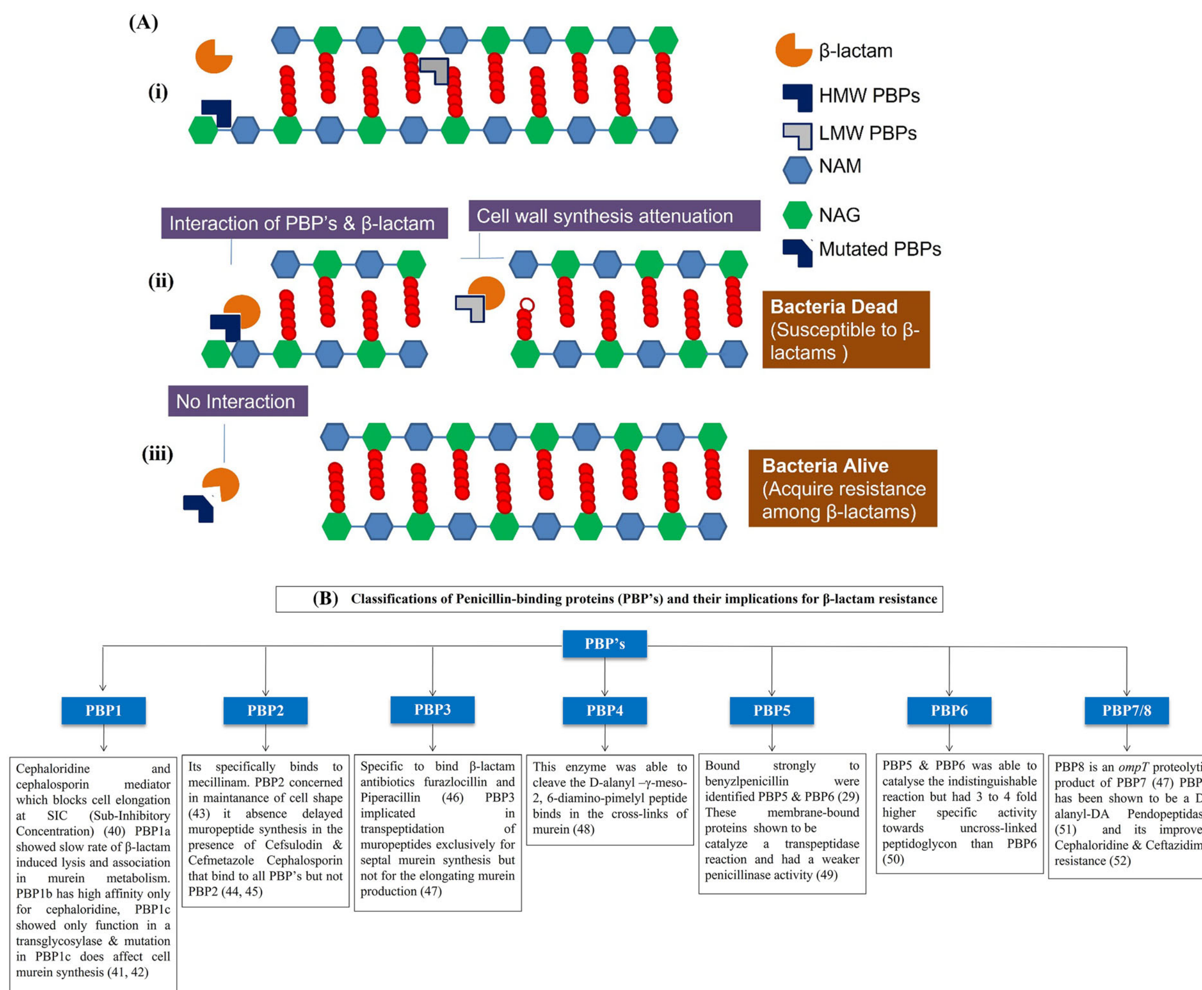

Fig. 1 **A** Cell wall biosynthesis involves the assembly of peptidoglycan into strands by glycosyltransferase function [linking the lipid-bound *N*-acetyl glucosamine (NAM) with the pentapeptide-bound *N*-acetyl muramic acid (NAG)] followed by cross-linking of the assembled peptidoglycan by transpeptidase activity. Peptidoglycan glycosyltransferases catalyze the glycosyltransferase, while transpeptidase activity is PBP mediated. The high-molecular-weight (HMW) PBS is involved in both glycosyltransferase and transpeptidase functions, while the lower-molecular-weight (LMW) PBP performs only the transpeptidase function. (i) Entry of β-lactam into the site of cell wall synthesis. (ii) PBP's arrest by β-lactam interaction. (iii) Structural alterations in PBPs due to mutation(s) affect their affinity to β-lactam, which therefore could not bind to PBPs; thus, cell wall synthesis continues. **B** Classifications of penicillin-blinding proteins (PBPs) and their implications

is based on identity of 20–21 amino acid positions between these two forms. In addition, there is a mixed type (*pbp5*-S/R; ampicillin MIC 4 mg/L) where, out of 20/21 positions, some amino acid substitutions resemble the susceptible PBP5, whereas a few substitutions are identical to resistance PBP5. These allelic differences in *pbp5* revealed evolutionary existence of two *E. faecium* clades; clade A (includes pathogenic isolates with *pbp5*-R and S-R versions) and B (human commensals/*pbp5*-S) [5]. Contemporary clinical isolates of enterococci display high-level resistance to β-lactams by overexpressing the low-binding-affinity PBP5, essentially precluding the use of β-lactams to treat infections by such isolates. When PBP5 of

Table 1 Summary of the different PBPs and their encoding genes, genomic position (in accordance with *E. coli* numbering), functions, and association with different antibiotics

PBPs	Encoding genes	Gene location	Function	Antibiotics activity
PBP1a PBP1c	*ponA* or *mrcA* *pbp1c*	75.9 min 56.9 min	Murein metabolism, catalyzed the polymerization of the glycan subunit and crosslinking of muropeptides [41, 42]	Cephaloridine and cephalosporin mediator blocks cell elongation [40]
PBP2	*pbpA*/*mrdA*	14 min	Maintenance of cell shape	Specifically binds only to mecillinam and not to cefsulodin, cefmetazole cephalosporin [45]
PBP3	*ftsI*	1.9 min	Cell division, septal ring formation, and transpeptidation of muropeptides [47]	Binds to furazlocillin and piperacillin [46]
PBP4	*dacB*	68 min	Catalysis of transpeptidase reaction	Indistinguishable
PBP5	*dacA*	14.2 min	Carboxypeptidase activity	Sensitive to penicillin due to variance of *dacA* (abolished DD-alanine carboxypeptidase activity) [49] and binds to benzylpenicillin [37]
PBP6 PBP6b	*dacC* *dacD*	44 min	Stabilization of murein at stationary phase	Indistinguishable
PBP7/8	*pbpG*	47.8 min	D-Alanyl-DAP-endopeptidase [51]	Increased cephaloridine and ceftazidime resistance [52]

E. faecium was crystallized with benzylpenicillin, the mechanistic details of β-lactam low affinity became clearer. Mutations of key amino acid residues decrease β-lactam access to the active site, with a loop playing a major role during the substrate restriction. *Enterococcus* play an important role in the horizontal gene transfer of large (~ 180–280 kb) chromosomal genetic platforms containing *pbp5* alleles [6]. In *E. faecium*, an alternate mechanism of peptidoglycan cross-linking also contributes to β-lactam (as well as vancomycin) resistance. Here, a PBP carboxypeptidase removes a D-alanine moiety from D-ala-D-ala dipeptide, and an L,D-peptidoglycan transpeptidase uses L-lys-D-ala dipeptide in the transpeptidation reaction to continue peptidoglycan synthesis even in the presence of β-lactams. Such L,D-transpeptidase activity in *E. faecalis* has not been reported in other species so far [7].

Staphylococcus aureus

Penicillinases produced by penicillin-resistant strains of *S. aureus* are among the first examples of β-lactamase-based resistance in Gram-positive bacteria. Methicillin, oxacillin, and other semisynthetic penicillins that are not hydrolyzed by penicillinases were therefore used to treat *S. aureus* infections. However, *S. aureus* acquired PBP2a, exhibiting methicillin resistance. These isolates are widely recognized as methicillin-resistant *S. aureus* (MRSA; "S" in ESKAPE). Methicillin-susceptible *S. aureus* (MSSA) contains eight PBPs: PBP1 (transpeptidase in cell division), PBP2 (bifunctional), PBP3 and PBP4 (transpeptidases), two transglycosylases, and two auxiliary transpeptidases. MRSA has nine PBPs (PBP2a in addition to the eight PBPs in MSSA). Among these, PBP1 and PBP2 are essential for MSSA and MRSAviability. In presence of β-lactams (sensed by trans-membrane

protein MecR), the *mecA* gene in MRSA produces PBP2a which is an alternate transpeptidase with an unaccessible β-lactams binding site, hence making β-lactams inactive against these populations of *S. aureus* [8]. PBP2a active site is not inhibited by β-lactams since it remains in a closed state and the active site catalytic residue lies deep inside the cavity. However, to carry out transpeptidation reaction, the active site needs to be opened. It has been demonstrated that PBP2a contains an allosteric non-penicillin-binding site and interaction of the substrate with this site triggers transpeptidation of protein [8]. Notably, *mecA*-negative *S. aureus* isolates also confer β-lactam resistance due to mutations in *pbp4*, *gdpP* and *acrB* genes [9]. However, the mechanism and clinical importance of such resistance requires further study.

PBP AND β-LACTAM RESISTANCE IN GRAM-NEGATIVE BACTERIA

β-Lactam resistance in Gram-negative bacteria is achieved either by the production of hydrolyzing enzymes (β-lactamases), target modifications (PBP mutations), restricted entry (impermeability), or efflux of active drug moiety. Resistance due to β-lactamases is highly developed and evolved as compared with other resistance mechanisms; however, co-presence of impermeability and efflux with β-lactamases are often found responsible for β-lactam resistance. Although less prevalent with less clinical relevance, a few reports have described the impact of PBP modifications in causing β-lactam resistance in Gram-negative bacteria.

Escherichia coli

Escherichia coli, model Gram-negative bacteria, are highly studied among Enterobacterales. Pathogenic *E. coli* can cause several community- and hospital-acquired infections. There are at least 12 PBPs in *E. coli*; in terms of relevance to effective β-lactam resistance, 2 PBPs (PBP2 and PBP3) are the most reported [10]. In addition, two aPBPs (PBP1a and PBP1b) are also targets of some β-lactams. Recently, in *E. coli* clinical isolates expressing diverse β-lactamases, a new type of PBP mutation (PBP3 insert) was detected that led to an increase in MICs of PBP3-targeting β-lactams. In *E. coli*, PBP3 is a cell division protein encoded by *ftsI* and is the target of several β-lactams, including cephalosporins, penicillins, and carbapenems [11]. PBP3 protein contains 588 amino acids with a catalytic serine at position 307 in the active site. At position 333, a novel YRIN or YRIK insert is reported that significantly elevates MICs (from 1- to 32-fold) of PBP3-targeting antibiotics, such as penicillins, monobactams, and cephalosporins but not carbapenems. This insertion is in close proximity to the PBP3 active site, but these residues do not interact with the ligand; instead, they lead to structural changes that obstruct entry of β-lactams (particularly for β-lactams that contain large side chains) into the binding pocket. PBP with this insertion retains its essential transpeptidase function but exhibits poor affinity to β-lactams. *Salmonella* express alternative PBPs (called "PBP2SAL" and "PBP3SAL") when placed in an acidic compartment, such as in the intracellular presence of these bacteria. These alternative PBPs are more resistant to some antibiotics, such as ceftriaxone or aztreonam, and might contribute to the relapses after therapy [12]. Modifications of the PBP targets should therefore be monitored because they represent an alternative mechanism to decrease β-lactam susceptibility [13, 14]. The PBP3 insertion-based resistance mechanism is so far observed only in *E. coli*, which is suggested to be due to less contribution of other resistance factors (such as efflux and impermeability) against β-lactams. It is mandatory to closely monitor the expansion of PBP3 insertion-based β-lactam resistance as the possibility of propagation of this resistance mechanism to other genera cannot be ruled out.

Klebsiella pneumoniae

Among Enterobacterales, *Klebsiella pneumoniae* ("K" in ESKAPE) is a prominent pathogen causing serious infections and conferring an appreciable level of β-lactam resistance. Throughout

antibiotic therapy and adaptation, *K. pneumoniae* has acquired several plasmids carrying genes that confer resistance to various classes of antimicrobial agents, leading to multidrug-resistant (MDR) phenotypes [15]. Against β-lactams, clinical isolates of *K. pneumoniae* possess a well-organized network of plasmid-encoded β-lactamases genes with the potential to resist all β-lactams efficiently. Furthermore, resistance against β-lactamase inhibitors has also emerged. Chromosomal resistance mechanisms, such as efflux pumps and cell membrane permeability, also contribute to the network [16]. Hence, PBP alterations in β-lactamase-producing *K. pneumoniae* isolates might contribute to increased β-lactam resistance [17].

Acinetobacter baumannii

Acinetobacter baumannii ("A" in ESKAPE) is an opportunistic hospital pathogen, and very limited treatment options are currently available against carbapenem-resistant isolates [18]. Besides other β-lactam resistance mechanisms, PBP mutations are also attributed to clinical resistance in *A. baumannii*, although such reports are scarce. This pathogen contains seven different PBPs: two aPBPs (PBP1a and PBP1b), two bPBPs (PBP2 and PBP3), and three cPBPs (PBP5/6, PBP6b, PBP7/8). In addition, a monofunctional transglycosylase is also present. Hot spot regions in all *A. baumannii* PBPs that are vulnerable mutations have been identified. However, most of the variations were identified as silent mutations and not associated with β-lactam resistance [19].

Pseudomonas aeruginosa

Among all Gram-negative pathogens, most examples of PBP-modification-based clinical resistance against β-lactams are reported in*Pseudomonas aeruginosa* ("P" in ESKAPE), another bug that causes difficult-to-treat hospital-associated infections, particularly in immunocompromised patients such as patients with cystic fibrosis. *P. aeruginosa* contains eight PBPs (aPBPs, PBP1a and PBP1b; bPBPs, PBP2, PBP3, and PBP3x; cPBPs, PBP4, PBP5, and PBP7); PBP3 is shown to be essential for *P. aeruginosa* growth. Clinical resistance against β-lactams due to mutations in PBP3 has been reported in several studies. Essentially, PBPs are sometimes considered the "killing site" of the antibiotic. Even conditional mutations of PBP3 led to cell division failures and higher susceptibility to β-lactams. PBP1a knockout led to motility retardation, and this observation, together with its localization at the cell poles, proposed its participation in flagellar function. Overall, these conclusions reveal that PBP3 represents the most capable target for drug discovery against *P. aeruginosa*, while other PBPs have less potential. The significance of PBP3 was also confirmed by the considerable reduction in MIC for some antibiotics in the PBP3 deletion strain. Certainly, this was the only deletion strain for which MICs were lower. Among the PBPs in *P. aeruginosa*, PBP3 has the maximum possibility to expand as a drug target. Essentially, they showed that the remaining PBPs (PBPs 1a and 1b and possibly also PBP2) are not likely to be productive targets [20]. In addition, mutation in PBP4 has been shown to cause hyperexpression of AmpC β-lactamase that leads to β-lactam resistance. Here, peptidoglycan synthesis precursors are not able to get recycled in the presence of PBP4 mutations and accumulate in periplasmic space. Eventually, it moved into the cytoplasm and through interaction with AmpR caused AmpC overexpression [21]. Though less common, the role of PBP4 mutations has also been reported in the induction of AmpC in *Enterobacter* spp. (last "E" in ESKAPE) isolates that resulted in resistance to β-lactam [22].

Not having too many clinically relevant mutations in PBPs is what keeps the use of β-lactams relevant in clinics since the problem of β-lactamases is relatively more manageable. To tackle the clinical situations imposed by diverse β-lactamases, β-lactamase inhibitors were introduced. Although protective, being β-lactams themselves, β-lactamases further evolved to resist the action of even these first-generation β-lactamase inhibitors. In recent times, non-β-lactam-based β-lactamase inhibitors have been used in combination antibiotics (with cephalosporins/carbapenems) and revamped to some extent the value of β-lactams.

The survey of available literature through the history of β-lactams clinical use suggests that, in Gram-negative bacteria, examples of PBP mutations capable of strong β-lactam resistance are not common. However, the recently reported example of PBP3 insert in clinical *E. coli* isolates is indicative of the fact that β-lactams are not so immune to PBP-based mutations, and in the future more such mutations may be encountered. Hence, what we need is to be ready with antibiotics/therapies that would be able to counteract PBP-modification-mediated resistance. In the next section, we discuss approaches that have been introduced (or are in preparation) in clinics to deal with PBP-mutation-driven β-lactam resistance in Gram-positive and Gram-negative bacteria.

Enterobacter Species

Members of the genus *Enterobacter* are ecological organisms found in water, sewage, soil, plants, or animal feces. They are opportunistic pathogens of plants and humans [23]. *Enterobacter* infections are mostly nosocomial, and therefore such infections are presented with broad resistance to third-generation cephalosporins, penicillins, and quinolones. Fourth-generation cephalosporins and carbapenems among β-lactams are effective enough to treat *Enterobacter* infections; however, carbapenems should only be used sparingly. Notably, third-generation cephalosporins and monobactams (e.g., aztreonam) pose a significant risk of in vivo derepression of AmpC β-lactamases, which might be caused by mutation(s) in the repressor protein, resulting in a high level of resistance to these antibiotics. Further, aminoglycosides are argued to be an otherwise excellent option to prevent this type of resistance [24, 25]. The use of fourth-generation cephalosporins (e.g., cefepime and cefpirome) appears to be very effective, mainly due to their action against AmpC-hyperproducing *Enterobacter* strains [26]. The use of the piperacillin–tazobactam mixture has recently been reported as an effective treatment option for *Enterobacter* spp. causing bloodstream infections [27]. A number of novel antibiotics have been tested against *Enterobacter* spp. The newly developed cefiderocol has exhibited a remarkable activity against *Enterobacter* spp. [28]. Different combinations of cephalosporins and β-lactamase inhibitors, such as cefepime–zidebactam, cefepime–tazobactam, ceftolozane–tazobactam, and ceftazidime–avibactam, have shown high-level antibacterial efficacy against these pathogens [29–31]. A key mutation (Gly112Asp) was previously identified in OmpK36 protein (eyelet region in the lumen) of a resistant isolate that caused a significant change in channel formation, altering its conductance and selectivity. Isolates with this porin mutation showed considerable β-lactam resistance [32, 33]. In *Enterobacter* spp., β-lactam exposure-related specific mutations in PBPs have not been observed. However, a series of mutations in key β-lactamase genes of *Enterococcus* are being reported [34]. In recent studies, several studies have reported existence of MDR *Enterobacter* isolates (*E. cloacae* and *E. aerogenes*), and in these isolates, the MDR profile was associated with the simultaneous presence of porin modifications, target mutations, β-lactamase production, and efflux overexpression [35, 36].

APPROACHES IN OVERCOMING PBP MODIFICATION-BASED RESISTANCE MECHANISMS

Association between Magnitude of Pharmacodynamic Activity and PBP Target Binding

β-Lactams are bactericidal drugs; however, the killing pattern (rapidity and extent of killing) differs among different β-lactams. For instance, carbapenems are known to show rapid as well as pronounced killing as compared with penicillins and cephalosporins. This difference is also reflected in the PK/PD requirement, typically measured from in vitro or in vivo PK/PD studies. While cephalosporins require 50–70% $fT > MIC$ for bactericidal activity (1 $\log_{10}$ kill), carbapenems evoke a similar response at much lower $fT > MIC$ (20–40%) [37], where fT is the percentage of time free drug remains above the

minimum inhibitory concentration. The relatively enhanced pharmacodynamic activity of carbapenems is attributable to their multiple PBP binding. The link between multiple PBP binding and enhancement of bacterial killing was experimentally shown. In *E. coli*, exclusively, cefsulodin binds to PBP1a/b, mecillinam binds to PBP2, and aztreonam binds to PBP3 [38]. The magnitude of killing effected by these agents in a time–kill study as a single drug or combinations (double or triple) were compared. The combination of cefsulodin and aztreonam or cesulodin and mecillinam or aztreonam and mecillinam showed augmented killing compared with that of alone drugs. When all three are combined, the magnitude of the kill is further improved. This observation established that multiple PBP inactivation leads to faster and more pronounced cidality. However, this experiment was performed employing a wild-type *E. coli*, and in case of a β-lactamase-expressing strain, these β-lactams will be inactivated and therefore cidal synergy is unlikely to occur.

ENHANCING THE PHARMACODYNAMIC ACTIVITY AGAINST β-LACTAMASE-PRODUCING STRAINS

The problem of β-lactamase production by bacteria is conventionally tackled by combining the β-lactam with a β-lactamase inhibitor. To explore the same, conceptually, the β-lactamase-positive strain was transformed into a β-lactamase-negative strain, thus allowing β-lactam to bind to its target PBP. Although this approach has been successfully used for many years (for instance, the use of piperacillin/tazobactam), contemporary Gram-negative isolates are capable of accumulating multiple various β-lactamases, including inhibitor non-susceptibility. Furthermore, these organisms, through mutational evolution, breed clones overexpressing β-lactamases and are selected upon exposure to β-lactam and β-lactamase combination. Even in the face of pharmacodynamics, since β-lactamase inhibitors do not possess standalone activity (no PBP binding), the PK/PD requirements of partner β-lactams remain unaltered. However, in recent years, a few Diazabicyclooctanes (DBO) class molecules, such as avibactam, relebactam, nacubactam, zidebactam, and ETX2514, have also been shown to bind PBPs with varying affinities (Table S1).

Earlier, we have mentioned that synergistic combinations are not utilizable when the organism expresses β-lactamases. However, this approach becomes valuable if one of the partners in the synergistic combination is completely stable to β-lactamases and thus able to offer unhindered PBP binding, which can be complemented with other partner that poses high PBP affinity albeit vulnerability to β-lactamases. This is the concept behind the β-lactam and β-lactam enhancer combinations, such as cefepime/zidebactam. In this combination, cefepime binds to PBP3 with high affinity. It also binds other PBPs such as PBP1a/b. In contrast, zidebactam, which is a novel DBO molecule, binds PBP2 potently in all clinically relevant Gram-negative bacteria [39]. Since DBOs are not typical β-lactams, they are not hydrolyzed by β-lactamases. Thus, the universal-β-lactamase-stable zidebactam binds to PBP2 in an unimpeded fashion, and when combined with cefepime, a synergistic (due to combined effect of PBP2 and PBP3 inhibition) strongly bactericidal activity is observed. The significance of this synergy in a cefepime-hydrolyzing and zidebactam non-inhibitable β-lactamase-producing organism is that the rapid binding of cefepime to its PBP target offsets the cefepime hydrolysis. The net effect is killing of the organism without the need for β-lactamase inhibition.

MULTIPLE PBP BINDING APPROACHES TO OVERCOME THE RESISTANCE DUE TO MUTATIONS IN PBP

The PBPs mutations described so far could be able to mount only a stepwise resistance rather than exponential resistance as in the case of β-

Table 2 Impact of currently followed β-lactam-/β-lactamase-based therapy against critical pathogens and their resistance strategy through PBP mutations

S. no.	Critical pathogen	Relevant PBP modification or mutations	Promising β-lactam/β-lactamase combinational therapy
1	*Escherichia coli*	PBP3 insert (YRIN/YRIK) [12, 53]	Aztreonam + avibactam
			Ceftazidime + avibactam
			Ceftriaxone + tazobactam
			Cefipime + taniborbactam
2	*Klebsiella pneumoniae*	PBP1, PBP2, and PBP3 [54]	Cefepime
			Mecilinam + amoxicilin
			Piperacillin, aztreonam, and ceftazidime
3	*Pseudomonas aeruginosa*	PBP1 and PBP3 [55, 56]	Ceftolozane + tazobactam
4	*Acinetobacter baumannii*	PBP3-H370Y	Sulbactam + durlobactam
		PBP3-Q488K and Y528H [57]	

lactamases. This means that mutations in PBP may lead to novel PBP variants whose β-lactam affinity may alter in a β-lactam-dependent manner. In that context, it is perceivable that multiple PBP binding agents/combinations of agents can effectively overcome PBP mutations compared with those agents that target a single PBP (Table 2). This is well explained in the unchanged activity of carbapenems against *E. coli* strains harboring altered PBP3 (due to four amino acid inserts). Since carbapenems bind to both PBP2 and PBP3 of *E. coli*, the potential loss in the affinity for altered PBP3 seems to be compensated by PBP2 binding.

CONCLUSION

Resistance to β-lactams continues to increase. In this review, we have discussed the role of PBP selectivity against a variety of β-lactam antibiotics among ESKAPE pathogens. This work provides a comprehensive overview of β-lactam resistance due to PBP mutations, a major β-lactam-resistance mechanism. The review also highlights the importance of selecting multiple PBP binding agents/combinations of agents to effectively overcome PBP mutations compared with those that target a single PBP.

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Data Availability. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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