β -Lactam Resistance Mechanisms: Gram-Positive Bacteria and *Mycobacterium tuberculosis*

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The value of the β -lactam antibiotics for the control of bacterial infection has eroded with time. Three Gram-positive human pathogens that were once routinely susceptible to β -lactam chemotherapy—*Streptococcus pneumoniae*, *Enterococcus faecium*, and *Staphylococcus aureus*—now are not. Although a fourth bacterium, the acid-fast (but not Gram-positive-staining) *Mycobacterium tuberculosis*, has intrinsic resistance to earlier β -lactams, the emergence of strains of this bacterium resistant to virtually all other antibiotics has compelled the evaluation of newer β -lactam combinations as possible contributors to the multidrug chemotherapy required to control tubercular infection. The emerging molecular-level understanding of these resistance mechanisms used by these four bacteria provides the conceptual framework for bringing forward new β -lactams, and new β -lactam strategies, for the future control of their infections.

Bacteria exemplify extraordinary diversity of size and shape (Young 2006, 2007). For the eubacteria, both the shape and integrity of the cell are intimately related to the chemical bonding pattern of the peptidoglycan polymer of their cell walls. Given this direct correlation, it is hardly surprising that many antibiotics target the enzymes that assemble the peptidoglycan (Schneider and Sahl 2010; Silhavy et al. 2010; Bugg et al. 2011; Silver 2013). Accordingly, bacteria have evolved a myriad of mechanisms to subvert these antibiotics.

Clinical bacterial resistance to antibiotics is often the acquisition of a primary resistance mechanism, abetted in important ways by secondary mechanisms. For the clinically important β -lactam antibiotics (these include the penicillin, cephalosporin, carbapenem, and

monobactam subfamilies), the primary resistance mechanisms used by Gram-positive bacteria are different from those used by the Gram-negative bacteria. The primary mechanism for the Gram-negative bacteria is expression of enzyme(s) that hydrolytically destroy the β -lactam, whereas for the Gram-positive bacteria the primary mechanism is target modification. This latter mechanism whereby structural changes to the specific enzyme targets of the β -lactam antibiotics render these enzymes less reactive to the β -lactam is identical to the mechanism used by the bacterial producers of the β -lactams (Ogawara 2015). The target of the β -lactams is a family of enzymes still known today by the name given to these enzymes—penicillin-binding proteins (PBPs)—dating from the discovery that these enzymes are inactivated, by covalent

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modification, by the β -lactams. The PBPs are the primary catalysts for the synthesis and remodeling of the peptidoglycan cell wall of bacteria. All bacteria have a family of PBPs, with some PBPs essential and others not essential. Recognition of a β -lactam structure by essential PBPs, leading to a mechanism-based loss of its enzymatic activity through the β -lactam inactivation, invariably culminates in cell lysis (Tomasz 1979). Although the evolutionary basis for the selection of target modification as the primary resistance mechanism for the Gram-positive bacteria is uncertain, the absence of an exterior membrane (and, thus, the absence of a control mechanism for exposure to antibiotics) in the Gram-positive bacteria surely contributes to this mechanism.

Although the peptidoglycan of all eubacteria has an identical core structure—a repeating disaccharide for the glycan strand with a peptide stem on the alternate saccharides of the glycan—each eubacteria tailors its peptidoglycan structure to accommodate the structural requirements for its shape, for the mechanisms for the reproduction *inter alia* of that shape, and for antibiotic resistance (Vollmer et al. 2008; Turner et al. 2014). The peptidoglycan of Gram-positive bacteria is a multilayer exoskeleton above their single membrane, whereas the peptidoglycan of the Gram-negative bacteria is a thinner (one- or two-layered) structure located in the periplasmic space between the two membranes. Two events define the structure of the peptidoglycan polymer: glycan strand elongation and cross-linking of the glycan strands by interconnection of the peptide stems on the alternate glycans. This latter event uses the terminal $-D-Ala-D-Ala$ structure of the stem as an acyl donor from one strand to an amine acceptor of an adjacent strand. The β -lactam antibiotics have nearly synonymous three-dimensional disposition to $-D-Ala-D-Ala$, but whereas the acyl-enzyme derived from the $-D-Ala-D-Ala$ structure is a catalytic species en route to a reaction, the acyl-enzyme derived from the β -lactam is not. The transpeptidase enzymes of peptidoglycan biosynthesis are inactivated by the β -lactam. Figure 1 summarizes the molecular events of peptidoglycan

synthesis, with focus on the transpeptidation event, in the context of the peptidoglycan structure found in *Streptococcus pneumoniae*. The compelling rationale for understanding these mechanisms is the timeless value of the β -lactams as therapeutic agents and the progressive emergence of resistance in both Gram-negative and -positive bacteria that were once routinely contained by β -lactam chemotherapy but are now clinically resistant.

THE ENZYME TARGETS OF THE β -LACTAM ANTIBIOTICS

The Enzymology of Peptidoglycan Assembly

The events of peptidoglycan synthesis involve a multiprotein and multienzyme assembly, whose composition and performance are distinctive to each bacterial species. The catalytic core of this assembly contains the enzyme catalysts of transglycosylation and transpeptidation. As β -lactams inactivate the enzyme catalysts of transpeptidation, these enzymes are collectively referred to as PBPs (Waxman and Strominger 1983; Sauvage et al. 2008; Spratt 2012). The use of “penicillin” in this terminology is historical: The penicillins were the first family of the β -lactam antibiotics, whereas the β -lactam antibiotics now used clinically include also the cephalosporins, carbapenems, and monobactams. All bacteria have a family of PBP enzymes classified by catalytic function and mass. The low-molecular-mass PBPs (also called class C PBPs) are primarily $-D-Ala-D-Ala$ carboxypeptidases and control the population of stems competent for cross-linking. The high-molecular-mass PBPs divide into two subclasses. The first (class A) is bifunctional, having (in separate domains) the two catalytic activities (transglycosylase and transpeptidase) required for peptidoglycan assembly. Class B PBPs are monofunctional catalysts of $D-Ala-D-Ala$ -dependent transpeptidation. Although all PBPs show signature active-site sequence motifs organized by a characteristic tertiary structure, in other respects (such as remaining sequence and thus nuance of domain function) they are distinctive, both with respect to each

Figure 1. Schematic for the D,D-cross-link of the peptidoglycan of *Streptococcus pneumoniae*. This schematic indicates the structural connectivity for the normal cross-linking of the peptidoglycan and does not represent three-dimensional structure. The peptidoglycan is assembled by sequential actions of the penicillin-binding proteins (PBPs). The presumed first action occurs at the glycosyl transferase active site wherein a glycan strand (1) is assembled by polymerization of lipid II as the substrate. The second action is cross-linking of the peptide stems (2) of adjacent glycan strands through transpeptidase catalysis by the PBPs. Completion of both steps gives the polymeric structure of the peptidoglycan cell wall. Transpeptidase catalysis is the step blocked by the β -lactam antibiotics. This schematic shows two glycan strands and the completed cross-link between them. Key additional features of the peptidoglycan are emphasized. The sequence of the terminal amino acids of the stem is $-L\text{-Lys-D-Ala-D-Ala}$ (in which the 3 identifies the penultimate D-Ala). In *S. pneumoniae*, an L-Ala-L-Ala dipeptide bridge (4) is added to the side-chain amine of this lysine. An L-Ser-L-Ala bridge is also encountered in this bacterium. An L-Ala-L-Ala bridge is also used by *Enterococcus faecium*, whereas *Staphylococcus aureus* uses a Gly₅ pentapeptide bridge. In all of these bacteria, the primary cross-linking event uses the central D-Ala of the stem as an acyl donor to a serine in the transpeptidase active site of the PBP, releasing the terminal D-Ala. This acyl-enzyme then transfers the acyl moiety to the terminal amino acid of the bridge, completing the cross-link (5). In this schematic, the upper glycan strand is the acyl-donor strand and the lower strand is the acyl-acceptor strand. The mechanisms controlling glycan strand length and the termination of glycan elongation (6) are poorly understood. In *S. pneumoniae*, a substantial portion of the N-acetyl groups of the glycan strand are hydrolytically removed (7) as a defensive measure against untoward peptidoglycan degradation, such as by lysozyme. An alternative pattern of cross-linking is used by *E. faecium* as a β -lactam resistance method. PBPs, Penicillin-binding proteins; NAM, N-acetylmuramic acid; NAG, N-acetyl-2-amino-2-deoxyglucosamine.

other and with respect to the PBPs of other (even closely related) bacterial species. A critical corollary is that individual β -lactam antibiotics show distinctive patterns for PBP inactivation at the (often) subsaturating conditions encoun-

tered in chemotherapy. This pattern determines the selection of the β -lactam to control infection and the development of β -lactam resistance. We examine the complexity of this nexus— involving β -lactam structure, PBP structure

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and function, and β -lactam resistance—for the four Gram-positive pathogens *S. pneumoniae*, *Enterococcus faecium*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis*.

THE MOLECULAR MECHANISMS OF β -LACTAM RESISTANCE BY *S. pneumoniae*

S. pneumoniae is a human commensal of the nasopharyngeal microbiota (Hakenbeck et al. 2012; Henriques-Normark and Tuomanen 2013; Fischetti and Ryan 2015). Notwithstanding the benefit of pneumococcal vaccines (which are themselves forces for clinical strain and resistance-determinant selections) (Angoulvant et al. 2015; van Tonder et al. 2015), invasive infection of *S. pneumoniae* among humans with compromised immune systems remains a significant cause of morbidity and mortality. In the 40 years since the first appearance of β -lactam resistance in *S. pneumoniae*, this bacterium has responded quickly to clinical interventions by recombination with other streptococci (Sauerbier et al. 2012; Jensen et al. 2015) to secure resistance not just to the β -lactams but to other clinically important antibiotics (Reinert 2009; Croucher et al. 2011). *S. pneumoniae* achieves β -lactam resistance through extensive and complementary “mosaic” mutation of three key PBP target enzymes with minimal fitness cost (Albarracín Orío et al. 2011) while retaining the genetic determinants that make this bacterium so successful for persistent human colonization (Nobbs et al. 2015). The challenge for successful chemotherapy against this bacterium is the invention of structure, whether antibiotic or vaccine, that subverts this balance. We address this challenge as it relates to β -lactam control of infection by *S. pneumoniae*, through consideration of the role of its PBPs in peptidoglycan biosynthesis and evaluation of the effect of the mutations to critical PBPs.

The PBPs of *S. pneumoniae*

S. pneumoniae is a fitting introduction to the topic of β -lactam resistance among the Gram-positive pathogens as this bacterium (compared with the others to be discussed) arguably presents our best (although far from complete)

understanding of Gram-positive peptidoglycan biosynthesis (Massidda et al. 2013). Three phases of peptidoglycan synthesis complete the ovococcal peptidoglycan of this bacterium. One phase is sidewall growth. The remaining two phases are septal growth and final septation. Septal growth occurs across the middle plane of the ovococcus and culminates with the synthesis of a distinctive circular growth—a surface annulus—of peptidoglycan growth immediately preceding septation (Wheeler et al. 2011). The regions of old and new peptidoglycan are distinctly demarcated, and *S. pneumoniae* grows without peptidoglycan turnover so as to presumably avoid detection of its peptidoglycan by the innate immune system (Boersma et al. 2015). *S. pneumoniae* apportions six PBPs to the task of constructing its peptidoglycan. As the different phases of peptidoglycan growth are interdependent (Philippe et al. 2015), functional complementarity (and redundancy) among these PBPs follows. Three (PBP1a, PBP1b, PBP2a) of the six PBPs are bifunctional class A PBPs, having both transglycosylase and transpeptidase activities. Two are class B transpeptidases (PBP2b and PBP2x), whereas the sixth PBP (PBP3) is a class C D,D -carboxypeptidase. The success of β -lactam chemotherapy in controlling *S. pneumoniae* infection establishes that certain of these PBPs, and certain events of peptidoglycan growth catalyzed by these PBPs, are essential to the bacterium. Unmasking this identity is central to the understanding of its β -lactam resistance.

The complexity of experimental design required for this task cannot be overstated. We emphasized previously the substantial sequence variability among the PBPs, apart from their signature catalytic motifs, within their conserved tertiary structure. Likewise, the β -lactam antibiotics also encompass structural diversity. Accordingly, the identification of β -lactam structure useful against a particular bacterium rests on the empirical discernment of the match between β -lactam structure and the structural space of the active site of the “essential” PBPs. It is thus not surprising that there is great breadth of β -lactam match, and mismatch, to the *S. pneumoniae* PBP ensemble (Kocaoglu

et al. 2015). Genetic analyses establish the monofunctional transpeptidases PBP2b (as a member of the elongosome complex used for peptidoglycan elongation) and PBP2x (as a member of the divisome complex synthesizing the peptidoglycan of the septum) as the two essential PBPs of *S. pneumoniae* (Berg et al. 2013; Philippe et al. 2014). Each of the bifunctional PBPs (PBP1a, PBP1b, PBP2) can be deleted individually. Paired PBP1a/PBP2 deletions as well as the triple deletion are lethal, whereas other pairwise deletions show morphological defects. Deletion of the low-molecular-mass PBP3 maintains viability but also at the cost of morphological defects (Hakenbeck et al. 2012). As PBP2b and PBP2x are monofunctional transpeptidases, completion of peptidoglycan synthesis requires their pairing with a transglycosylase. As a result of the functional redundancy among PBP1a, PBP1b, and PBP2, the identities for such pairings (and roles) are uncertain. Indeed, although the elongosome and divisome complexes colocalize at mid-cell at the start of the growth and division events of peptidoglycan synthesis, the PBPs of these complexes subsequently follow individual patterns of spatial localization (Land et al. 2013; Tsui et al. 2014). Resistance to β -lactams by *S. pneumoniae* is the consequence of mutations to either PBP2b or PBP2x. High-level resistance to the penicillins requires abetting mutations in PBP1a (Zapun et al. 2008).

The β -Lactam-Resistant PBPs of *S. pneumoniae*

Notwithstanding the preservation of the lysine-serine signature motifs in both the PBP2b and PBP2x D,D -transpeptidases, PBP2x is structurally distinct from PBP2b. PBP2b is a “typical” four-domain class A/B PBP: a short amino-terminal cytoplasmic tail; a transmembrane anchor; and protruding into the periplasm, a protein–protein interaction “pedestal” domain followed by the catalytic domain (Contreras-Martel et al. 2009). The cytoplasmic tail and transmembrane domains are also functional, presumably for protein–protein recognition (Berg et al. 2014). In addition to equivalents

of these four domains, PBP2x has two additional carboxy-terminal domains—termed “PBP and serine-threonine kinase-associated” (PASTA) domains—that follow the catalytic domain. The PASTA domains are suggested to respond to peptidoglycan structure under phosphorylation control (Maestro et al. 2011; Morlot et al. 2013). The importance of the PASTA domains for modulation of the catalytic domain of PBP2x (Dias et al. 2009; Maurer et al. 2012) and for its septal localization (Peters et al. 2014) has been shown.

The key questions with respect to β -lactam resistance are the mutation(s) to the essential PBPs, the mechanistic relevance of the mutations, and the additional contributors to *S. pneumoniae* β -lactam resistance. A recent genetic analysis addressed the last question. Genome-wide association of single nucleotide polymorphisms identified, in addition to the genes for PBP1a, PBP2b, and PBP2x, contributions from the *mraW* and *mraY* genes within the peptidoglycan synthesis pathway, as well as genes in the cell-division pathway (*ftsL*, *gpsB*), genes encoding chaperones (*clpL*, *clpX*), and a gene in the recombination pathway (*recU*) (Chewapreecha et al. 2014). The gene associations with penicillins were not coincident for the cephalosporins. The polymorphisms were distributed both in vaccine-targeted and non-vaccine-targeted lineages, suggested to explain why vaccination has failed to reduce β -lactam resistance (Hakenbeck 2014). A key determinant with respect to PBP mutation is the β -lactam used for resistance selection. For example, cefotaxime selectively inactivates the PBP2x (it is unreactive to PBP2b) of susceptible *S. pneumoniae* (Kocaoglu et al. 2015). A single-point mutation in the catalytic domain of PBP2x achieves clinical resistance to cefotaxime—but not to penicillins—as a result of a fourfold increase in the minimal-inhibitory concentration (MIC) of cefotaxime (Coffey et al. 1995). The more common experience is exemplified by the comparative structures of the PBP2b isolated from a penicillin-susceptible (minimum inhibitory concentration [MIC] of 0.01 $\mu\text{g/mL}$) and a PBP2b isolated from a highly penicillin-resistant (MIC of 6 $\mu\text{g/mL}$) clini-

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cal strain of *S. pneumoniae*. This latter enzyme had 58 mutations (Fig. 2A), presenting a mosaic pattern of alteration across both the pedestal and catalytic domains (Contreras-Martel et al. 2009). The differentiation between mutations that are incidental to resistance and those that contribute to resistance cannot be made solely from structural analysis (Hakenbeck et al. 2012). Although genetic analyses of the mosaic changes underscore notable “point” mutations

to PBP2b and PBP2x, as exemplified recently (Ip et al. 2015), the realization of β -lactam resistance has a greater dimension than that seen as amino acid change alone.

The intertwining of protein structure, catalytic function, and resistance pathways is exemplified by the “piperacillin paradox” observed for *S. pneumoniae* (Philippe et al. 2015). Piperacillin (a penicillin) is extensively used in the clinic against *S. pneumoniae*, and, although

Figure 2. (A) The mosaic pattern of resistance mutations within the periplasm-located domains of *S. pneumoniae* PBP2b (PDB Code 2WAE; Contreras-Martel et al. 2009). The enzyme crystallizes as a monomer. The 58 residues that undergo mutation during the transformation of this enzyme from a β -lactam-susceptible to a β -lactam-resistant state are shown in space-filling depictions. The catalytic serine used in the acyl-transfer reactions of transpeptidation is depicted in green in a space-filling representation. Although mutations in the catalytic domain predominate, several distal mutations are implicated as critical (as evidenced by the frequency of their appearance). In general, the differentiation between mutations that are incidental and those mutations that contribute directly to resistance by favorably altering the loop “breathing motions” required to enable access to this serine is exceptionally challenging. (B) The structure of the periplasm-located domains of *S. aureus* PBP2a with a bound quinazolinone (non- β -lactam) allosteric effector (PDB Code 4CJN; Bouley et al. 2015). The enzyme crystallizes as a dimer. The allosteric effector has intrinsic antibacterial activity and is depicted in green in a space-filling depiction (*lower right* of the structure). A single molecule of the allosteric effector is bound in a groove between the allosteric domain and a so-called pedestal domain. The orientation of this PBP2a dimer is approximately 90° relative to that of the PBP2b of A. The two catalytic serines are shown in green in a space-filling depiction. The mauve-colored arrows direct attention to their location. These serines are ~ 60 Å distant from the allosteric site. The structural change that occurs in the allosteric transformation that controls access to these active-site serines is understood (Otero et al. 2013).

it has greater potency against PBP2x (half minimal inhibitory concentration (IC_{50}) of 0.02 $\mu\text{g/mL}$ in susceptible *S. pneumoniae*), it selects preferentially for resistance mutations in PBP2b (IC_{50} of 0.18 $\mu\text{g/mL}$) (Kocaoglu et al. 2015). The proposed explanation for this paradox is that partial loss of PBP2b function in elongation is sufficient to arrest growth, whereas the more complete loss of PBP2x function in septation does not. Acquisition of a low-affinity PBP2b, thus, suffices to maintain cell multiplication (Philippe et al. 2015).

The molecular basis for the low affinity in the PBP targets has been extensively explored by crystallography. Correlation of the observed mutations to the locations within these structures shows preference for mutation at or near the active site. The cumulative effect of these mutations is interpreted as a conformation adjustment within the cleft that opens so as to favor substrate over the β -lactam inactivator. This concept is discussed with respect to the particular structures of *S. pneumoniae* PBP1a (Contreras-Martel et al. 2006; Job et al. 2008), PBP1b (Macheboeuf et al. 2005), PBP2b (Contreras-Martel et al. 2009), and PBP2x (Gordon et al. 2000; Dessen et al. 2001; Chesnel et al. 2003; Carapito et al. 2006; Maurer et al. 2008; Yamada et al. 2008). In selected cases, the molecular basis of the mutation is understood. Cephalosporins have poor PBP2b affinity but good PBP2x affinity and, thus, select PBP2x mutations. In response to cefotaxime challenge, under both laboratory and clinical conditions, mutation within the active site of PBP2x of the noncatalytic threonine-550 residue to alanine confers cefotaxime resistance (T550A, 20-fold decrease in acylation). The basis for cefotaxime resistance in these PBP2x mutants is interpreted as a result of the loss of a key hydrogen bond that is used for recognition of the cephalosporins (Gordon et al. 2000). The T550A PBP2x is, however, more susceptible to inactivation by penicillins (Mouz et al. 1999). Computational evaluations are now used in which such simple correlations are not possible, as with clinical mosaic mutations (Ge et al. 2012; Ramalingam et al. 2013). An aspect missing from these analyses is the effect of mutations on the transpep-

tidase reaction itself (as in vitro PBP assay of this reaction is not possible) and on the integration of the PBP into the elongosome and divisome complexes (Zerfass et al. 2009).

Antibiotic resistance often corresponds to multiple adaptation mechanisms and, here as well, *S. pneumoniae* shows its capability for β -lactam resistance. Its *murMN* operon encodes transferases that add an L-Ala-L-Ala (or L-Ser-L-Ala) cross-bridge extension to the L-Lys residue of the peptidoglycan stem (Filipe et al. 2002). These extensions contribute to β -lactam resistance (Hakenbeck et al. 2012), possibly by improving the efficacy of PBP2b-dependent transpeptidation (Berg et al. 2013). MurM/MurN-dependent cross-bridge extension is also important for proper control of pneumolysin release from the peptidoglycan to support virulence (Greene et al. 2015). An additional enzyme of peptidoglycan biosynthesis, MurE (the catalyst of L-Lys addition to the stem in the course of lipid II biosynthesis), also enhances—for unknown reasons—the β -lactam resistance of *S. pneumoniae*, as it also does for *S. aureus* (Todorova et al. 2015). *S. pneumoniae* further exemplifies the increasing recognition that antibiotic discovery in the future will require evaluations beyond that of the interaction of the antibiotic with its target. Although we recognize that all targets (and especially the PBPs) function within a confluence of regulated metabolic pathways, we equally well recognize that we understand neither the key components of these pathways nor how these components interrelate. For example, the roles for Ser/Thr kinase control of not just PBP2x, but of the peptidoglycan biosynthetic pathway (Dias et al. 2009) and the pathways for cell growth and division (Falk and Weisblum 2013; Fleurie et al. 2014), remain essentially unknown. An example of the value provided by a whole bacterium perspective on antibiotic selection is the study of the growth response of antibiotic-susceptible *S. pneumoniae* following exposure to antibiotics that were either bacteriostatic, bactericidal, or bactericidal as a result of lysis (i.e., β -lactams) (Sorg and Veening 2015). Exposure of *S. pneumoniae* to these antibiotics at the “F10” concentration of the antibiotic (in which

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F10 is the antibiotic concentration that achieves a 10-fold suppression of growth over a 10-h period and is a concentration typically somewhat greater than the MIC) confirmed the advantage of a bactericidal over a bacteriostatic mechanism. However, remarkable differences were seen among different antibiotics. Among the three β -lactams compared (ampicillin, MIC of 0.018 $\mu\text{g/mL}$; penicillin G, MIC of 0.015 $\mu\text{g/mL}$; and cephalexin, MIC of 0.22 $\mu\text{g/mL}$), ampicillin was the most efficacious. The basis for its superiority was interpreted in terms of its possession of a narrow concentration range for efficacy (corresponding to a narrow mutant selection window) coinciding with suppression of heterogeneous phenotype selection. In addition, the β -lactam data further suggested a relationship between heteroresistance and cell morphology (Sorg and Veening 2015). These observations, although fully consistent with the uniqueness of β -lactam structure coinciding with a unique profile for inhibition among the PBP family (Kocaoglu et al. 2015), affirm the growing recognition of the limitation of the MIC value (alone) as the criterion to define antibiotic efficacy.

THE MOLECULAR MECHANISMS OF β -LACTAM RESISTANCE BY *E. faecium*

Before the introduction of the antibiotics, the enterococci were innocuous commensal bacteria of the gut and were infrequently associated with infection (Arias and Murray 2012; Hendrickx et al. 2013; Werner et al. 2013; Kristich et al. 2014). Coincident with the introduction of the antibiotics, rapid genetic diversification culminated in the emergence 30 years ago of the enterococci as insidious, multidrug-resistant nosocomial pathogens. Whereas the key pathogen at the time of this emergence was *Enterococcus faecalis*, infections by *E. faecium* and *E. faecalis* now are equally prevalent. A key observation made during this transition was that the enterococci had intrinsic resistance to the cephalosporin class of β -lactam antibiotics. The transition of these bacteria from high- to low-penicillin susceptibility—to the point today in which nosocomial infection by the en-

terococci in the United States is presumed to be both β -lactam- and vancomycin-resistant—has been addressed from the vantages of genomics, structural biology, and enzymology (Palmer et al. 2010; Hendrickx et al. 2013; Lebreton et al. 2013; Werner et al. 2013). The origin of the β -lactam resistance of *E. faecium* (the more pathogenic of the two, in large part as a result of its greater β -lactam resistance) exemplifies the multifactorial resistance mechanisms now used by resistant bacteria.

Despite the identity of *S. pneumoniae*, the enterococci, and *S. aureus* as Gram-positive cocci, the structural similarities of their peptidoglycans, and the in vitro ability to functionally interchange the peptidoglycan biosynthetic pathways of these cocci (Arbeloa et al. 2004a), each chooses a different PBP mechanism to attain β -lactam resistance. The ability of the enterococci to assimilate pathways in support of antibiotic resistance and virulence was exemplified sharply by the appearance of vancomycin resistance as a result of the acquisition of the self-resistance mechanism used by vancomycin-producing bacteria. Vancomycin resistance results from the remodeling of peptidoglycan synthesis so as to replace the vancomycin-binding D-Ala-D-Ala stem terminus normally used for transpeptidation (Cattoir and Leclercq 2013). β -Lactam resistance by *E. faecium* involves a low-affinity PBP for catalysis of transpeptidation. Mutation of the essential (and intrinsically cephalosporin-unreactive) PBP5 of *E. faecium* gives the requisite low-affinity enzyme PBP5fm (Zorzi et al. 1996; Arbeloa et al. 2004b; Lebreton et al. 2013; Pietta et al. 2014). PBP5fm pairs with one of several transglycosylases to complete peptidoglycan synthesis (Rice et al. 2009). The basis for the low affinity, visualized from the vantage of the location of the mutations in the PBP5fm structure, is suggested as restricted motion in the loop that controls by an opening motion access to the active site (Sauvage et al. 2002). This mechanism parallels the observation for the key PBPs of *S. pneumoniae* and also parallels the resistance mechanism of PBP2a of *S. aureus*.

The contribution of PBP5fm to the β -lactam resistance of *E. faecium* is central

but not exclusive. All bacteria revamp their metabolism in response to cell-wall stress. For the enterococci, these additional changes include mitigation of reactive species to attenuate the bactericidal effect of antibiotics in general (Ladjouzi et al. 2013; Djorić and Kristich 2015), consistent with the emerging hypotheses for the bactericidal effect of antibiotics (Lobritz et al. 2015). A genome-wide evaluation of ampicillin resistance in *E. faecium* confirmed the central role of PBP5fm and identified a supporting contribution arising from an alternative mechanism for peptidoglycan cross-linking (Zhang et al. 2012). Here, the cooperative activity of a PBP carboxypeptidase (to remove the terminal D-Ala from the peptide stem) and an L,D-peptidoglycan transpeptidase, enabling use of the L-Lys-D-Ala stem segment as the acyl-donor for peptidoglycan cross-linking and, thus, the bypass of the β -lactam-sensitive use of D-Ala-D-Ala as the acyl donor

(Fig. 3). An identical enzyme function contributes to β -lactam resistance in *M. tuberculosis*. An L,D-transpeptidase is encoded in yet other Gram-positive bacterium (such as *Clostridium difficile*) but is not present in *S. pneumoniae* and *E. faecalis*. The operation of this pathway is under both two-component and Ser/Thr kinase control (Sacco et al. 2014). The structure, mechanism, and versatility (with respect to peptidoglycan structure) of this L,D-transpeptidase are characterized in Mainardi et al. (2005), Biarrotte-Sorin et al. (2006), Cremniter et al. (2006), and Magnet et al. (2007). Notwithstanding substantive catalytic differences compared with the PBPs (a catalytic cysteine, rather than a serine), this L,D-transpeptidase is inactivated by carbapenems via acylation of the catalytic cysteine. Penicillins do not inactivate and cephalosporins weakly inactivate (Dubée et al. 2012; Lecoq et al. 2013; Triboulet et al. 2013, 2015).

Figure 3. Schematic for the L,D-cross-link of the peptidoglycan of *E. faecium*. The peptidoglycan found in susceptible strains of *E. faecium* has D,D-cross-linking, wherein a -D-Ala-D-Ala-derived acyl moiety is transferred to the α -amine of the *iso*-D-Asx (depicted as *iso*-Asn) residue of the stem bridge. Following in vitro selection for ampicillin resistance, *E. faecium* that is fully resistant to both β -lactams and vancomycin is obtained by peptidoglycan synthesis using a non-PBP-dependent L,D-cross-link. Here, trimming of terminal D-Ala of the cross-link the stem permits the use of the -L-Lys-D-Ala moiety of the stem for acyl transfer to the α -amine of the *iso*-D-Asx to achieve the cross-link. The six-position of the MurNAc saccharide is acetylated, as occurs frequently in the enterococci as a lysozyme-resistance adaptation (Pfeffer et al. 2006).

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The minimal fitness cost of these resistance pathways (Foucault et al. 2010; Starikova et al. 2013; Gilmore et al. 2015) and the ability of the enterococci to further assimilate resistance to new therapeutic agents (such as daptomycin) account for the modern challenge of enterococci chemotherapy (Kristich et al. 2014). Later cephalosporins against *E. faecalis* and β -lactam-containing antibiotic combinations against *E. faecium* exemplify the direction for future β -lactam therapy of enterococcal infection (Henry et al. 2013; Hindler et al. 2015; Smith et al. 2015a,b; Werth et al. 2015).

THE MOLECULAR MECHANISMS OF β -LACTAM RESISTANCE BY *S. aureus*

S. aureus is a Gram-positive coccus and human commensal (Missiakas and Schneewind 2015). Invasive infection by β -lactam-resistant *S. aureus* following surgery and increasingly within the community to the soft tissue remains as difficult a challenge for chemotherapeutic control today as in prior decades (de Lencastre et al. 2007; Stryjewski and Corey 2014; Knox et al. 2015). New β -lactams, again acting to compromise the integrity of the peptidoglycan of the cell wall, remain a critical means of surmounting the resistance mechanisms used by *S. aureus* (Holmes and Howden 2014; Peyrani and Ramirez 2015). These mechanisms correspond to a complexity of regulatory mechanisms and center on an acquired PBP known today as PBP2a. This acquired PBP2a completes the synthesis of the peptidoglycan of *S. aureus* following incapacitation of its other PBPs by a β -lactam. The cell wall of *S. aureus* is thicker than many other Gram-positive bacteria, and, indeed, increased cell-wall thickness is a resistance mechanism used by *S. aureus* against other cell-wall targeting antibiotics, notably vancomycin (Cazares-Dominguez et al. 2015). Its peptidoglycan is exceptionally cross-linked (as much as 80% of the available stem peptide). Growth of the peptidoglycan of *S. aureus* involves synthesis of a cross-wall septum to create a pair of nascent, hemisphere-shaped bacteria. Rapid remodeling of the peptidoglycan provides the spherical shape of the mature bacterium. Microscopic

analysis of this transformation suggests a concentric growth pattern, followed by loss of the concentric features coinciding with the peptidoglycan remodeling (Turner et al. 2010, 2014; Bailey et al. 2014). At the molecular level, a distinctive feature of the peptidoglycan of β -lactam-resistant *S. aureus* is the presence of a pentaglycine cross-bridge extension to lysine of the peptidoglycan stem. Addition of this pentaglycine extension involves catalysis by the enzymes of the Fem (“factors enhancing methicillin” resistance) pathway (Dare and Ibba 2012). A comparison of the mature cell wall of *S. aureus* and the cell wall made by *S. aureus* having disruptions in the Fem pathway shows distinct differences in the polymeric structure (Kim et al. 2015; Singh et al. 2015). Nonetheless, the difference at the molecular level for the correlation of the pentaglycine extension to methicillin resistance is not known.

There is no uncertainty for the core mechanism of β -lactam resistance by *S. aureus*. This bacterium acquired two resistance mechanisms, with each acquisition occurring early in the 50-year history of this resistance (Chambers 2005; Moellering 2012; Peacock and Paterson 2015; Tong et al. 2015). Following the clinical introduction of the earliest penicillins, penicillin-resistant *S. aureus* appeared as a result of expression of a penicillin-specific β -lactamase. This β -lactamase was, and remains today, a “penicillinase” of modest catalytic ability by comparison to the pan- β -lactam capability of the β -lactamases now endemic among the Gram-negative bacteria. This penicillinase nonetheless provided resistance to *S. aureus* against these early penicillin structures. In response, medicinal chemists discovered that penicillins substituted with sterically large aryl groups, exemplified by methicillin, were poor substrates of the penicillinase. The therapeutic value of methicillin was not long-lived. *S. aureus*, in short order, acquired methicillin resistance by acquisition from environmental cocci of a new PBP (Zhou et al. 2008; Antignac and Tomasz 2009; Tsubakishita et al. 2010). This acquired transpeptidase (PBP2a) integrates into the enzyme assembly for peptidoglycan synthesis as a β -lactam-insensitive catalyst. PBP2a

distinguishes between the peptidoglycan as a substrate and against the β -lactam as an inactivator. As this ability extends to all structural classes of β -lactams (not just methicillin), this second resistance mechanism has persevered as a powerful resistance mechanism against all but the newest guises of β -lactam structure. The empirically derived structures of the anti-MRSA (methicillin-resistant *S. aureus*) cephalosporins, exemplified by ceftobiprole and ceftaroline (Fernandez et al. 2014; Stryjewski and Corey 2014), are successful antibiotics against *S. aureus* (and other Gram-positive bacteria) as a direct result of their ability to evade this structural discrimination by PBP2a. However, although the framework for our discussion is PBP2a, we also provide a perspective on the role of the auxiliary mechanisms.

The central question is the uniqueness of PBP2a. Methicillin-sensitive *S. aureus* encodes eight enzymes for peptidoglycan synthesis. MRSA has nine (now including PBP2a). The core eight enzymes are PBP1 (a monofunctional transpeptidase, active in cell division and separation), PBP2 (a bifunctional transglycosylase and transpeptidase), PBP3 (a transpeptidase), PBP4 (a low-molecular-mass transpeptidase), two monofunctional transglycosylases, and two “auxiliary” transpeptidases (FmtA and FmtB). Genetic deletion of these activities, most recently using a MRSA strain, shows that only two—PBP1 and PBP2—are required for the normal growth and normal shape of *S. aureus*, albeit with loss of β -lactam resistance, increased lysozyme susceptibility, and decreased virulence (Reed et al. 2015). Assembly of the MRSA peptidoglycan in the presence of the β -lactam challenge requires cooperative PBP2-dependent transglycosylation and PBP2a-dependent transpeptidation (Pinho et al. 2001). The presumption that the peptidoglycan benefits from cross-linking by a second transpeptidase is supported by evidence implicating both PBP4 and FmtA. PBP4 is involved in peripheral wall peptidoglycan remodeling (Leski and Tomasz 2005; Loskill et al. 2014; Qiao et al. 2014; Gautam et al. 2015). PBP4 is essential for β -lactam resistance in the community strains of MRSA (Memmi et al. 2008). A β -lactam (cefex-

itin) with high PBP4 affinity is synergistic with oxacillin (Memmi et al. 2008). Although the catalytic role of FmtA remains to be fully clarified, its presumed function is transpeptidation under conditions of cell-wall stress (Qamar and Golemi-Kotra 2012).

Peptidoglycan biosynthesis is highly regulated at numerous levels, including by kinase phosphorylation, stress-response pathways, and the transmembrane delivery of lipid II as the substrate for its PBPs (Lages et al. 2013). Notwithstanding the catalytic competence of PBP2a in MRSA, PBP2a expression is induced following the irreversible acylation of the exposed cell-surface domain of the transmembrane sensor protein MecR by a β -lactam antibiotic. PBP2a is made only when circumstances demand its presence. The MecR protein is structurally and functionally homologous to a BlaR sensor protein, which itself controls expression of the *S. aureus* penicillinase. Cross talk between the two pathways adds additional complexity to the poorly understood and multi-event cascades ultimately inducing PBP2a (and/or penicillinase) expression (Oliveira and de Lencastre 2011; Amoroso et al. 2012; Llarrull and Mobashery 2012; Peacock and Paterson 2015; Staude et al. 2015). A molecular-level understanding of these pathways is anticipated to identify new targets for antibiotic synergy with β -lactams. An important advance with respect to PBP2a is the discovery that its active site is under allosteric control with respect to two loop motions that open the active site in response to its peptidoglycan substrate (Otero et al. 2013; Fishovitz et al. 2014). β -Lactams that appropriately mimic peptidoglycan structure, such as ceftaroline, have good MRSA activity by their ability to effect this allosteric opening. The appreciable distance between the newly discovered allosteric site and transpeptidase active site is evident from the crystal structure of the non- β -lactam bound to PBP2a (Fig. 2B). As a consequence of their allosteric-induced opening of the active site, the concentrations achieved by these β -lactams in vivo coincide with the concentration required for PBP2a inactivation (Fishovitz et al. 2014, 2015). Subversion of the allosteric mechanism also has been achieved in vitro us-

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ing a threefold β -lactam combination (Gonzales et al. 2015). Non- β -lactam allosteric effectors capable of β -lactam synergy have also been identified (Bouley et al. 2015).

Genomic technologies identify additional loci that synergize with β -lactams (Roemer et al. 2012), including within the peptidoglycan biosynthesis pathway (Mann et al. 2013), the FtsZ-dependent organization of the peptidoglycan biosynthetic machinery (Tan et al. 2012), and the coordination of the synthesis of the wall teichoic acids with that of the peptidoglycan (Atilano et al. 2010; Pasquina et al. 2013; Wang et al. 2013; Sewell and Brown 2014; Winstel et al. 2014). The targets identified within the wall teichoic acid pathway may have special significance as the synergistic pairing of intervention against both biosynthetic pathways may extend to other Gram-positive bacteria (Hendrickx et al. 2013). The translation of successful in vitro combination therapy into successful clinical therapy is never straightforward (Bush 2015). Nonetheless, intervention at coupled binding sites (such as by simultaneous occupancy of the allosteric site and active site of PBP2a by two β -lactams) and at intersecting pathways (such as synergy between simultaneous disruption of peptidoglycan and teichoic acid biosynthesis by two separate inhibitors) is emerging as a credible (if not yet viable, apart from β -lactam- β -lactamase pairs) strategy to control resistant bacterial pathogens.

THE MOLECULAR MECHANISMS OF β -LACTAM RESISTANCE BY *M. tuberculosis*

The non-Gram-positive staining mycobacteria possess a cell envelope structure that is fundamentally different from and structurally more complex compared with the cell envelope of either the Gram-positive or -negative bacterium (Jackson et al. 2013; Alderwick et al. 2015; Nataraj et al. 2015). A consequence of the nuanced layers of this cell envelope is impermeability to antibiotic structure. Indeed, the challenge of chemotherapeutic control of mycobacterial infection is legendary (Chakraborty and Rhee 2015). Important additional factors contributing toward the β -lactam insensitivity of

M. tuberculosis (in addition to impermeability) are the expression of efflux transporters, the versatility of this bacterium with respect to the synthesis of alternate peptidoglycan structures, and expression of a robust—sufficiently so, as to represent a possible means of detection of *M. tuberculosis* infection (Cheng et al. 2014)— β -lactamase. For these reasons, the β -lactams have never been among the many antibiotics used to control *M. tuberculosis* infection. Nonetheless, the combination of the emergence of highly resistant *M. tuberculosis* strains (Seung et al. 2015) with recent studies showing some promise for β -lactams against *M. tuberculosis* (Hugonnet et al. 2009; Hazra et al. 2014; Wivagg et al. 2014) has justified reconsideration of the β -lactams.

Although *M. tuberculosis* has the expected PBP family for peptidoglycan biosynthesis (Prigozhin et al. 2014), as a matter of routine, it uses both PBP-catalyzed (and, thus, β -lactam-sensitive) D,D-transpeptidation and the β -lactam-insensitive L,D-transpeptidation for this task (Goffin and Ghuyssen 2002). The intrinsic β -lactam unreactivity by some of these PBPs (Bansal et al. 2015), a greater dependence on L,D-transpeptidation in the presence of β -lactams (Gupta et al. 2010; Kumar et al. 2012; Schoonmaker et al. 2014), and reactivity as BlaC substrates explain the historic therapeutic failure of the penicillin β -lactams. There is, however, promise for carbapenem combinations. As discussed previously for *E. faecium*, the carbapenems not only inactivate these L,D-transpeptidases (Lavollay et al. 2008; Triboulet et al. 2011; Cordillot et al. 2013; Schoonmaker et al. 2014; Brammer Basta et al. 2015; Wivagg et al. 2016) but have excellent activity against several of the essential PBPs of *M. tuberculosis* (Chambers et al. 2005; Kumar et al. 2012) and are poor substrates for its BlaC β -lactamase (Tremblay et al. 2010; Hazra et al. 2014; Horita et al. 2014). The sensitivity of BlaC-type enzymes to inactivation by clavulanate (Tremblay et al. 2008) and the diazabicyclooctanone class (Xu et al. 2012; Dube et al. 2015) indicates promise for β -lactam pairing. What remains to be seen is whether clinical use would give a BlaC mutation that diminishes the efficacy of clavulanate (Ve-

ziris et al. 2011; Feiler et al. 2013; Kurz et al. 2013; Egesborg et al. 2015; Soroka et al. 2015), and whether a diazabicyclooctanone derivative will be identified having adequate clinical reactivity. Among the exploratory pairings reported are meropenem-clavulanate, faropenem-clavulanate, amoxicillin-clavulanate, ceftaroline-clavulanate, ceftaroline-avibactam, and meropenem-sulbactam (Hugonnet et al. 2009; Gonzalo and Drobniewski 2013; Solapure et al. 2013; Dhar et al. 2015; Dubée et al. 2015; Zhang et al. 2015). Although some data for these pairings are promising, it is certain that such pairs will require incorporation into a multidrug regimen. The composition of such regimen may correspond to proven, emerging, or new drugs as may be identified against new targets (some of unknown identity) as recognized by genomic synthetic lethality screening (Lun et al. 2014).

CONCLUSION

The power of the β -lactams as antibiotics has been diminished, but surely not lost, by the breadth of resistance mechanisms now encountered in both the Gram-positive and -negative bacteria. The most recent generations of cephalosporin and carbapenem structures (in some cases, now paired with β -lactamase inhibitors), in particular, show promise against many of the most resistance-capable bacteria now encountered. This outcome argues that the structural space of the β -lactams needed to subvert target-based (and other) resistance mechanisms, as has been discussed here for the Gram-positive bacteria, is not exhausted. We now understand that the target modification of the Gram-positive PBPs does not stand alone but is supported by underlying pathways that may secure synergy with β -lactams if cocompromised. Even a bacterium historically regarded as impervious to the β -lactams, *M. tuberculosis*, may be made vulnerable. Yet, even with the molecular dissection of the resistant targets and resistance pathways, the future exploration of the structural space around the β -lactams will still demand investment in empirical structure-activity development at a time when commercial interest in empirical antibiotic discovery has waned. This

obligation is no less true for the complementary targets of these bacteria. We arguably are entering an era in which the most intractable bacteria will be treated with multiantibiotic regimens, as always has been the case for *M. tuberculosis*. It is small comfort to offer assurance that when the impasse with respect to investing in antibiotic discovery and development is surmounted, the targets and strategies to secure the future place of yet-undiscovered β -lactam antibiotics will be in place. The substance of this review attests to this assurance, although we are compelled to omit the identity of such structures.

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REFERENCES

- Albarracin Orio AG, Pinas GE, Cortes PR, Cian MB, Eche-nique J. 2011. Compensatory evolution of *pbp* mutations restores the fitness cost imposed by β -lactam resistance in *S. pneumoniae*. *PLoS Pathog* 7: e1002000.
- Alderwick LJ, Harrison J, Lloyd GS, Birch HL. 2015. The mycobacterial cell wall—Peptidoglycan and arabinogalactan. *Cold Spring Harb Perspec Med* 5: a021113.
- Amoroso A, Boudet J, Berzigotti S, Duval S, Teller N, Mengin-Lecreux D, Luxen A, Simorre JP, Joris B. 2012. A peptidoglycan fragment triggers β -lactam resistance in *B. licheniformis*. *PLoS Pathog* 8: e1002571.
- Angoulvant F, Cohen R, Doit C, Elbez A, Werner A, Béchet S, Bonacorsi S, Varon E, Levy C. 2015. Trends in antibiotic resistance of *S. pneumoniae* and *H. influenzae* isolated from nasopharyngeal flora in children with acute otitis media in France before and after 13 valent pneumococcal conjugate vaccine introduction. *BMC Infect Dis* 15: 236.
- Antignac A, Tomasz A. 2009. Reconstruction of the phenotypes of methicillin-resistant *S. aureus* by replacement of the staphylococcal cassette chromosome *mec* with a plasmid-borne copy of *S. sciuri pbpD* gene. *Antimicrob Agents Chemother* 53: 435–441.
- Arbeloa A, Hugonnet JE, Sentilhes AC, Josseume N, Dubost L, Monsempes C, Blanot D, Brouard JP, Arthur M. 2004a. Synthesis of mosaic peptidoglycan cross-bridges by hybrid peptidoglycan assembly pathways in Gram-positive bacteria. *J Biol Chem* 279: 41546–41556.
- Arbeloa A, Segal H, Hugonnet JE, Josseume N, Dubost L, Brouard JP, Gutmann L, Mengin-Lecreux D, Arthur M. 2004b. Role of class A PBPs in PBP5-mediated β -lactam resistance in *E. faecalis*. *J Bacteriol* 186: 1221–1228.
- Arias CA, Murray BE. 2012. The rise of the *Enterococcus*: Beyond vancomycin resistance. *Nat Rev Microbiol* 10: 266–278.

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- Atilano ML, Pereira PM, Yates J, Reed P, Veiga H, Pinho MG, Filipe SR. 2010. Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *S. aureus*. *Proc Natl Acad Sci* **107**: 18991–18996.
- Bailey RG, Turner RD, Mullin N, Clarke N, Foster SJ, Hobbs JK. 2014. The interplay between cell wall mechanical properties and the cell cycle in *S. aureus*. *Biophys J* **107**: 2538–2545.
- Bansal A, Kar D, Murugan RA, Mallick S, Dutta M, Pandey SD, Chowdhury C, Ghosh AS. 2015. A putative low-molecular mass (LMM) PBP of *M. smegmatis* exhibits prominent physiological characters of DD-carboxypeptidase and β -lactamase. *Microbiology* **161**: 1081–1091.
- Berg KH, Stamsås GA, Straume D, Håvarstein LS. 2013. Effects of low PBP2b levels on cell morphology and peptidoglycan composition in *S. pneumoniae* R6. *J Bacteriol* **195**: 4342–4354.
- Berg KH, Straume D, Håvarstein LS. 2014. The function of the transmembrane and cytoplasmic domains of pneumococcal PBP2x and PBP2b extends beyond that of simple anchoring devices. *Microbiology* **160**: 1585–1598.
- Biarrotte-Sorin S, Hugonnet JE, Delfosse V, Mainardi JL, Gutmann L, Arthur M, Mayer C. 2006. Crystal structure of a novel β -lactam-insensitive peptidoglycan transpeptidase. *J Mol Biol* **359**: 533–538.
- Boersma MJ, Kuru E, Rittichier JT, VanNieuwenhze MS, Brun YV, Winkler ME. 2015. Minimal peptidoglycan (PG) turnover in wild-type and pg hydrolase and cell division mutants of *S. pneumoniae* D39 growing planktonically and in host-relevant biofilms. *J Bacteriol* **197**: 3472–3485.
- Bouley R, Kumarasiri M, Peng Z, Otero LH, Song W, Suckow MA, Schroeder VA, Wolter WR, Lastochkin E, Antunes NT, et al. 2015. Discovery of antibiotic (E)-3-(3-carboxyphenyl)-2-(4-cyanostyryl)quinazolin-4(3H)-one. *J Am Chem Soc* **137**: 1738–1741.
- Brammer Basta LA, Ghosh A, Pan Y, Jakoncic J, Lloyd EP, Townsend CA, Lamichhane G, Bianchet M. 2015. A loss of a functionally and structurally distinct LD-transpeptidase, LdtMt5, compromises cell wall integrity in *M. tuberculosis*. *J Biol Chem* **290**: 25670–25685.
- Bugg TD, Braddick D, Dowson CG, Roper DI. 2011. Bacterial cell wall assembly: Still an attractive antibacterial target. *Trends Biotechnol* **29**: 167–173.
- Bush K. 2015. Antibiotics: Synergistic MRSA combinations. *Nat Chem Biol* **11**: 832–833.
- Carapito R, Chesnel L, Vernet T, Zapun A. 2006. Pneumococcal β -lactam resistance due to a conformational change in PBP2x. *J Biol Chem* **281**: 1771–1777.
- Cattoir V, Leclercq R. 2013. Twenty-five years of shared life with vancomycin-resistant enterococci: Is it time to divorce? *J Antimicrob Chemother* **68**: 731–742.
- Cazares-Dominguez V, Cruz-Cordova A, Ochoa SA, Escalona G, Arellano-Galindo J, Rodriguez-Leviz A, Hernandez-Castro R, Lopez-Villegas EO, Xicohtencatl-Cortes J. 2015. Vancomycin-tolerant, methicillin-resistant *S. aureus* reveals the effects of vancomycin on cell wall thickening. *PLoS ONE* **10**: e0118791.
- Chakraborty S, Rhee KY. 2015. Tuberculosis drug development: History and evolution of the mechanism-based paradigm. *Cold Spring Harb Perspect Med* **5**: a021147.
- Chambers HF. 2005. Community-associated MRSA—Resistance and virulence converge. *N Engl J Med* **352**: 1485–1487.
- Chambers HF, Turner J, Schecter G, Kawamura M, Hopewell PC. 2005. Imipenem for treatment of tuberculosis in mice and humans. *Antimicrob Agents Chemother* **49**: 2816–2821.
- Cheng Y, Xie H, Sule P, Hassounah H, Graviss EA, Kong Y, Cirillo JD, Rao J. 2014. Fluorogenic probes with substitutions at the 2 and 7 positions of cephalosporin are highly BlaC-specific for rapid *M. tuberculosis* detection. *Angew Chem, Int Ed* **53**: 9360–9364.
- Chesnel L, Pernot L, Lemaire D, Champelovier D, Croizé J, Dideberg O, Vernet T, Zapun A. 2003. The structural modifications induced by the M339F substitution in PBP2x from *St. pneumoniae* further decreases the susceptibility to β -lactams of resistant strains. *J Biol Chem* **278**: 44448–44456.
- Chewapreecha C, Marttinen P, Croucher NJ, Salter SJ, Harris SR, Mather AE, Hanage WP, Goldblatt D, Nosten FH, Turner C, et al. 2014. Comprehensive identification of single nucleotide polymorphisms associated with β -lactam resistance within pneumococcal mosaic genes. *PLoS Genet* **10**: e1004547.
- Coffey TJ, Daniels M, McDougal LK, Dowson CG, Tenover FC, Spratt BG. 1995. Genetic analysis of clinical isolates of *S. pneumoniae* with high-level resistance to expanded-spectrum cephalosporins. *Antimicrob Agents Chemother* **39**: 1306–1313.
- Contreras-Martel C, Job V, Di Guilmi AM, Vernet T, Dideberg O, Dessen A. 2006. Crystal structure of PBP1a reveals a mutational hotspot implicated in β -lactam resistance in *S. pneumoniae*. *J Mol Biol* **355**: 684–696.
- Contreras-Martel C, Dahout-Gonzalez C, Martins Ados S, Kotnik M, Dessen A. 2009. PBP active site flexibility as the key mechanism for β -lactam resistance in pneumococci. *J Mol Biol* **387**: 899–909.
- Cordillot M, Dubee V, Triboulet S, Dubost L, Marie A, Hugonnet JE, Arthur M, Mainardi JL. 2013. In vitro cross-linking of *M. tuberculosis* peptidoglycan by L,D-transpeptidases and inactivation of these enzymes by carbapenems. *Antimicrob Agents Chemother* **57**: 5940–5945.
- Cremniter J, Mainardi JL, Josseume N, Quincampoix JC, Dubost L, Hugonnet JE, Marie A, Gutmann L, Rice LB, Arthur M. 2006. Novel mechanism of resistance to glycopeptide antibiotics in *E. faecium*. *J Biol Chem* **281**: 32254–32262.
- Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, van der Linden M, McGee L, von Gottberg A, Song JH, Ko KS, et al. 2011. Rapid pneumococcal evolution in response to clinical interventions. *Science* **331**: 430–434.
- Dare K, Ibba M. 2012. Roles of tRNA in cell wall biosynthesis. *Wiley Interdiscip Rev RNA* **3**: 247–264.
- de Lencastre H, Oliveira D, Tomasz A. 2007. Antibiotic resistant *S. aureus*: A paradigm of adaptive power. *Curr Opin Microbiol* **10**: 428–435.
- Dessen A, Mouz N, Gordon E, Hopkins J, Dideberg O. 2001. Crystal structure of PBP2x from a highly penicillin-resistant *S. pneumoniae* clinical isolate: A mosaic framework containing 83 mutations. *J Biol Chem* **276**: 45106–45112.
- Dhar N, Dubée V, Ballell L, Cuinet G, Hugonnet JE, Signorino-Gelo F, Barros D, Arthur M, McKinney JD. 2015.

- Rapid cytotoxicity of *M. tuberculosis* by faropenem, an orally bioavailable β-lactam antibiotic. *Antimicrob Agents Chemother* **59**: 1308–1319.
- Dias R, Felix D, Canica M, Trombe MC. 2009. The highly conserved serine threonine kinase StkP of *S. pneumoniae* contributes to penicillin susceptibility independently from genes encoding PBPs. *BMC Microbiol* **9**: 121.
- Djorić D, Kristich CJ. 2015. Oxidative stress enhances cephalosporin resistance of *E. faecalis* through activation of a two-component signaling system. *Antimicrob Agents Chemother* **59**: 159–169.
- Dubé V, Arthur M, Fief H, Triboulet S, Mainardi JL, Gutmann L, Sollogoub M, Rice LB, Etheve-Quelejeu M, Hugonnet JE. 2012. Kinetic analysis of *E. faecium* L,D-transpeptidase inactivation by carbapenems. *Antimicrob Agents Chemother* **56**: 3409–3412.
- Dubé V, Bernut A, Cortes M, Lesne T, Dorcène D, Lefebvre AL, Hugonnet JE, Gutmann L, Mainardi JL, Herrmann JL, et al. 2015. β-Lactamase inhibition by avibactam in *M. abscessus*. *J Antimicrob Chemother* **70**: 1051–1058.
- Egesborg P, Carletini H, Volpato JP, Doucet N. 2015. Combinatorial active-site variants confer sustained clavulanate resistance in BlaC β-lactamase from *M. tuberculosis*. *Protein Sci* **24**: 534–544.
- Falk SP, Weisblum B. 2013. Phosphorylation of the *S. pneumoniae* cell wall biosynthesis enzyme MurC by a eukaryotic-like ser/thr kinase. *FEMS Microbiol Lett* **340**: 19–23.
- Feiler C, Fisher AC, Boock JT, Marrichi MJ, Wright L, Schmidpeter PA, Blankenfeldt W, Pavelka M, Delisa MP. 2013. Directed evolution of *M. tuberculosis* β-lactamase reveals gatekeeper residue that regulates antibiotic resistance and catalytic efficiency. *PLoS ONE* **8**: e73123.
- Fernandez R, Paz LI, Rosato RR, Rosato AE. 2014. Ceftazolin is active against heteroresistant methicillin-resistant *Staphylococcus aureus* clinical strains despite associated mutational mechanisms and intermediate levels of resistance. *Antimicrob Agents Chemother* **58**: 5736–5746.
- Filipe SR, Severina E, Tomasz A. 2002. The *murMN* operon: A functional link between antibiotic resistance and antibiotic tolerance in *S. pneumoniae*. *Proc Natl Acad Sci* **99**: 1550–1555.
- Fischetti VA, Ryan P. 2015. *Streptococcus*. In *Practical handbook of microbiology*, 3rd ed. (ed. Goldman E, Green LH), pp. 411–427. CRC, Boca Raton, FL.
- Fishovitz J, Rojas-Altuve A, Otero LH, Dawley M, Carrasco-Lopez C, Chang M, Hermoso JA, Mobashery S. 2014. Disruption of allosteric response as an unprecedented mechanism of resistance to antibiotics. *J Am Chem Soc* **136**: 9814–9817.
- Fishovitz J, Taghizadeh N, Fisher JF, Chang M, Mobashery S. 2015. The Tipper-Strominger hypothesis and triggering of allostery in PBP2a of methicillin-resistant *S. aureus* (MRSA). *J Am Chem Soc* **137**: 6500–6505.
- Fleurie A, Manuse S, Zhao C, Campo N, Cluzel C, Lavergne JP, Freton C, Combet C, Guiral S, Soufi B, et al. 2014. Interplay of the serine/threonine-kinase StkP and the paralogs DivIVA and GpsB in pneumococcal cell elongation and division. *PLoS Genet* **10**: e1004275.
- Foucault ML, Depardieu F, Courvalin P, Grillot-Courvalin C. 2010. Inducible expression eliminates the fitness cost of vancomycin resistance in *Enterococci*. *Proc Natl Acad Sci* **107**: 16964–16969.
- Gautam S, Kim T, Spiegel DA. 2015. Chemical probes reveal an extraseptal mode of cross-linking in *S. aureus*. *J Am Chem Soc* **137**: 7441–7447.
- Ge Y, Wu J, Xia Y, Yang M, Xiao J, Yu J. 2012. Molecular dynamics simulation of the complex PBP-2x with drug cefuroxime to explore the drug resistance mechanism of *S. suis* R61. *PLoS ONE* **7**: e35941.
- Gilmore MS, Rauch M, Ramsey MM, Himes PR, Varahan S, Manson JM, Lebreton F, Hancock LE. 2015. Pheromone killing of multidrug-resistant *E. faecalis* V583 by native commensal strains. *Proc Natl Acad Sci* **112**: 7273–7278.
- Goffin C, Ghuyssen JM. 2002. Biochemistry and comparative genomics of SxxK superfamily acyltransferases offer a clue to the mycobacterial paradox: Presence of penicillin-susceptible target proteins versus lack of efficiency of penicillin as therapeutic agent. *Microbiol Mol Biol Rev* **66**: 702–738.
- Gonzales PR, Pesesky MW, Bouley R, Ballard A, Biddy BA, Suckow MA, Wolter WR, Schroeder VA, Burnham CD, Mobashery S, et al. 2015. Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA. *Nat Chem Biol* **11**: 855–861.
- Gonzalo X, Drobniewski F. 2013. Is there a place for β-lactams in the treatment of multidrug-resistant/extensively drug-resistant tuberculosis? Synergy between meropenem and amoxicillin/clavulanate. *J Antimicrob Chemother* **68**: 366–369.
- Gordon E, Mouz N, Duée E, Dideberg O. 2000. The crystal structure of the penicillin-binding protein 2x from *S. pneumoniae* and its acyl-enzyme form: Implication in drug resistance. *J Mol Biol* **299**: 477–485.
- Greene NG, Narciso AR, Filipe SR, Camilli A. 2015. Peptidoglycan branched stem peptides contribute to *S. pneumoniae* virulence by inhibiting pneumolysin release. *PLoS Pathog* **11**: e1004996.
- Gupta R, Lavollay M, Mainardi JL, Arthur M, Bishai WR, Lamichhane G. 2010. The *M. tuberculosis* protein LdtMt2 is a nonclassical transpeptidase required for virulence and resistance to amoxicillin. *Nat Med* **16**: 466–469.
- Hakenbeck R. 2014. Discovery of β-lactam-resistant variants in diverse pneumococcal populations. *Genome Med* **6**: 72.
- Hakenbeck R, Bruckner R, Denapate D, Maurer P. 2012. Molecular mechanisms of β-lactam resistance in *S. pneumoniae*. *Future Microbiol* **7**: 395–410.
- Hazra S, Xu H, Blanchard JS. 2014. Tebipenem, a new carbapenem antibiotic, is a slow substrate that inhibits the β-lactamase from *M. tuberculosis*. *Biochemistry* **53**: 3671–3678.
- Hendrickx APA, van Schaik W, Willems RJL. 2013. The cell wall architecture of *E. faecium*: From resistance to pathogenesis. *Future Microbiol* **8**: 993–1010.
- Henriques-Normark B, Tuomanen EI. 2013. The pneumococcus: Epidemiology, microbiology, and pathogenesis. *Cold Spring Harb Perspect Med* **3**: a010215.
- Henry X, Verlaine O, Amoroso A, Coyette J, Frere JM, Joris B. 2013. Activity of ceftazolin against *E. faecium* PBP5. *Antimicrob Agents Chemother* **57**: 6358–6360.
- Hindler JA, Wong-Beringer A, Charlton CL, Miller SA, Klesidis T, Carvalho M, Sakoulas G, Nonejuie P, Pogliano J, Nizet V, et al. 2015. In vitro activity of daptomycin in

J.F. Fisher and S. Mobashery

- combination with β -lactams, gentamicin, rifampin, and tigecycline against daptomycin-nonsusceptible enterococci. *Antimicrob Agents Chemother* **59**: 4279–4288.
- Holmes NE, Howden BP. 2014. What's new in the treatment of serious MRSA infection? *Curr Opin Infect Dis* **27**: 471–478.
- Horita Y, Maeda S, Kazumi Y, Doi N. 2014. In vitro susceptibility of *M. tuberculosis* isolates to an oral carbapenem alone or in combination with β -lactamase inhibitors. *Antimicrob Agents Chemother* **58**: 7010–7014.
- Hugonnet JE, Tremblay IW, Boshoff HI, Barry CE, Blanchard JS. 2009. Meropenem-clavulanate is effective against extensively drug-resistant *M. tuberculosis*. *Science* **323**: 1215–1218.
- Ip M, Ang I, Liyanapathirana V, Ma H, Lai R. 2015. Genetic analyses of PBP determinants in multidrug-resistant *S. pneumoniae* serogroup 19 CC320/271 clone with high-level resistance to third-generation cephalosporins. *Antimicrob Agents Chemother* **59**: 4040–4045.
- Jackson M, McNeil MR, Brennan PJ. 2013. Progress in targeting cell envelope biogenesis in *Mycobacterium tuberculosis*. *Future Microbiol* **8**: 855–875.
- Jensen A, Valdórrson O, Frimodt-Møller N, Hollingshead S, Kilian M. 2015. Commensal streptococci serve as a reservoir for β -lactam resistance genes in *S. pneumoniae*. *Antimicrob Agents Chemother* **59**: 3529–3540.
- Job V, Carapito R, Vernet T, Dessen A, Zapun A. 2008. Common alterations in PBP1a from resistant *S. pneumoniae* decrease its reactivity toward β -lactams: Structural insights. *J Biol Chem* **283**: 4886–4894.
- Kim SJ, Chang J, Singh M. 2015. Peptidoglycan architecture of Gram-positive bacteria by solid-state NMR. *Biochim Biophys Acta* **1848**: 350–362.
- Knox J, Uhlemann AC, Lowy FD. 2015. *S. aureus* infections: Transmission within households and the community. *Trends Microbiol* **23**: 437–444.
- Kocaoglu O, Tsui HCT, Winkler ME, Carlson EE. 2015. Profiling of β -lactam selectivity for penicillin-binding proteins in *S. pneumoniae* D39. *Antimicrob Agents Chemother* **59**: 3548–3555.
- Kristich CJ, Rice LB, Arias CA. 2014. Enterococcal infection—Treatment and antibiotic resistance. In *Enterococci: From commensals to leading causes of drug resistant infection* (ed. Gilmore MS, Clewell DB, Ike Y, Shankar N), pp. 1–46. Massachusetts Eye and Ear Infirmary, Boston.
- Kumar P, Arora K, Lloyd JR, Lee IY, Nair V, Fischer E, Boshoff HI, Barry CE. 2012. Meropenem inhibits D,D-carboxypeptidase activity in *M. tuberculosis*. *Mol Microbiol* **86**: 367–381.
- Kurz SG, Wolff KA, Hazra S, Bethel CR, Hujer AM, Smith KM, Xu Y, Tremblay LW, Blanchard JS, Nguyen L, et al. 2013. Can inhibitor resistant substitutions in the *M. tuberculosis* β -lactamase BlaC lead to clavulanate resistance? A biochemical rationale for the use of β -lactam β -lactamase inhibitor combinations. *Antimicrob Agents Chemother* **57**: 6085–6096.
- Ladjouzi R, Bizzini A, Lebreton F, Sauvageot N, Rincé A, Benachour A, Hartke A. 2013. Analysis of the tolerance of pathogenic *Enterococci* and *S. aureus* to cell wall active antibiotics. *J Antimicrob Chemother* **68**: 2083–2091.
- Lages MC, Beilharz K, Morales Angeles D, Veening J-W, Scheffers DJ. 2013. The localization of key *Bacillus subtilis* penicillin binding proteins during cell growth is determined by substrate availability. *Environ Microbiol* **15**: 3272–3281.
- Land AD, Tsui HC, Kocaoglu O, Vella SA, Shaw SL, Keen SK, Sham LT, Carlson EE, Winkler ME. 2013. Requirement of essential Pbp2x and GpsB for septal ring closure in *S. pneumoniae* D39. *Mol Microbiol* **90**: 939–955.
- Lavollay M, Arthur M, Fourgeaud M, Dubost L, Marie A, Veziris N, Blanot D, Gutmann L, Mainardi JL. 2008. The peptidoglycan of stationary-phase *M. tuberculosis* predominantly contains cross-links generated by L,D-transpeptidation. *J Bacteriol* **190**: 4360–4366.
- Lebreton F, van Schaik W, McGuire AM, Godfrey P, Griggs A, Mazumdar V, Corander J, Cheng L, Saif S, Young S, et al. 2013. Emergence of epidemic multidrug-resistant *E. faecium* from animal and commensal strains. *MBio* **4**: e00534-13.
- Lecoq L, Dubée V, Triboulet S, Bougault C, Hugonnet JE, Arthur M, Simorre JP. 2013. Structure of *E. faecium* L,D-transpeptidase acylated by ertapenem provides insight into the inactivation mechanism. *ACS Chem Biol* **8**: 1140–1146.
- Leski TA, Tomasz A. 2005. Role of PBP2 in the antibiotic susceptibility and cell wall cross-linking of *S. aureus*: Evidence for the cooperative functioning of PBP2, PBP4, and PBP2A. *J Bacteriol* **187**: 1815–1824.
- Llarrull LI, Mobashery S. 2012. Dissection of events in the resistance to β -lactam antibiotics mediated by the protein BlaR1 from *S. aureus*. *Biochemistry* **51**: 4642–4649.
- Lobritz MA, Belenky P, Porter CBM, Gutierrez A, Yang JH, Schwarz EG, Dwyer DJ, Khalil AS, Collins JJ. 2015. Antibiotic efficacy is linked to bacterial cellular respiration. *Proc Natl Acad Sci* **112**: 8173–8180.
- Loskill P, Pereira PM, Jung P, Bischoff M, Herrmann M, Pinho MG, Jacobs K. 2014. Reduction of the peptidoglycan crosslinking causes a decrease in stiffness of the *S. aureus* cell envelope. *Biophys J* **107**: 1082–1089.
- Lun S, Miranda D, Kubler A, Guo H, Maiga MC, Winglee K, Pelly S, Bishai WR. 2014. Synthetic lethality reveals mechanisms of *M. tuberculosis* resistance to β -lactams. *mBio* **5**: e01767.14.
- Macheboeuf P, Di Guilmi AM, Job V, Vernet T, Dideberg O, Dessen A. 2005. Active site restructuring regulates ligand recognition in class A PBPs. *Proc Natl Acad Sci* **102**: 577–582.
- Maestro B, Novakova L, Hesek D, Lee M, Leyva E, Mobashery S, Sanz JM, Branny P. 2011. Recognition of peptidoglycan and β -lactam antibiotics by the extracellular domain of the Ser/Thr protein kinase StkP from *S. pneumoniae*. *FEBS Lett* **585**: 357–363.
- Magnet S, Arbeloa A, Mainardi JL, Hugonnet JE, Fourgeaud M, Dubost L, Marie A, Delfosse V, Mayer C, Rice LB, et al. 2007. Specificity of L,D-transpeptidases from Gram-positive bacteria producing different peptidoglycan chemotypes. *J Biol Chem* **282**: 13151–13159.
- Mainardi JL, Fourgeaud M, Hugonnet JE, Dubost L, Brouard JP, Ouazzani J, Rice LB, Gutmann L, Arthur M. 2005. A novel peptidoglycan cross-linking enzyme for a β -lactam-resistant transpeptidation pathway. *J Biol Chem* **280**: 38146–38152.

- Mann PA, Muller A, Xiao L, Pereira PM, Yang C, Lee SH, Wang H, Trzeciak J, Schneeweis J, Dos Santos MM, et al. 2013. Murgocil is a highly bioactive staphylococcal-specific inhibitor of the peptidoglycan glycosyltransferase enzyme MurG. *ACS Chem Biol* **8**: 2442–2451.
- Massidda O, Novakova L, Vollmer W. 2013. From models to pathogens: How much have we learned about *S. pneumoniae* cell division? *Environ Microbiol* **15**: 3133–3157.
- Maurer P, Koch B, Zerfass I, Krauss J, van der Linden M, Frère JM, Contreras-Martel C, Hakenbeck R. 2008. PBP2x of *S. pneumoniae*: Three new mutational pathways for remodelling an essential enzyme into a resistance determinant. *J Mol Biol* **376**: 1403–1416.
- Maurer P, Todorova K, Sauerbier J, Hakenbeck R. 2012. Mutations in *S. PBP2x*: Importance of the C-terminal PBP and serine/threonine kinase-associated domains for β-lactam binding. *Microb Drug Resist* **18**: 314–321.
- Memmi G, Filipe SR, Pinho MG, Fu Z, Cheung A. 2008. *S. aureus* PBP4 is essential for β-lactam resistance in community-acquired methicillin-resistant strains. *Antimicrob Agents Chemother* **52**: 3955–3966.
- Missiakas D, Schneewind O. 2015. *S. aureus* and related staphylococci. In *Practical handbook of microbiology*, 3rd ed. (ed. Goldman E, Green LH), pp. 383–409. CRC, Boca Raton, FL.
- Moellering RC Jr. 2012. MRSA: The first half century. *J Antimicrob Chemother* **67**: 4–11.
- Morlot C, Bayle L, Jacq M, Fleurie A, Tourcier G, Galisson F, Vernet T, Grangeasse C, Di Guilmi AM. 2013. Interaction of PBP2x and Ser/Thr protein kinase StkP, two key players in *S. pneumoniae* R6 morphogenesis. *Mol Microbiol* **90**: 88–102.
- Mouz N, Di Guilmi AM, Gordon E, Hakenbeck R, Dideberg O, Vernet T. 1999. Mutations in the active site of penicillin-binding protein PBP2x from *S. pneumoniae*. Role in the specificity for β-lactam antibiotics. *J Biol Chem* **274**: 19175–19180.
- Nataraj V, Varela C, Javid A, Singh A, Besra GS, Bhatt A. 2015. Mycolic acids: Deciphering and targeting the Achilles' heel of the tubercle bacillus. *Mol Microbiol* **98**: 7–16.
- Nobbs AH, Jenkinson HF, Everett DB. 2015. Generic determinants of *Streptococcus* colonization and infection. *Infect Genet Evol* **33**: 361–370.
- Ogawara H. 2015. PBPs in *Actinobacteria*. *J Antibiot* **68**: 223–245.
- Oliveira DC, de Lencastre H. 2011. Methicillin-resistance in *S. aureus* is not affected by the overexpression in *trans* of the *mecA* gene repressor: A surprising observation. *PLoS ONE* **6**: e23287.
- Otero LH, Rojas-Altuve A, Llarrull LI, Carrasco-López C, Kumarasiri M, Lastochkin E, Fishovitz J, Dawley M, Hessek D, Lee M, et al. 2013. How allosteric control of *S. aureus* PBP2a enables methicillin resistance and physiological function. *Proc Natl Acad Sci* **110**: 16808–16813.
- Palmer KL, Kos VN, Gilmore MS. 2010. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. *Curr Opin Microbiol* **13**: 632–639.
- Pasquina LW, Santa Maria JP, Walker S. 2013. Teichoic acid biosynthesis as an antibiotic target. *Curr Opin Microbiol* **16**: 531–537.
- Peacock SJ, Paterson GK. 2015. Mechanisms of methicillin resistance in *S. aureus*. *Annu Rev Biochem* **84**: 577–601.
- Peters K, Schweizer I, Beilharz K, Stahlmann C, Veening JW, Hakenbeck R, Denapate D. 2014. *S. pneumoniae* PBP2x mid-cell localization requires the C-terminal PASTA domains and is essential for cell shape maintenance. *Mol Microbiol* **92**: 733–755.
- Peyrani P, Ramirez J. 2015. What is the best therapeutic approach to methicillin-resistant *Staphylococcus aureus* pneumonia. *Curr Opin Infect Dis* **28**: 164–170.
- Pfeffer JM, Strating H, Weadge JT, Clarke AJ. 2006. Peptidoglycan O-acetylation and autolysin profile of *E. faecalis* in the viable but nonculturable state. *J Bacteriol* **188**: 902–908.
- Philippe J, Vernet T, Zapun A. 2014. The elongation of ovococci. *Microb Drug Resist* **20**: 215–221.
- Philippe J, Gallet B, Morlot C, Denapate D, Hakenbeck R, Chen Y, Vernet T, Zapun A. 2015. Mechanism of β-lactam action in *S. pneumoniae*: The piperacillin paradox. *Antimicrob Agents Chemother* **59**: 609–621.
- Pietta E, Montealegre MC, Roh JH, Cocconcelli PS, Murray BE. 2014. *E. faecium* PBP5-S/R, the missing link between PBP5-S and PBP5-R. *Antimicrob Agents Chemother* **58**: 6978–6981.
- Pinho MG, Filipe SR, de Lencastre H, Tomasz A. 2001. Complementation of the essential peptidoglycan transpeptidase function of PBP2 by the drug resistance protein PBP2A in *S. aureus*. *J Bacteriol* **183**: 6525–6531.
- Prigozhin DM, Krieger IV, Huizar JP, Mavric D, Waldo GS, Hung L, Sacchetti JC, Terwilliger TC, Alber T. 2014. Subfamily-specific adaptations in the structures of two PBPs from *M. tuberculosis*. *PLoS ONE* **9**: e116249.
- Qamar A, Golemi-Kotra D. 2012. Dual roles of FmtA in *S. aureus* cell wall biosynthesis and autolysis. *Antimicrob Agents Chemother* **56**: 3797–3805.
- Qiao Y, Lebar MD, Schirner K, Schaefer K, Tsukamoto H, Kahne D, Walker S. 2014. Detection of lipid-linked peptidoglycan precursors by exploiting an unexpected transpeptidase reaction. *J Am Chem Soc* **136**: 14678–14681.
- Ramalingam J, Vennila J, Subbiah P. 2013. Computational studies on the resistance of PBP2B of wild-type and mutant strains of *S. pneumoniae* against β-lactam antibiotics. *Chem Biol Drug Des* **82**: 275–289.
- Reed P, Atilano ML, Alves R, Hoiczky E, Sher X, Reichmann NT, Pereira PM, Roemer T, Filipe SR, Pereira-Leal JB, et al. 2015. *S. aureus* survives with a minimal peptidoglycan synthesis machine but sacrifices virulence and antibiotic resistance. *PLoS Pathog* **11**: e1004891.
- Reinert RR. 2009. The antimicrobial resistance profile of *S. pneumoniae*. *Clin Microbiol Infect* **15**: 7–11.
- Rice LB, Carias LL, Rudin S, Hutton R, Marshall S, Hassan M, Josseume N, Dubost L, Marie A, Arthur M. 2009. Role of class A PBPs in the expression of β-lactam resistance in *E. faecium*. *J Bacteriol* **191**: 3649–3656.
- Roemer T, Davies J, Giaever G, Nislow C. 2012. Bugs, drugs and chemical genomics. *Nat Chem Biol* **8**: 46–56.
- Sacco E, Cortes M, Josseume N, Rice LB, Mainardi JL, Arthur M. 2014. Serine/threonine protein phosphatase-mediated control of the peptidoglycan cross-linking L,D-transpeptidase pathway in *E. faecium*. *mBio* **5**: e01446.14.

J.F. Fisher and S. Mobashery

- Sauerbier J, Maurer P, Rieger M, Hakenbeck R. 2012. *S. pneumoniae* R6 interspecies transformation: Genetic analysis of penicillin resistance determinants and genome-wide recombination events. *Mol Microbiol* **86**: 692–706.
- Sauvage E, Kerff F, Fonze E, Herman R, Schoot B, Marquette JP, Taburet Y, Prevost D, Dumas J, Leonard G, et al. 2002. The 2.4-Å crystal structure of the PBP-binding protein PBP5fm from *E. faecium* in complex with benzylpenicillin. *Cell Mol Life Sci* **59**: 1223–1232.
- Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. 2008. The PBPs: Structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev* **32**: 234–258.
- Schneider T, Sahl HG. 2010. An oldie but a goodie—Cell wall biosynthesis as antibiotic target pathway. *Int J Med Microbiol* **300**: 161–169.
- Schoonmaker MK, Bishai WR, Lamichhane G. 2014. Non-classical transpeptidases of *M. tuberculosis* alter cell size, morphology, the cytosolic matrix, protein localization, virulence, and resistance to β -lactams. *J Bacteriol* **196**: 1394–1402.
- Seung KJ, Keshavjee S, Rich ML. 2015. Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. *Cold Spring Harb Perspect Med* **5**: a017863.
- Sewell EWC, Brown ED. 2014. Taking aim at wall teichoic acid synthesis: New biology and new leads for antibiotics. *J Antibiot (Tokyo)* **67**: 43–51.
- Silhavy TJ, Kahne D, Walker S. 2010. The bacterial cell envelope. *Cold Spring Harb Perspect Biol* **2**: a000414.
- Silver LL. 2013. Viable screening targets related to the bacterial cell wall. *Ann NY Acad Sci* **1277**: 29–53.
- Singh M, Kim SJ, Sharif S, Preobrazhenskaya M, Schaefer J. 2015. REDOR constraints on the peptidoglycan lattice architecture of *S. aureus* and its FemA mutant. *Biochim Biophys Acta* **1848**: 363–368.
- Smith JR, Barber KE, Raut A, Aboutaleb M, Sakoulas G, Rybak MJ. 2015a. β -Lactam combinations with daptomycin provide synergy against vancomycin-resistant *E. faecalis* and *E. faecium*. *J Antimicrob Chemother* **70**: 1738–1743.
- Smith JR, Barber KE, Raut A, Rybak MJ. 2015b. β -Lactams enhance daptomycin activity against vancomycin-resistant *E. faecalis* and *E. faecium* in in vitro pharmacokinetic/pharmacodynamic models. *Antimicrob Agents Chemother* **59**: 2842–2848.
- Solapure S, Dinesh N, Shandil R, Ramachandran V, Sharma S, Bhattacharjee D, Ganguly S, Reddy J, Ahuja V, Panduga V, et al. 2013. In vitro and in vivo efficacy of β -lactams against replicating and slowly growing/nonreplicating *M. tuberculosis*. *Antimicrob Agents Chemother* **57**: 2506–2510.
- Sorg RA, Veening JW. 2015. Microscale insights into pneumococcal antibiotic mutant selection windows. *Nat Commun* **6**: 8773.
- Soroka D, Li de la Sierra-Gallay I, Dubée V, Triboulet S, van Tilbeurgh H, Compain F, Ballell L, Barros D, Mainardi JL, Hugonnet JE, et al. 2015. Hydrolysis of clavulanate by *Mycobacterium tuberculosis* β -lactamase BlaC harboring a canonical SDN motif. *Antimicrob Agents Chemother* **59**: 5714–5720.
- Spratt BG. 2012. The 2011 Garrod lecture: From penicillin-binding proteins to molecular epidemiology. *J Antimicrob Chem* **67**: 1578–1588.
- Starikova I, Al-Haroni M, Werner G, Roberts AP, Sørum V, Nielsen KM, Johnsen PJ. 2013. Fitness costs of various mobile genetic elements in *E. faecium* and *E. faecalis*. *J Antimicrob Chem* **68**: 2755–2765.
- Staud MW, Frederick TE, Natarajan SV, Wilson BD, Tanner CE, Ruggiero ST, Mobashery S, Peng JW. 2015. Investigation of signal transduction routes within the sensor/transducer protein BlaR1 of *S. aureus*. *Biochemistry* **54**: 1600–1610.
- Stryjewski ME, Corey GR. 2014. Methicillin-resistant *S. aureus*: An evolving pathogen. *Clin Infect Dis* **58**: S10–S19.
- Tan CM, Therien AG, Lu J, Lee SH, Caron A, Gill CJ, Lebeau-Jacob C, Benton-Perdomo L, Monteiro JM, Pereira PM, et al. 2012. Restoring methicillin-resistant *S. aureus* susceptibility to β -lactam antibiotics. *Sci Transl Med* **4**: 126ra35.
- Todorova K, Maurer P, Rieger M, Becker T, Bui NK, Gray J, Vollmer W, Hakenbeck R. 2015. Transfer of penicillin resistance from *S. oralis* to *S. pneumoniae* identifies *murE* as resistance determinant. *Mol Microbiol* **97**: 866–880.
- Tomasz A. 1979. The mechanism of the irreversible antimicrobial effects of penicillins: How the β -lactams kill and lyse bacteria. *Annu Rev Microbiol* **33**: 113–137.
- Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. 2015. *S. aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* **28**: 603–661.
- Tremblay LW, Hugonnet JE, Blanchard JS. 2008. Structure of the covalent adduct formed between *M. tuberculosis* β -lactamase and clavulanate. *Biochemistry* **47**: 5312–5316.
- Tremblay LW, Fan F, Blanchard JS. 2010. Biochemical and structural characterization of *M. tuberculosis* β -lactamase with the carbapenems ertapenem and doripenem. *Biochemistry* **49**: 3766–3773.
- Triboulet S, Arthur M, Mainardi JL, Veckerlé C, Dubée V, Nguekam-Moumi A, Gutmann L, Rice LB, Hugonnet JE. 2011. Inactivation kinetics of a new target of β -lactam antibiotics. *J Biol Chem* **286**: 22777–22784.
- Triboulet S, Dubée V, Lecoq L, Bougault C, Mainardi JL, Rice LB, Etcheve-Quelquejeu M, Gutmann L, Marie A, Dubost L, et al. 2013. Kinetic features of L,D-transpeptidase inactivation critical for β -lactam antibacterial activity. *PLoS ONE* **8**: e67831.
- Triboulet S, Bougault CM, Laguri C, Hugonnet JE, Arthur M, Simorre J-P. 2015. Acyl acceptor recognition by *Enterococcus faecium* L,D-transpeptidase Ldt_{fm}. *Mol Microbiol* **98**: 90–100.
- Tsubakishita S, Kuwahara-Arai K, Sasaki T, Hiramatsu K. 2010. Origin and molecular evolution of the determinant of methicillin resistance in *Staphylococci*. *Antimicrob Agents Chemother* **54**: 4352–4359.
- Tsui HCT, Boersma MJ, Vella SA, Kocaoglu O, Kuru E, Peceny JK, Carlson EE, VanNieuwenhze MS, Brun YV, Shaw SL, et al. 2014. Pbp2x localizes separately from Pbp2b and other peptidoglycan synthesis proteins during later stages of cell division of *S. pneumoniae* D39. *Mol Microbiol* **94**: 21–40.

- Turner RD, Ratcliffe EC, Wheeler R, Golestanian R, Hobbs JK, Foster SJ. 2010. Peptidoglycan architecture can specify division planes in *S. aureus*. *Nat Commun* **1**: 1025.
- Turner RD, Vollmer W, Foster SJ. 2014. Different walls for rods and balls: The diversity of peptidoglycan. *Mol Microbiol* **91**: 862–874.
- van Tonder AJ, Bray JE, Roalfe L, White R, Zancolli M, Quirk SJ, Haraldsson G, Jolley KA, Maiden MCJ, Bentley SD, et al. 2015. Genomics reveals the worldwide distribution of multidrug-resistant serotype 6E pneumococci. *J Clin Microbiol* **53**: 2271–2285.
- Veziris N, Truffot C, Mainardi JL, Jarlier V. 2011. Activity of carbapenems combined with clavulanate against murine tuberculosis. *Antimicrob Agents Chemother* **55**: 2597–2600.
- Vollmer W, Blanot D, de Pedro MA. 2008. Peptidoglycan structure and architecture. *FEMS Microbiol Rev* **32**: 149–167.
- Wang H, Gill CJ, Lee SH, Mann P, Zuck P, Meredith TC, Murgolo N, She X, Kales S, Liang L, et al. 2013. Discovery of wall teichoic acid inhibitors as potential anti-MRSA β -lactam combination agents. *Chem Biol* **20**: 272–284.
- Waxman DJ, Strominger JL. 1983. PBPs and the mechanism of action of β -lactam antibiotics. *Annu Rev Biochem* **52**: 825–869.
- Werner G, Coque TM, Franz CMA, Grohmann E, Hegstad K, Jensen L, van Schaik W, Weaver K. 2013. Antibiotic resistant *Enterococci*—Tales of a drug resistance gene trafficker. *Int J Med Microbiol* **303**: 360–379.
- Werth BJ, Barber KE, Tran KNT, Nonejuie P, Sakoulas G, Pogliano J, Rybak MJ. 2015. Ceftobiprole and ampicillin increase daptomycin susceptibility of daptomycin-susceptible and -resistant VRE. *J Antimicrob Chemother* **70**: 489–493.
- Wheeler R, Mesnage S, Boneca IG, Hobbs JK, Foster SJ. 2011. Super-resolution microscopy reveals cell wall dynamics and peptidoglycan architecture in ovococcal bacteria. *Mol Microbiol* **82**: 1096–1109.
- Winstel V, Xia G, Peschel A. 2014. Pathways and roles of wall teichoic acid glycosylation in *S. aureus*. *Int J Med Microbiol* **304**: 215–221.
- Wivagg CN, Bhattacharyya RP, Hung DT. 2014. Mechanisms of β -lactam killing and resistance in the context of *M. tuberculosis*. *J Antibiot* **67**: 645–654.
- Wivagg CN, Wellington S, Gomez JE, Hung DT. 2016. Loss of a class A penicillin-binding protein alters β -lactam susceptibilities in *M. tuberculosis*. *ACS Infect Dis* **2**: 104–110.
- Xu H, Hazra S, Blanchard JS. 2012. NXL104 irreversibly inhibits the β -lactamase from *M. tuberculosis*. *Biochemistry* **51**: 4551–4557.
- Yamada M, Watanabe T, Baba N, Takeuchi Y, Ohsawa F, Gomi S. 2008. Crystal structures of biapenem and tebipenem complexed with PBPs 2X and 1A from *S. pneumoniae*. *Antimicrob Agents Chemother* **52**: 2053–2060.
- Young KD. 2006. The selective value of bacterial shape. *Microbiol Mol Biol Rev* **70**: 660–703.
- Young KD. 2007. Bacterial morphology: Why have different shapes? *Curr Opin Microbiol* **10**: 596–600.
- Zapun A, Contreras-Martel C, Vernet T. 2008. PBPs and β -lactam resistance. *FEMS Microbiol Rev* **32**: 361–385.
- Zerfass I, Hakenbeck R, Denapaite D. 2009. An important site in PBP2x of penicillin-resistant clinical isolates of *S. pneumoniae*: Mutational analysis of Thr338. *Antimicrob Agents Chemother* **53**: 1107–1115.
- Zhang X, Paganelli FL, Bierschen D, Kuipers A, Bonten MJM, Willems RJL, van Schaik W. 2012. Genome-wide identification of ampicillin resistance determinants in *E. faecium*. *PLoS Genet* **8**: e1002804.
- Zhang D, Wang Y, Lu J, Pang Y. 2015. In vitro activity of β -lactams in combination with β -lactamase inhibitors against multidrug-resistant *Mycobacterium tuberculosis* isolates. *Antimicrob Agents Chemother* **60**: 393–399.
- Zhou Y, Antignac A, Wu SW, Tomasz A. 2008. PBPs and cell wall composition in β -lactam-sensitive and -resistant strains of *S. sciuri*. *J Bacteriol* **190**: 508–514.
- Zorzi W, Zhou XY, Dardenne O, Lamotte J, Raze D, Pierre J, Gutmann L, Coyette J. 1996. Structure of the low-affinity PBP5fm in wild-type and highly penicillin-resistant strains of *E. faecium*. *J Bacteriol* **178**: 4948–4957.

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