Trends in Microbiology

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Review

Can β-Lactam Antibiotics Be Resurrected to Combat MRSA?

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The use of β-lactam antibiotics to treat infections caused by *Staphylococcus aureus* has been severely compromised by the acquisition by horizontal gene transfer of a gene that encodes the β-lactam-insensitive penicillin-binding protein PBP2a. This allows methicillin-resistant *S. aureus* (MRSA) to proliferate in the presence of β-lactam antibiotics. Paradoxically the dependence on PBP2a for the essential transpeptidase activity in cell wall peptidoglycan biosynthesis is the 'Achilles heel' of MRSA. Compounds that disrupt the divisome, wall teichoic acid, and functional membrane microdomains act synergistically with β-lactams against MRSA. These include drugs such as statins that are widely used in human medicine. The antibiotics vancomycin and daptomycin are also synergistic with β-lactams, and combinations have been employed to treat persistent MRSA infections. An additional benefit of exposing MRSA to β-lactams could be a reduction in virulence mediated by interfering with the global regulator Agr. The mechanistic basis of synergy is discussed, and the possibility that β-lactams can be resurrected to combat MRSA infections is explored.

Highlights

While the penicillin-binding protein PBP2a allows MRSA to synthesize peptidoglycan in the presence of β-lactam antibiotics, PBP2a is also its 'Achilles heel'.

Compounds that interfere with the stability of PBP2a by inhibiting functional membrane microdomains, wall teichoic acid, or the PrsA chaperone render MRSA susceptible to β-lactam antibiotics.

Compounds that interfere with protein secretion or the divisome act synergistically with β-lactam antibiotics.

Daptomycin acts synergistically with β-lactams that target PBP1 and have been used together to treat persistent MRSA infections.

Daptomycin-resistant MRSA strains are sensitized to β-lactams that target PBP2 because of failure to link to the membrane the PrsA chaperone lipoprotein needed for PBP2a stability.

β-Lactams can reduce the virulence of MRSA by making it more susceptible to host antimicrobial peptides and by reducing toxin expression.

β-Lactam Antibiotic Resistance in *S. aureus*

β-Lactams are the most successful chemical class of antibiotic ever used to treat infections in humans. This is due to their wide spectrum of activity, oral availability, excellent pharmacokinetics, lack of toxicity, and bactericidal action. They would be the drugs of choice for treating serious invasive infections caused by *S. aureus* [1]. However, their usefulness has been compromised by the development of resistance [2]. Resistance to penicillin was observed soon after its introduction and involved expression of β-lactamase. Following the development of the β-lactamase-insensitive penicillin derivative methicillin and the isoxazoyl penicillins (e.g., oxacillin), strains acquired SCC*mec* cassettes by horizontal transfer which encoded a penicillin-binding protein (PBP2a) that could not be inactivated by β-lactams [3]; these are called MRSA strains.

The first MRSA strains were mainly confined to hospitals and carried type I, II, or III SCC*mec* cassettes [2,3]. The hospital-associated HA-MRSA strains express high levels of resistance to oxacillin and typically cause invasive infections manifested as bacteraemia in hospitalized patients who are immunocompromised or who have undergone surgery [4]. During the past 15 years strains carrying a type IV SCC*mec* cassette have emerged in the community and are particularly widespread in North America, Australia, and New Zealand [5], while community-associated MRSA (CA-MRSA) strains bearing a type V SCC*mec* cassette have emerged in Asia [6]. CA-MRSA strains typically express a low level of resistance to oxacillin and cause serious skin and soft-tissue infections in individuals who were previously healthy.

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The clinical usefulness of the broad-spectrum β-lactam amoxycillin was extended by coadministration with the β-lactamase inhibitor clavulanic acid [7]. Other successful combinations of β-lactam and β-lactamase inhibitor are ampicillin–sulbactam and piperacillin–tazobactam. Thus there are precedents for resensitizing resistant bacteria to β-lactams by combination of a β-lactam with a molecule that enhances its activity.

Several molecules have been identified that potentiate β-lactam activity towards MRSA. These include drugs that are already licensed for treating human diseases which fortuitously inhibit targets that compromise the integrity of PBP2a making bacteria reliant upon the β-lactam-susceptible housekeeping PBP2 [8–10]. Daptomycin is used to treat serious MRSA infections, particularly since strains have emerged that are insensitive to the previous drug of choice, vancomycin (VISA). β-Lactams and daptomycin act synergistically, and combinations have been used to treat persistent invasive MRSA/VISA infections [11]. In addition, MRSA that have acquired resistance to daptomycin paradoxically become susceptible to β-lactams – which has also allowed successful treatment of persistent infection [12].

This review examines the mechanistic basis of synergy between β-lactams and molecules that interfere with factors that are required for the integrity of PBP2a – viz. wall teichoic acid and functional membrane microdomains – or which disrupt the integrity of the divisome; it also examines the synergy that occurs between β-lactams that target different PBPs.

Cell Division in MRSA

Cell division is initiated by the assembly of the divisome complex at the nascent septum at the mid-cell [13,14] (Figure 1). The membrane-bound FtsA protein acts as an anchor for FtsZ, the tubulin-like protein that forms the Z-ring. The divisome is a machine that coordinates the formation of the septum with the synthesis of peptidoglycan in the new cell wall that eventually separates the dividing cells. FtsZ undergoes slow 'treadmilling' at the new mid-cell and initiates the initial invagination of the membrane at the new septum [15]. The lipid II flippase MurJ then arrives at the divisome (Figure 2). Lipid II is translocated to the outer face of the membrane which acts as a signal for recruiting PBP2 (and presumably PBP2a in MRSA). Massive synthesis of peptidoglycan begins, which becomes the driving force for cytokinesis.

Peptidoglycan synthesis in *S. aureus* involves several penicillin-binding proteins [16] (Figure 1). PBP1 has a functional transpeptidase domain but its main role is to initiate peptidoglycan biosynthesis in coordination with the assembly of the divisome [17]. PBP2 is an essential bifunctional transglycosylase–transpeptidase [18], while the normally dispensable PBP4 provides the final cross-linking to peptidoglycan [18].

In MRSA, synthesis of new cell wall peptidoglycan in the presence of β-lactam antibiotics that block the active site of PBP2 transpeptidase requires PBP2 to act in concert with the imported SCC*mec*-encoded PBP2a [19]. PBP2 provides the transglycosylase activity, and PBP2a provides the cross-linking transpeptidase [19]. Peptidoglycan formed under these circumstances in HA-MRSA strains is functional but cross-linking is incomplete. In CA-MRSA strains, cross-linking is inadequate and requires the activity of PBP4 otherwise cells become dependent on PBP2 and susceptible to oxacillin [20]. The transpeptidase active site of PBP2a is buried in a deep cleft that is inaccessible to all β-lactam antibiotics except the fifth-generation cephalosporin ceftaroline fosamil, which has the ability to activate the allosteric binding site which opens the active transpeptidase site to a second molecule [21–23].

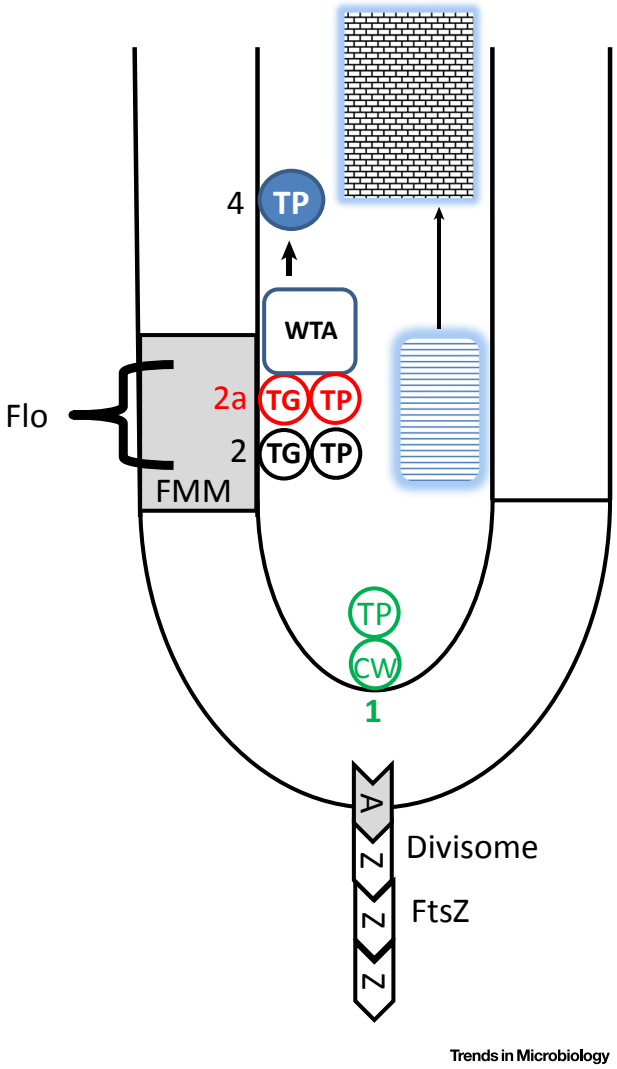

Figure 1. Events Occurring at the Cell Division Septum in Methicillin-Resistant *Staphylococcus aureus* (MRSA). Cell division is initiated by the assembly of the divisome at a site on the membrane at the mid-cell. Key components are shown in the expanding septum, including FtsA, FtsZ, and PBP1. The bifunctional transglycosylase (TG) transpeptidase (TP) domains of the membrane-anchored PBP2 and PBP2a are shown attached to a functional membrane microdomain (FMM) rich in staphyloxanthin (grey box) and incorporating dimers of flotillin (Flo), while PBP1 is shown in association with the divisome (CW, cell wall synthesis initiation domain). This is inferred from [9]. Wall teichoic acid (WTA) synthesized locally acts as a signal for the attendance of PBP4 which completes cross-linking of peptidoglycan symbolized in the blue boxes. The black boxes with cross-hatching symbolize the incompletely cross-linked peptidoglycan produced by PBP2/PBP2a and the final product produced by PBP4.

Wall teichoic acid (WTA) is intimately involved in peptidoglycan biosynthesis by MRSA. Firstly, WTA acts as a platform/scaffold for PBP2a [24]. Secondly, newly synthesized WTA is a temporal signal and marker for the localization of PBP4, the transpeptidase which completes the cross-linking of peptidoglycan formed by PBP2/PBP2a.

Functional membrane microdomains (FMMs) are lipid rafts that are enriched in the triterpenoid carotenoid staphyloxanthin and contain the protein flotillin. FMMs promote efficient oligomerization of protein complexes involved in export, secretion, and signalling [9]. Although not essential for cell division *in vitro*, FMMs enhance the process in MRSA by promoting the oligomerization and stability of PBP2 and PBP2a. There are fewer FMMs in mutants defective in staphyloxanthin biosynthesis, and the remaining FMMs are less likely to occur at the septum.

As well as allowing *S. aureus* to proliferate in the presence of β-lactam antibiotics, paradoxically PBP2a itself is the 'Achilles heel' of MRSA. The *mecA* gene that encodes PBP2a was imported from other species of staphylococci on mobile genetic elements [25,26]. Unlike PBP2, it

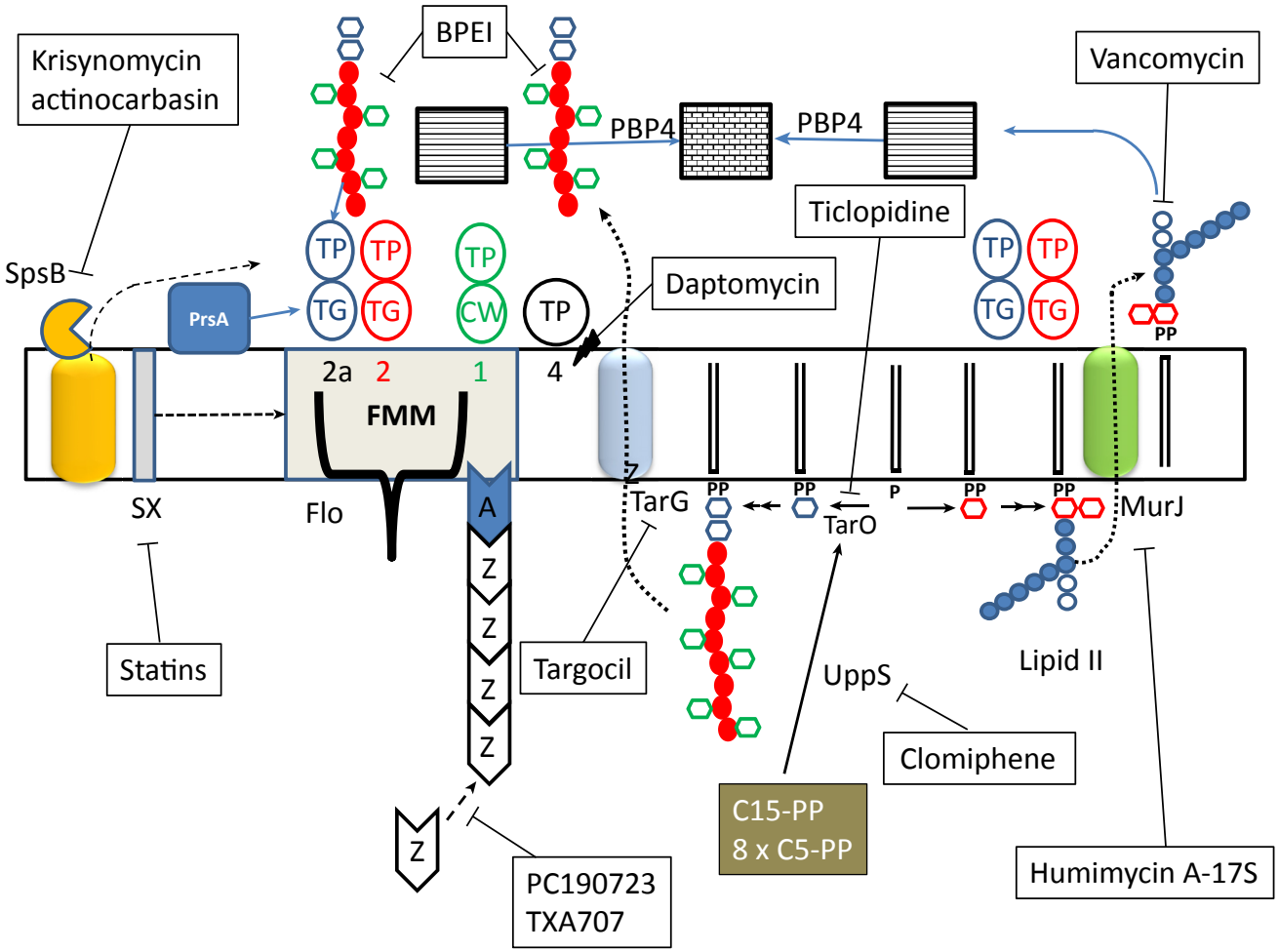

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Figure 2. Targets for Inhibitors That Act Synergistically with β-Lactams against Methicillin-Resistant *Staphylococcus aureus* (MRSA). Summary of events occurring at the cell division septum in MRSA and the site of action of molecules that impair the process and render cells susceptible to β-lactams. They key events at the divisome shown in Figure 1 are incorporated at the centre of the diagram with a functional membrane microdomain (FMM) in a staphyloxanthin (SX)-rich lipid raft (grey box) incorporating dimers of flotillin (Flo). The housekeeping penicillin-binding proteins PBP1, PBP2, and PBP4 are shown and their transpeptidase (TP), transglycosylase (TG), and cell-wall-initiating (CW) domains are indicated. Also included is PBP2a. Factors that also support PBP2a are the PrsA chaperone and glycosylated wall teichoic acid (WTA) shown (red circles, polyribitol phosphate; blue hexagons, hexoses attaching ribitol phosphate attached to C55 isoprenoid precursor, double black line; green hexagons, β-*O*-GlcNAc). The TarG flippase is shown as a light blue oval. The right hand side summarizes the synthesis of the peptidoglycan precursor lipid II (red hexagons, *N*-acetylmuramic acid and *N*-acetylglucosamine; blue circles, amino acids, including D-alanines, unfilled) which is flipped to the exterior face of the cytoplasmic membrane by the MurJ 'flippase' (green oval) and is then incorporated into peptidoglycan by PBP2 and PBP2a. The left hand side shows the Sec secretion system (yellow oval) and the SpsB signal peptidase. The targets for molecules that act synergistically with β-lactams are indicated. The black boxes with cross-hatching symbolize the incompletely cross-linked peptidoglycan produced by PBP2/PBP2a and the final product produced by PBP4.

requires a chaperone, the peptidyl-prolyl *cis–trans* isomerase PrsA in order to fold correctly [27], and, as mentioned above, it requires WTAs and FMMs which provide a scaffold for its localization to, and retention at, the septum.

Molecules That Potentiate β-Lactam Antibiotic Activity

The imported PBP2a allows MRSA to proliferate in the presence of β-lactam antibiotics. However, MRSA is vulnerable to combinations of a β-lactam and molecules that destabilize the divisome or disrupt the platform/scaffold required for PBP2a integrity.

Statins Inhibit Functional Membrane Microdomains

Drugs in the statin family are widely used to treat hyperlipidaemia in the prevention of cardiovascular disease [28]. Statins inhibit the enzyme 3-hydroxy 3-methylglutamyl coenzyme A reductase (HMCR) in the mevalonate pathway [29]. Mevalonate is a precursor of the lipid cholesterol. There are considerable clinical and epidemiological data to indicate that patients receiving statin therapy have a significantly better outcome from serious infections including sepsis, pneumonia, and bacteraemia [30]. Two large meta-analyses demonstrated protective effects, but considerable differences occurred in statin exposure prior to, or during, hospitalization [31,32]. A recent cohort study in the USA of *S. aureus* bacteraemia patients concluded that continued statin therapy after hospitalization was beneficial but that there was no benefit if treatment was discontinued after hospitalization or if treatment started after hospitalization [33]. Similar results were seen in a controlled trial of sepsis patients [34].

There is considerable evidence to indicate that statins have immunomodulatory effects which could result in a reduced cytokine storm during sepsis. In mice and humans administered with lipopolysaccharide (LPS), statins reduce the level of proinflammatory cytokines TNF-α, IL-1β, and IL-6 as well as the expression of TLR2 and TLR4 on monocytes [35,36]. Also peripheral blood mononuclear cells from humans taking statins have lower responses to LPS *ex vivo* [37].

Statins reduce the uptake and killing of *S. aureus* by human neutrophils but paradoxically increase the level of killing following prolonged exposure. Statin treatment increases the release of phagocyte DNA which form extracellular traps containing bactericidal proteases, antimicrobial peptides, and histones [38]. In a murine model of pneumonia caused by *S. aureus*, more extracellular traps were evident in statin-treated animals – which could explain the lower level of bacteria in the lungs.

In *S. aureus*, statins inhibit the synthesis of staphyloxanthin, an unphosphorylated membrane saccharolipid that gives the bacterial colonies their golden yellow colour [39]. Staphyloxanthin is an antioxidant that helps to protect *S. aureus* cells from the oxidative burst following uptake by phagocytic cells [40]. Statins inhibit staphyloxanthin biosynthesis by targeting staphylococcal HMCR which prevents synthesis of the staphyloxanthin precursor mevalonate.

Very recently it has become apparent that the cytoplasmic membrane of prokaryotes has lipid raft-like domains called FMMs [9] (Figures 1 and 2). These are rich in isoprenoid lipids (staphyloxanthin in the case of *S. aureus*) combined with dimers of the membrane protein flotillin (FloA) [41]. FMMs act as scaffolds for assembly of multimeric protein complexes involved in secretion and signalling. Interestingly, FMMs are also important for the integrity of PBP2a in MRSA [9]. Extracted FMMs contain PBP2a, while FloA and PBP2a were shown to interact by two-hybrid and pull-down experiments. Inhibition of staphyloxanthin synthesis with the statin zaragozic acid resulted in reduced PBP2a oligomerization in the membrane and rendered the MRSA cells susceptible to β-lactam antibiotics *in vitro* and in a mouse infection model.

Thus, statins are excellent candidates for combination with β-lactams to treat MRSA infections. Not only do they sensitize MRSA to β-lactams but they also reduce virulence by promoting extracellular trap formation which enhances bacterial killing, and reduce excessive production of proinflammatory cytokines.

FtsZ Inhibitors

PC190723 is the forerunner of a series of compounds that bind to the tubulin-like protein FtsZ and block formation of the Z-ring at the septum [42]. TXA709 is a prodrug derivative with

improved oral availability and pharmacokinetics [43]. The inhibitors are potent and rapidly bactericidal. Cells continue to grow and expand in the presence of the FtsZ inhibitors but they cannot divide and eventually lyse.

A genetic knockdown screen was employed to identify genes that are required for resistance to β-lactams in MRSA strains [44]. It revealed components of the divisome, including FtsZ, to be important. It was shown that the FtsZ inhibitor PC190723 acted synergistically with β-lactam antibiotics (Figure 2). Fluorescence microscopy with green fluorescent protein (GFP) fusions revealed that FtsZ and PBP2a colocalized at the septum and that treatment with PC190723 caused both to become dislocalized. Synergy stems from a reduction in the concentration of β-lactams needed to inhibit residual housekeeping PBPs during perturbation of the integrity of the septum/divisome [10,44]. Indeed, the degree of synergy with a particular β-lactam depends on its affinity for PBP2 [10].

One difficulty with PC190723 and TXA709 is the high frequency of resistance that can be selected in a single step [42,45]. Mutants occur primarily at two glycine residues, substitutions at which block access of the drug to its binding site. While the high mutation frequency will likely preclude development of these drugs for monotherapy, several factors indicate that combination with a β-lactam could be successful: (i) the frequency of mutation to PC190723 was 10-fold lower in the presence of a β-lactam; (ii) PC190723-resistant mutants had greatly increased susceptibility to β-lactams (imipenem MIC $\geq$0.5 mg/ml compared to 32 $\geq$mg/ml); and (iii) resistant mutants were less virulent in a mouse infection model [45].

Another member of this chemical class, TXA6101, has structural flexibility that allows it to avoid steric clashes with G193 and G196 substitutions that are responsible for resistance to PC190723 and TXA707/709 [46]. The molecule retains activity against conventional FtsZ-resistant mutants and has an 8-fold lower MIC against wild-type *S. aureus*.

Signal Peptidase Inhibitors

The cyclic depsipeptide antibiotic krisynomycin and the lipoglycopeptide actinocarbasin showed synergistic activity with the carbapenem imipenem against the HA-MRSA strain COL and a panel of clinical MRSA isolates [47]. Their molecular target is the essential cell-surface-located signal peptidase SpsB that cleaves the N terminal secretory signal sequence from proteins as they pass through the Sec secretion pathway (Figure 2). It is likely that reduced secretion of PBP2a and other proteins involved in cell wall peptidoglycan biosynthesis and cell division renders MRSA more dependent on housekeeping PBPs and thus susceptible to β-lactam antibiotics. *In vivo* efficacy was demonstrated in a deep tissue wound infection model and in a bacteraemia/kidney abscess model. An added bonus of inhibiting protein secretion is likely to be a reduction in secretion of cell-wall-associated and extracellular virulence factors and that pathogenesis is likely to be compromised.

WTA Inhibitors

WTA is a polymer of ribitol phosphate that is covalently linked to peptidoglycan during cell wall synthesis. It is a major component of the cell wall of *S. aureus* and has important roles in determining cell shape and host colonization [48,49]. The precursor is assembled in the cytoplasm using the C55 undecaprenyl lipid carrier that is also used in assembling the peptidoglycan precursor lipid II [50,51] (Figure 2). The first step in the WTA pathway is catalysed by TarO which adds GlucNAc to C55-P to form C55-PP-GlcNAc (reviewed in [50,51]). The completed WTA precursor is flipped to the outer face of the cytoplasmic membrane by the membrane-bound TarG protein. WTA is not essential for cell survival *in vitro*, but cells lacking

WTA cannot complete cell division efficiently, lack fitness, and have reduced virulence [52]. MRSA lacking WTA becomes sensitive to β-lactam antibiotics because PBP2a relies on glycosylated WTA to act as a scaffold/platform [24,53]. Specifically, the addition of β-*O*-GlcNAc residues to the WTA backbone catalyzed by the glycosyltransferase encoded by TarS is required for PBP2a function [24]. Furthermore, newly synthesized WTA is a temporal and spatial signal for the correct localization of PBP4 at the septum [54]. In MRSA where peptidoglycan synthesis is catalysed inefficiently by PBP2/PBP2a, PBP4 is required to complete cross-linking [20]. If PBP4 is disrupted, cells become susceptible to β-lactams that target PBP2. Thus, inhibitors of WTA synthesis act synergistically with β-lactams both by misdirecting PBP4 and also by destabilizing PBP2a [53] (Figure 2).

The antibiotic tunicamycin has modest activity towards MRSA but acts synergistically with β-lactams [55]. It inhibits enzymes, including TarO in WTA biosynthesis, that transfer hexose-1-phosphates to membrane-bound lipid carriers to synthesize lipid-PP-hexoses. This was the first study to indicate the link between WTA and peptidoglycan biosynthesis and paved the way for the discovery of other molecules that potentiate β-lactams against MRSA.

Targocil inhibits the latter stages of WTA synthesis by blocking the transporter TarG [51,56] (Figure 2). Although it apparently lacks synergy with β-lactams against MRSA in standard chequerboard assays, it does potentiate β-lactams is a mouse infection model where impaired fitness and loss of virulence will magnify the synergistic effect.

Repurposing drugs that have been approved for other clinical indications is particularly attractive because of their well established safety, bioavailability, and pharmacokinetics. Screens of previously approved drugs for molecules with the ability to act synergistically with β-lactams against MRSA identified ticlopidine and clomiphene.

Ticlopidine is an antiplatelet drug that inhibits ADP-dependent platelet activation [57]. Like tunicamycin, it inhibits the synthesis of C55-PP-GlcNac by TarO (Figure 2), and although lacking antimicrobial activity itself ticlopidine potentiates the activity of β-lactams that target PBP2. Clomiphene is a widely used and safe nonsteroidal oestrogen receptor modulator [58]. It was identified in an antagonism screen for molecules that suppressed the activity of the TarG inhibitor targocil [59]. Clomiphene has modest antibacterial activity (MIC 8 μg/ml) but potentiates the activity of β-lactams against MRSA. It inhibits undecaprenyl diphosphate synthase (UppS) that is involved in C55 isoprenoid biosynthesis and thus inhibits both WTA and peptidoglycan biosynthesis (Figure 2). These molecules could be useful lead compounds for development of more potent synergistic combinations.

Branched polyethylenimines bind directly to WTA in the cell wall through phosphate–amide electrostatic interactions [60] (Figure 2). They act synergistically with β-lactams *in vitro* most likely by preventing correct localization of PBP2a and PBP4 at the septum. The polyethylenimines should be evaluated for *in vivo* efficacy, lack of toxicity, and appropriate pharmacokinetics to determine if they have potential to be used therapeutically.

MurJ Flippase Inhibitors

The MurJ is a conserved essential membrane protein that flips lipid II from the cytoplasmic face of the membrane to the outer face where it provides the substrate for peptidoglycan biosynthesis and acts as a signal for recruitment of PBP2 to the divisome [15] (Figure 2). Molecules that block MurJ activity, including the *N*-acylated linear heptapeptide antibiotic humimycin A

[61] and two unrelated synthetic chemicals [62], have antibacterial activity and act synergistically with β-lactams against MRSA.

Daptomycin

Daptomycin is a lipopeptide antibiotic that complexes with Ca^{2+} to form oligomers that insert into bacterial membranes by binding to negatively charged phosphatidylglycerol head groups [63,64]. This results in depolarization, permeabilization, leakage of ions, and ultimately cell death. Daptomycin is an important weapon in the treatment of serious infections caused by MRSA and is the preferred option for invasive infections caused by strains with elevated MICs to vancomycin [65]. β-Lactam antibiotics enhance the bactericidal activity of daptomycin and have been employed clinically to treat bacteraemia that is refractory to conventional therapy. Evaluation of data from several centres suggests that the outcome of endovascular and bone/joint infections can be improved by using this combination, but these data are only from case reports and observational studies (reviewed in [66]). An ongoing randomized clinical trial is designed to provide statistically significant results [67].

The mechanistic basis of synergy between β-lactams and daptomycin is due to inhibitory activity of the β-lactam towards PBP1 [68] (Figure 2). Indeed, carbapenems such as imipenem that specifically target PBP1 have greater synergy than those β-lactams that bind PBPs 1–4 and have a lower affinity for PBP1 than carbapenems [69].

PBP1 is primarily responsible for the initiation of cell division rather than peptidoglycan biosynthesis per se [17] (Figures 1 and 2). The *pbpA* gene that encodes PBP1 is part of the cell wall/cell division cluster and is induced in response to daptomycin-induced membrane damage [3,68]. The initial response of cells to daptomycin is to increase septation and sites of cell division. This compensatory response to daptomycin-induced stress renders cells particularly susceptible to PBP1-targetting carbapenems [68].

Daptomycin Resistance

Daptomycin treatment failure often occurs due to acquisition of gain-of-function mutations affecting the MprF protein, a membrane-bound enzyme that converts phosphatidylglycerol to lysyl-phosphatidylglycerol [63,64]. The introduction of positively charged lysine residues at the outer face of the membrane serves to repel Ca^{2+}-daptomycin and prevents it from entering the membrane. Another consequence of the lack of phosphatidylglycerol is reduced lipidation of the lipobox cysteines by Lgt acetyltransferase needed for anchorage of lipoproteins to the outer face of the membrane [70]. These include the peptidyl-prolyl *cis–trans* isomerase chaperone PsrA that is required for the correct folding of PBP2a [27,71] (Figure 2). Thus, daptomycin-insensitive mutants of MRSA paradoxically become susceptible to β-lactams that target PBP2–the so-called 'see saw' effect – due to the failure to express the PrsA chaperone [12]. Indeed, invasive *S. aureus* infections that fail to respond to daptomycin have been successfully treated with β-lactams [72,73].

Ceftaroline fosamil is a fifth-generation cephalosporin that has been approved for treating community-acquired bacterial pneumonia and serious skin and soft-tissue infections [23]. Infections such as infective endocarditis and osteomyelitis that involve a high density of MRSA with persistent bacteraemia are often recalcitrant to treatment with vancomycin or daptomycin. Ceftaroline acts synergistically with daptomycin against MRSA, including VISA and daptomycin-insensitive strains [74–76]. A small number of cases that did not respond to conventional monotherapy have been treated successfully (salvage therapy) with a combination of daptomycin and ceftaroline [77]. *In vitro* studies showed that ceftaroline enhanced

daptomycin-induced membrane depolarization and enhanced killing by the antimicrobial peptide cathelicidin [76].

Vancomycin

Vancomycin is a glycopeptide antibiotic that is the mainstay for treatment of invasive MRSA infections in hospital patients. It binds to the terminal D-alanine residues of lipid II and prevents transglycosylation and transpeptidation by PBP2 and PBP2a [78] (Figure 2). Treatment failure is due to the emergence during prolonged exposure of strains that have acquired several mutations in chromosomal genes that affect cell wall biosynthesis and homeostasis. These variants have a vancomycin MIC just above the breakpoint and are called vancomycin-intermediate *Staphylococcus aureus* (VISA) [79,80]. The development of VISA is complex and can occur by many different pathways. The underlying mechanism of nonsusceptibility is an increase in thickness and altered architecture of the cell wall [79]. This requires the antibiotic to travel further to encounter lipid II. Also, the reduced cross-linking provides false targets that sequester the drug.

Several studies have reported that vancomycin acts synergistically with β-lactams against MRSA and VISA (reviewed in [66]). The mechanistic basis of synergy has not been investigated but could be due to a combination of (i) decreased expression of PBPs, (ii) disrupted function of PBPs, and (iii) β-lactam activity reducing the thickness of the cell wall allowing easier access of vancomycin [81].

Clinical data on the combination of vancomycin and β-lactams has been confined to a restrospective cohort study and a controlled randomized trial which demonstrated trends towards successful outcomes [66]. A larger trial that includes daptomycin/β-lactam synergy (see above) is ongoing [67].

Synergistic Combinations of β-Lactam Antibiotics

In addition to the synergistic combinations described above, combinations of β-lactams that target different PBPs act synergistically against some MRSA strains.

Oxacillin and Cefoxitin

The combination of a β-lactam antibiotic that targets PBP4 (cefoxitin) with one that blocks the transpeptidase activity of PBP2 (oxacillin) is synergistic against the CA-MRSA strain LAC but not against the HA-MRSA strain COL [20,82]. CA-MRSA express a lower level of resistance to oxacillin, compared to HA-MRSA, and the peptidoglycan synthesized by the combination of PBP2 and PBP2a during exposure to oxacillin is poorly cross-linked and requires completion by PBP4. Indeed, a mutant of CA-MRSA lacking PBP4 becomes susceptible to oxacillin whereas a similar mutant of COL remains resistant [20]. When PBP4 is inactivated by cefoxitin, CA-MRSA cells become vulnerable to oxacillin because the lower level of PBP2a provides insufficient cross-linking, and they require the activity of PBP2 to survive.

The SCC*mec* type I element of COL was exchanged for the type IV element of CA-MRSA strain MW2 by genetic manipulation [83]. The construct did not express the low level of oxacillin resistance seen in MW2, which indicates that critical factors that control the level of, or activity of, PBP2a are not expressed by genes contained within the SCC*mec* element. The reciprocal swap of the CA-MRSA SCC*mec* type IV element for the type I element of COL was not reported.

The combination of oxacillin with cefoxitin has not been tested in animal infection models. The clinical usefulness of the combination is yet to be explored and will be compromised by the need for clinicians to know if the infection is caused by a CA-MRSA strain. This might be appropriate in geographic regions such as the USA, Australia, and New Zealand, where CA-MRSA strains are widespread, but not in regions such as Europe where CA-MRSA is less important.

Meropenem, Tazobactam, and Piperacillin

The combination of a carbapenem (meropenem) with the ureidopenicillin piperacillin and the β-lactamase inhibitor tazobactam has synergistic activity towards HA-MRSA strains [84]. The explanation for this effect is that meropenem inhibits PBP1 as well as activating the allosteric site in PBP2a to open up the buried transpeptidase active site to a second inhibitory molecule of the carbapenem and/or to piperacillin. Meanwhile, tazobactam inhibits β-lactamase which allows the β-lactamase-susceptible piperacillin to block the transpeptidase activity of PBP2 [84]. The combination was effective in reducing mortality in a neutropenic mouse peritonitis model of *S. aureus* infection.

This combination should be examined in more detail by probing the mechanistic basis of synergy and determining if it applies to a well characterized collection of MRSA strains, including the predominant HA-MRSA and CA-MRSA. More rigorous animal infection model studies should be performed as well as the pharmacokinetics of administering a combination of three drugs. Investigating whether the β-lactamase-susceptible piperacillin could be exchanged for oxacillin could make it possible to dispense with tazobactam.

Other Effects of β-Lactam Antibiotics

Nafcillin (and presumably other penicillin derivatives such as oxacillin) renders *S. aureus* more susceptible to damage by cationic antimicrobial peptides and consequently to enhanced killing following opsonization by human neutrophils *in vitro* and in whole blood [85]. The mechanistic basis of this phenomenon deserves thorough investigation. It is likely to be related to the finding that exposing CA-MRSA to subinhibitory concentrations of oxacillin reduces the expression of toxins and renders the cells more susceptible to opsonophagocytosis, phenomena that involve altered expression of the global virulence regulator Agr [86]. (Incidentally, a molecule that blocks binding of the Agr response regulator to its operator sites in the *agr* locus and reduces density-dependent induction of expression of virulence factors also sensitizes MRSA to β-lactams [87]. However, the mechanistic basis of this phenomenon is unclear.) A word of caution [88] is due because the naficillin study was performed with hospital-associated MRSA strains that caused bacteraemia and endovascular infections and which have high MICs to β-lactams. In contrast, the oxacillin-toxin study was conducted with a single CA-MRSA strain that has a low MIC to β-lactams and predominantly causes skin and soft-tissue infections. High-toxin-expressing CA-MRSA strains struggle to grow well in serum and often acquire mutations that reduce toxin expression during a bloodstream infection which increases their fitness *in vivo* [89,90]. Also, there have been contradictory reports of the effects of β-lactams on toxin expression, suggesting that effects are strain-specific and dependent on *in vitro* growth conditions [86,88].

Concluding Remarks and Future Prospects

This review has discussed the mechanisms of action of a diverse array of molecules that act synergistically with β-lactam antibiotics and have the potential to resurrect this important class of drugs to combat MRSA infections in the hospital and the community.

Outstanding Questions

Will clinical trials prove the effectiveness of synergistic combinations, paving the way for licensing by regulatory authorities?

Do PBPs act in multiprotein complexes as suggested by studies with functional membrane microdomains?

What is the nature of the signal for PBP4 localization to the divisome?

What is the mechanistic basis of oxacillin reducing toxin expression controlled by Agr in CA-MRSA?

Does inhibition of virulence by β-lactams extend to HA-MRSA?

Synergy stems from the vulnerability of PBP2a due to its dependence on functional membrane microdomains, WTA, and the PrsA chaperone. Molecules that interfere with any of these elements have the effect of impairing PBP2a and exposing sensitive housekeeping PBPs to β-lactam attack.

Clinical evidence that combination therapy could be effective is limited and comes from studies with vancomycin and daptomycin. So far these are restricted to case reports and observational studies, so the outcome of a large clinical trial is eagerly awaited. Preclinical studies with other synergistic molecules must be completed, including testing for activity against the diversity of CA-MRSA and HA-MRSA. Then properly empowered clinical trials like those with vancomycin and daptomycin will be required (see Outstanding Questions).

Unexpected beneficial consequences of exposing MRSA to some of the molecules discussed above could be a reduction in expression of virulence factors and rendering bacteria more susceptible to host defences. The positive effect of long-term statin use on the outcome of serious infections and sepsis was detected in patients who were not being treated with β-lactams. It will be interesting to determine if β-lactam/statin synergy gives a positive outcome in infected patients beginning therapy who were not previously exposed to statins compared to those previously on long-term statins therapy.

In conclusion, there is every reason to be hopeful that β-lactam therapy of MRSA infections by combination with a synergistic molecule can become a bona fide treatment option and that this important class of antibiotics can be re-employed against serious MRSA infections.

References

1. Llarrull, L.I. *et al.* (2010) The future of the beta-lactams. *Curr. Opin. Microbiol.* 13, 551–557
2. Chambers, H.F. and Deleo, F.R. (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat. Rev. Microbiol.* 7, 629–641
3. Peacock, S.J. and Paterson, G.K. (2015) Mechanisms of methicillin resistance in *Staphylococcus aureus*. *Annu. Rev. Biochem.* 84, 577–601
4. Stryjewski, M.E. and Corey, G.R. (2014) Methicillin-resistant *Staphylococcus aureus*: an evolving pathogen. *Clin. Infect. Dis.* 58 (Suppl. 1), S10–S19
5. Otto, M. (2010) Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. *Annu. Rev. Microbiol.* 64, 143–162
6. Chen, C.J. and Huang, Y.C. (2014) New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin. Microbiol. Infect.* 20, 605–623
7. Drawz, S.M. and Bonomo, R.A. (2010) Three decades of beta-lactamase inhibitors. *Clin. Microbiol. Rev.* 23, 160–201
8. Wang, H. *et al.* (2013) Discovery of wall teichoic acid inhibitors as potential anti-MRSA beta-lactam combination agents. *Chem. Biol.* 20, 272–284
9. Garcia-Fernandez, E. *et al.* (2017) Membrane microdomain disassembly inhibits MRSA antibiotic resistance. *Cell* 171, 1354–1367 e20
10. Ferrer-Gonzalez, E. *et al.* (2017) beta-Lactam antibiotics with a high affinity for PBP2 act synergistically with the FtsZ-targeting agent TXA707 against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 61, e00863-17
11. Mehta, S. *et al.* (2012) beta-Lactams increase the antibacterial activity of daptomycin against clinical methicillin-resistant *Staphylococcus aureus* strains and prevent selection of daptomycin-resistant derivatives. *Antimicrob. Agents Chemother.* 56, 6192–6200
12. Renzoni, A. *et al.* (2017) Molecular bases determining daptomycin resistance-mediated resensitization to beta-lactams (seesaw effect) in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 61, e01634-16
13. Du, S. and Lutkenhaus, J. (2017) Assembly and activation of the *Escherichia coli* divisome. *Mol. Microbiol.* 105, 177–187
14. Lutkenhaus, J. *et al.* (2012) Bacterial cytokinesis: from Z ring to divisome. *Cytoskeleton (Hoboken)* 69, 778–790
15. Monteiro, J.M. *et al.* (2018) Peptidoglycan synthesis drives an FtsZ-treadmilling-independent step of cytokinesis. *Nature* 554, 528–532
16. Fuda, C.C. *et al.* (2005) Beta-lactam resistance in *Staphylococcus aureus*: the adaptive resistance of a plastic genome. *Cell. Mol. Life Sci.* 62, 2617–2633
17. Pereira, S.F. *et al.* (2009) Evidence for a dual role of PBP1 in the cell division and cell separation of *Staphylococcus aureus*. *Mol. Microbiol.* 72, 895–904
18. Reed, P. *et al.* (2015) *Staphylococcus aureus* survives with a minimal peptidoglycan synthesis machine but sacrifices virulence and antibiotic resistance. *PLoS Pathog.* 11, e1004891
19. Pinho, M.G. *et al.* (2001) An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10886–10891
20. Memmi, G. *et al.* (2008) *Staphylococcus aureus* PBP4 is essential for beta-lactam resistance in community-acquired methicillin-resistant strains. *Antimicrob. Agents Chemother.* 52, 3955–3966
21. Lim, D. and Strynadka, N.C. (2002) Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat. Struct. Biol.* 9, 870–876
22. Otero, L.H. *et al.* (2013) How allosteric control of *Staphylococcus aureus* penicillin binding protein 2a enables methicillin resistance and physiological function. *Proc. Natl. Acad. Sci. U. S. A.* 110, 16808–16813

23. Laudano, J.B. (2011) Ceftaroline fosamil: a new broad-spectrum cephalosporin. *J. Antimicrob. Chemother.* 66 (Suppl. 3), iii11–8
24. Brown, S. *et al.* (2012) Methicillin resistance in *Staphylococcus aureus* requires glycosylated wall teichoic acids. *Proc. Natl. Acad. Sci. U. S. A.* 109, 18909–18914
25. Rolo, J. *et al.* (2017) Evidence for the evolutionary steps leading to *mecA*-mediated beta-lactam resistance in staphylococci. *PLoS Genet.* 13, e1006674
26. Rolo, J. *et al.* (2017) Evolutionary origin of the staphylococcal cassette chromosome mec (SCCmec). *Antimicrob. Agents Chemother.* 61, e02302-16
27. Jousselin, A. *et al.* (2015) The *Staphylococcus aureus* chaperone PrsA is a new auxiliary factor of oxacillin resistance affecting penicillin-binding protein 2A. *Antimicrob. Agents Chemother.* 60, 1656–1666
28. Smith, S.C., Jr and Grundy, S.M. (2014) 2013 ACC/AHA guideline recommends fixed-dose strategies instead of targeted goals to lower blood cholesterol. *J. Am. Coll. Cardiol.* 64, 601–612
29. Shitara, Y. and Sugiyama, Y. (2006) Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug–drug interactions and interindividual differences in transporter and metabolic enzyme functions. *Pharmacol. Ther.* 112, 71–105
30. Hennessy, E. *et al.* (2016) Is there potential for repurposing statins as novel antimicrobials? *Antimicrob. Agents Chemother.* 60, 5111–5121
31. Janda, S. *et al.* (2010) The effect of statins on mortality from severe infections and sepsis: a systematic review and meta-analysis. *J. Crit. Care* 25, 656 e7–e22
32. Ma, Y. *et al.* (2012) Systematic review and meta-analysis on the association between outpatient statins use and infectious disease-related mortality. *PLoS One* 7, e51548
33. Caffrey, A.R. *et al.* (2017) Evidence to support continuation of statin therapy in patients with *Staphylococcus aureus* bacteremia. *Antimicrob. Agents Chemother.* 61, e02228-16
34. Kruger, P. *et al.* (2013) A multicenter randomized trial of atorvastatin therapy in intensive care patients with severe sepsis. *Am. J. Respir. Crit. Care Med.* 187, 743–750
35. Chaudhry, M.Z. *et al.* (2008) Statin (cerivastatin) protects mice against sepsis-related death via reduced proinflammatory cytokines and enhanced bacterial clearance. *Surg. Infect. (Larchmt)* 9, 183–194
36. Niessner, A. *et al.* (2006) Simvastatin suppresses endotoxin-induced upregulation of toll-like receptors 4 and 2 *in vivo*. *Atherosclerosis* 189, 408–413
37. Rosenson, R.S. *et al.* (1999) Inhibition of proinflammatory cytokine production by pravastatin. *Lancet* 353, 983–984
38. Chow, O.A. *et al.* (2010) Statins enhance formation of phagocyte extracellular traps. *Cell Host Microbe* 8, 445–454
39. Liu, C.I. *et al.* (2008) A cholesterol biosynthesis inhibitor blocks *Staphylococcus aureus* virulence. *Science* 319, 1391–1394
40. Clauditz, A. *et al.* (2006) Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. *Infect. Immun.* 74, 4950–4953
41. Lopez, D. and Kolter, R. (2010) Functional microdomains in bacterial membranes. *Genes Dev.* 24, 1893–1902
42. Haydon, D.J. *et al.* (2008) An inhibitor of FtsZ with potent and selective anti-staphylococcal activity. *Science* 321, 1673–1675
43. Kaul, M. *et al.* (2015) TXA709, an FtsZ-targeting benzamide prodrug with improved pharmacokinetics and enhanced *in vivo* efficacy against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 59, 4845–4855
44. Tan, C.M. *et al.* (2012) Restoring methicillin-resistant *Staphylococcus aureus* susceptibility to beta-lactam antibiotics. *Sci. Transl. Med.* 4, 126ra35
45. Kaul, M. *et al.* (2016) Combining the FtsZ-targeting prodrug TXA709 and the cephalosporin cefdinir confers synergy and reduces the frequency of resistance in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 60, 4290–4296
46. Fujita, J. *et al.* (2017) Structural flexibility of an inhibitor overcomes drug resistance mutations in *Staphylococcus aureus* FtsZ. *ACS Chem. Biol.* 12, 1947–1955
47. Therien, A.G. *et al.* (2012) Broadening the spectrum of beta-lactam antibiotics through inhibition of signal peptidase type I. *Antimicrob. Agents Chemother.* 56, 4662–4670
48. Neuhaus, F.C. and Baddiley, J. (2003) A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. *Microbiol. Mol. Biol. Rev.* 67, 686–723
49. Weidenmaier, C. and Peschel, A. (2008) Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nat. Rev. Microbiol.* 6, 276–287
50. Pasquina, L.W. *et al.* (2013) Teichoic acid biosynthesis as an antibiotic target. *Curr. Opin. Microbiol.* 16, 531–537
51. Swoboda, J.G. *et al.* (2010) Wall teichoic acid function, biosynthesis, and inhibition. *ChemBioChem* 11, 35–45
52. Weidenmaier, C. *et al.* (2004) Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nat. Med.* 10, 243–245
53. Sewell, E.W. and Brown, E.D. (2014) Taking aim at wall teichoic acid synthesis: new biology and new leads for antibiotics. *J. Antibiot. (Tokyo)* 67, 43–51
54. Atilano, M.L. *et al.* (2010) Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. U. S. A.* 107, 18991–18996
55. Campbell, J. *et al.* (2011) Synthetic lethal compound combinations reveal a fundamental connection between wall teichoic acid and peptidoglycan biosyntheses in *Staphylococcus aureus*. *ACS Chem. Biol.* 6, 106–116
56. Swoboda, J.G. *et al.* (2009) Discovery of a small molecule that blocks wall teichoic acid biosynthesis in *Staphylococcus aureus*. *ACS Chem. Biol.* 4, 875–883
57. Farha, M.A. *et al.* (2013) Inhibition of WTA synthesis blocks the cooperative action of PBPs and sensitizes MRSA to beta-lactams. *ACS Chem. Biol.* 8, 226–233
58. Homburg, R. (2005) Clomiphene citrate – end of an era? A mini-review. *Hum. Reprod.* 20, 2043–2051
59. Farha, M.A. *et al.* (2015) Antagonism screen for inhibitors of bacterial cell wall biogenesis uncovers an inhibitor of undecaprenyl diphosphate synthase. *Proc. Natl. Acad. Sci. U. S. A.* 112, 11048–11053
60. Foxley, M.A. *et al.* (2017) Targeting wall teichoic acid *in situ* with branched polyethylenimine potentiates beta-lactam efficacy against MRSA. *ACS Med. Chem. Lett.* 8, 1083–1088
61. Chu, J. *et al.* (2018) Human microbiome inspired antibiotics with improved beta-lactam synergy against MDR *Staphylococcus aureus*. *ACS Infect. Dis.* 4, 33–38
62. Huber, J. *et al.* (2009) Chemical genetic identification of peptidoglycan inhibitors potentiating carbapenem activity against methicillin-resistant *Staphylococcus aureus*. *Chem. Biol.* 16, 837–848
63. Miller, W.R. *et al.* (2016) Mechanism of action and resistance to daptomycin in *Staphylococcus aureus* and enterococci. *Cold Spring Harb. Perspect. Med.* 6, a026997
64. Bayer, A.S. *et al.* (2013) Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. *Ann. N. Y. Acad. Sci.* 1277, 139–158
65. Moore, C.L. *et al.* (2012) Daptomycin versus vancomycin for bloodstream infections due to methicillin-resistant *Staphylococcus aureus* with a high vancomycin minimum inhibitory concentration: a case-control study. *Clin. Infect. Dis.* 54, 51–58
66. Bartash, R. and Nori, P. (2017) Beta-lactam combination therapy for the treatment of *Staphylococcus aureus* and *Enterococcus* species bacteremia: a summary and appraisal of the evidence. *Int. J. Infect. Dis.* 63, 7–12
67. Tong, S.Y. *et al.*, and group Cs, the Australasian Society for Infectious Diseases Clinical Research N (2016) CAMERA2 – combination antibiotic therapy for methicillin-resistant

Staphylococcus aureus infection: study protocol for a randomised controlled trial. *Trials* 17, 170

68. Berti, A.D. *et al.* (2015) Penicillin binding protein 1 is important in the compensatory response of *Staphylococcus aureus* to daptomycin-induced membrane damage and is a potential target for beta-lactam-daptomycin synergy. *Antimicrob. Agents Chemother.* 60, 451–458
69. Berti, A.D. *et al.* (2013) beta-Lactam antibiotics targeting PBP1 selectively enhance daptomycin activity against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 57, 5005–5012
70. Stoll, H. *et al.* (2005) *Staphylococcus aureus* deficient in lipidation of prelipoproteins is attenuated in growth and immune activation. *Infect. Immun.* 73, 2411–2423
71. Jousselin, A. *et al.* (2012) The posttranslocational chaperone lipoprotein PrsA is involved in both glycopeptide and oxacillin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 56, 3629–3640
72. Dhand, A. *et al.* (2011) Use of antistaphylococcal beta-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant *Staphylococcus aureus*: role of enhanced daptomycin binding. *Clin. Infect. Dis.* 53, 158–163
73. Moise, P.A. *et al.* (2013) Multicenter evaluation of the clinical outcomes of daptomycin with and without concomitant beta-lactams in patients with *Staphylococcus aureus* bacteremia and mild to moderate renal impairment. *Antimicrob. Agents Chemother.* 57, 1192–1200
74. Werth, B.J. *et al.* (2014) Evaluation of ceftaroline, vancomycin, daptomycin, or ceftaroline plus daptomycin against daptomycin-nonsusceptible methicillin-resistant *Staphylococcus aureus* in an *in vitro* pharmacokinetic/pharmacodynamic model of simulated endocardial vegetations. *Antimicrob. Agents Chemother.* 58, 3177–3181
75. Barber, K.E. *et al.* (2015) Evaluation of ceftaroline alone and in combination against biofilm-producing methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to daptomycin and vancomycin in an *in vitro* pharmacokinetic/pharmacodynamic model. *Antimicrob. Agents Chemother.* 59, 4497–4503
76. Werth, B.J. *et al.* (2013) Ceftaroline increases membrane binding and enhances the activity of daptomycin against daptomycin-nonsusceptible vancomycin-intermediate *Staphylococcus aureus* in a pharmacokinetic/pharmacodynamic model. *Antimicrob. Agents Chemother.* 57, 66–73
77. Sakoulas, G. *et al.* (2014) Antimicrobial salvage therapy for persistent staphylococcal bacteremia using daptomycin plus ceftaroline. *Clin. Ther.* 36, 1317–1333
78. Zeng, D. *et al.* (2016) Approved glycopeptide antibacterial drugs: mechanism of action and resistance. *Cold Spring Harb. Perspect. Med.* 6, a026989
79. Howden, B.P. *et al.* (2010) Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin. Microbiol. Rev.* 23, 99–139
80. Gardete, S. and Tomasz, A. (2014) Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *J. Clin. Invest.* 124, 2836–2840
81. Ortwine, J.K. *et al.* (2013) Reduced glycopeptide and lipopeptide susceptibility in *Staphylococcus aureus* and the 'seesaw effect': taking advantage of the back door left open? *Drug Resist. Update* 16, 73–79
82. Banerjee, R. *et al.* (2013) Combinations of cefoxitin plus other beta-lactams are synergistic *in vitro* against community associated methicillin-resistant *Staphylococcus aureus*. *Eur. J. Clin. Microbiol. Infect. Dis.* 32, 827–833
83. Reichmann, N.T. and Pinho, M.G. (2017) Role of SCCmec type in resistance to the synergistic activity of oxacillin and cefoxitin in MRSA. *Sci. Rep.* 7, 6154
84. Gonzales, P.R. *et al.* (2015) Synergistic, collaterally sensitive beta-lactam combinations suppress resistance in MRSA. *Nat. Chem. Biol.* 11, 855–861
85. Sakoulas, G. *et al.* (2014) Nafcillin enhances innate immune-mediated killing of methicillin-resistant *Staphylococcus aureus*. *J. Mol. Med. (Berl)* 92, 139–149
86. Waters, E.M. *et al.* (2017) Redeploying beta-lactam antibiotics as a novel antivirulence strategy for the treatment of methicillin-resistant *Staphylococcus aureus* infections. *J. Infect. Dis.* 215, 80–87
87. Kuo, D. *et al.* (2015) Novel quorum-quenching agents promote methicillin-resistant *Staphylococcus aureus* (MRSA) wound healing and sensitize MRSA to beta-lactam antibiotics. *Antimicrob. Agents Chemother.* 59, 1512–1518
88. Bayer, A.S. and Xiong, Y.Q. (2017) Redeploying beta-lactams against *Staphylococcus aureus*: repurposing with a purpose. *J. Infect. Dis.* 215, 11–13
89. Laabei, M. *et al.* (2015) Evolutionary trade-offs underlie the multi-faceted virulence of *Staphylococcus aureus*. *PLoS Biol.* 13, e1002229
90. Das, S. *et al.* (2016) Natural mutations in a *Staphylococcus aureus* virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation. *Proc. Natl. Acad. Sci. U. S. A.* 113, E3101–E3110