Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

*Curr Top Microbiol Immunol*. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

*Curr Top Microbiol Immunol*. 2017 ; 404: 1–44. doi:10.1007/82_2015_5021.

# Envelope Structures of Gram-Positive Bacteria

**Mithila Rajagopal**[1,2] and **Suzanne Walker**[1,*]

[1]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA, 02115

[2]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts, USA, 02138

## Abstract

Gram-positive organisms, including the pathogens *Staphylococcus aureus, Streptococcus pneumoniae* and *Enterococcus faecalis*, have dynamic cell envelopes that mediate interactions with the environment and serve as the first line of defense against toxic molecules. Major components of the cell envelope include peptidoglycan, which is a well-established target for antibiotics, teichoic acids, capsular polysaccharides, surface proteins, and phospholipids. These components can undergo modification to promote pathogenesis, decrease susceptibility to antibiotics and host immune defenses, and enhance survival in hostile environments. This chapter will cover the structure, biosynthesis and important functions of major cell envelope components in Gram-positive bacteria. Possible targets for new antimicrobials will be noted.

## 1. Introduction

The cell envelope is a complex, dynamic, multilayered structure that serves to protect bacteria from their unpredictable and often hostile surroundings. The cell envelopes of most bacteria fall into one of two major groups. Gram-negative bacteria have an inner, cytoplasmic membrane surrounded by a thin layer of peptidoglycan (PG) and an outer membrane containing lipopolysaccharide. The outer membrane functions as a permeability barrier to control the influx and egress of ions, nutrients and environmental toxins, and it also contributes to osmoprotection. Gram-positive bacteria lack a protective outer membrane but the PG layers are many times thicker than those in Gram-negative organisms (Silhavy *et al.* 2010; Vollmer *et al.* 2008). Embedded in the inner membrane and attached to the PG layers are long anionic polymers called teichoic acids (TAs), which play multiple roles in cell envelope physiology as well as pathogenesis (Brown *et al.* 2013; Percy and Gründling 2014; Schneewind and Missiakas 2014). Membrane-embedded and wall-associated proteins serve as environmental sensors, regulate passage of nutrients and ions across the cytoplasmic membrane, facilitate efflux of toxins and other molecules, modulate surface adhesion, and participate in enzymatic synthesis, degradation, and remodeling of the cell envelope during growth and division, and in response to environmental stress (Buist *et al.* 2008; Kovacs-Simon *et al.* 2011; Navarre and Schneewind 1999; Stock *et al.* 2000; Zhen *et al.* 2009).

*To whom correspondence should be addressed: suzanne_walker@hms.harvard.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Other important cell envelope components in Gram-positive organisms include capsular polysaccharides (CPS), which are covalently attached to PG, and extracellular polysaccharides, which form an amorphous outer layer (Arciola *et al.* 2015; Yother 2011).

The importance of the cell envelope for bacterial survival makes it a target for antibiotics, and several classes of clinically used antibiotics inhibit biosynthesis of PG, resulting in osmotic rupture. Other antibiotics damage the membrane barrier (Walsh 2003). Because resistance to clinically used antibiotics has become widespread, there is a push to better understand cell envelope biogenesis and regulation, and to identify new cell envelope targets that can be exploited in the development of next generation antibiotics. In this chapter, we will focus on important cell envelope components of Gram-positive pathogens using *Staphylococcus aureus* as a focal point, except where other Gram-positive pathogens are better studied. Attention will also be given to the non-pathogenic *Bacillus subtilis* because its genetic tractability and other biological characteristics have led to its adoption as the principal Gram-positive model organism.

## 2. Cell membrane

Gram-positive organisms are surrounded by bilayer membranes that can vary substantially in composition but typically include large amounts of phosphatidylglycerol and cardiolipin. In *Bacillus* species, phosphatidylethanolamine is abundant as well (Clejan *et al.* 1986; Haque and Russell 2004; Minnikin and Abdolraimzadeh 1974). Many Gram-positive species express at least one type of aminoacylatedphosphatidylglycerol (Epand *et al.* 2007; Parsons and Rock 2014). For example, in *S. aureus*, lysyl-phosphatidylglycerol is found in significant amounts, particularly during logarithmic growth (Ernst *et al.* 2009). This phospholipid is synthesized by a polytopic membrane protein, MprF, which catalyzes the transfer of lysine from lysyl-tRNA to phosphatidylglycerol on the inner leaflet of the membrane and then translocates this species to the outer leaflet of the membrane (Ernst *et al.* 2009; Kristian *et al.* 2003). Lysyl-phosphatidylglycerol reduces susceptibility to antimicrobial peptides produced during host infection (Peschel *et al.* 2001) and also provides protection against aminoglycosides, bacitracin, daptomycin, and some β-lactams (Nishi *et al.* 2004; Komatsuzawa *et al.* 2001). Daptomycin-resistant *S. aureus* clinical isolates frequently contain mutations that increase MprF expression or translocase activity (Friedman *et al.* 2006; Julian *et al.* 2007). Other species of Gram-positives have MprF homologs that have been implicated in similar functions (Ernst and Peschel 2011). It is thought that the positive charges of lysyl-phosphatidylglycerol serve to repel positively charged antibiotics or antibiotic-metal complexes (Ernst and Peschel 2011; Nishi *et al.* 2004).

The composition of both the head groups and the fatty acyl chains in membrane phospholipids can change rapidly in response to environmental conditions, such as low pH, osmotic stress, or temperature extremes (Zhang and Rock 2008). For example, branched chain fatty acid content in membranes can vary substantially depending on growth conditions. Membrane lipid composition affects membrane viscosity, which modulates membrane permeability and can influence both solute transport and protein interactions.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



Membrane lipid homeostasis is thus a crucial process and interfering with it can compromise viability (de Mendoza 2014; Zhang and Rock 2008).

In addition to the lipid components, the cell membrane contains the lipid anchor component of lipoteichoic acid (LTA), and includes numerous transmembrane and lipoproteins with functions in cell envelope synthesis, transport of cell envelope precursors and nutrients, and export of toxic compounds (Fig. 1). Among these transmembrane proteins are the sensory components of several two component sensing systems that regulate the cell's response to external stimuli, including cell density and presence of damaging toxins. For instance, the amount of lysyl-phosphatidylglycerol in *S. aureus* is regulated by a complex of proteins that includes a two-component signalling system, GraRS, and a two-component ABC-transporter-like system, VraFG. This complex, which senses and responds to a variety of stimuli, including the presence of antimicrobial peptides, also regulates D-alanylation of TAs (Falord *et al.* 2011; Li *et al.* 2007a; Li *et al.* 2007b; Yang *et al.* 2012). Modulating the negative charge density of the cell envelope through lysinylation of phosphatidylglycerol and D-alanylation of TAs decreases susceptibility of *S. aureus* to antimicrobial peptides produced during host infection and increases resistance to cationic antibiotics administered to treat infection (Ernst and Peschel 2011; Brown *et al.* 2013; Revilla-Guarinos *et al.* 2014; Bayer *et al.* 2013).

## 3. Peptidoglycan

Gram-positive bacteria are surrounded by many layers of peptidoglycan (PG), which form a protective shell that is 30–100 nm thick (Silhavy *et al.* 2010). The PG layers are covalently modified with carbohydrate polymers including wall teichoic acids (WTAs) or functionally related anionic glycopolymers as well as CPS. The PG layers also scaffold numerous proteins, some of which are bound non-covalently through interactions with PG-binding modules such as LysM domains (Buist *et al.* 2008) while others are covalently attached by sortases (Schneewind and Missiakas 2012). Some wall-associated proteins play important roles in cell envelope remodeling during growth and division, whereas others scavenge nutrients and metals from the environment or serve as adhesins that promote surface binding and colonization (Navarre and Schneewind 1999). PG has numerous important functions but perhaps the most important is that it stabilizes the cell membrane, enabling it to withstand high internal osmotic pressures. This function is critical for cell survival because the turgor pressure pushing against the cell membrane can reach 20 atmospheres in some Gram-positive bacteria (Mitchell and Moyle 1956; Norris and Sweeney 1993). Since PG is essential for viability and the biosynthetic pathway is highly conserved in Gram-positive and Gram-negative organisms, PG biosynthesis is a target for many clinically used antibiotics, including β-lactams, which are the most successful class of antibiotics in history, and vancomycin, which is still widely used to treat serious Gram-positive infections, including methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

### 3.1 Peptidogylcan structure

PG is composed of linear chains of repeating disaccharide units cross-linked via peptide side chains (Fig. 2). The disaccharide subunit is completely conserved and consists of *N*-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

acetylglucosamine (GlcNAc) coupled through a β-1,4-linkage to *N*-acetylmuramic acid (MurNAc) (Schleifer and Kandler 1972). The average chain length of the glycan strands can vary considerably across species. In *S. aureus*, the glycan strands are relatively short, averaging 6–18 disaccharide units (Boneca *et al.* 2000; Ward 1973) while in *B. subtilis*, the glycan chains are much longer. Early measurements of *B. subtilis* glycan strands indicated an average chain length of 54–96 disaccharide units, but more recent experiments using atomic force microscopy to probe size exclusion-purified glycan strands have suggested that glycan chains can reach 5000 disaccharide units in length (Hayhurst *et al.* 2008; Ward 1973). The longer glycan chains found in *B. subtilis* may be a result of the cylindrical shape, which results in a substantially greater stress imparted on the cylindrical walls compared with the poles (Hayhurst *et al.* 2008).

MurNAc, a sugar unique to bacteria, contains a C3 lactate group. In nascent (uncrosslinked) PG of Gram-positive organisms, this group is bonded to the N-terminus of a linear peptide consisting of five amino acids. The first, L-alanine, is typically followed by D-isoglutamine, and the terminal dipeptide is D-Ala-D-Ala. Position 3 of the pentapeptide chain is either L-lysine or *meso*-diaminopimelic acid (*m*-DAP), with the former being found in *S. aureus, Streptococcus pneumoniae, Enterococcus faecalis*, and *Enterococcus faecium*, and the latter being found in *B. subtilis* (Schleifer and Kandler 1972). The ε-amino group of L-Lys is typically coupled to one or more additional amino acids. In *S. aureus*, for example, L-lysine is coupled to pentaglycine, although serine can also be incorporated in some strains (De Jonge *et al.* 1993; Schleifer and Kandler 1972). *S. pneumoniae* and *E. faecalis* contain dipeptide substituents consisting of L-Ala-L-Ser or L-Ala-L-Ala, respectively (De Jonge *et al.* 1996; Schleifer and Kandler 1972; Severin and Tomasz 1996). *S. pneumoniae* PG is unusual in that it can be a mixture of either dipeptide-subtituted or un-subtituted stem peptides (Garcia-Bustos *et al.* 1987; Severin and Tomasz 1996). *E. faecium* contains a D-aspartate substituent (Patti *et al.* 2008; Vollmer *et al.* 2008). Canonical glycan strand crosslinking occurs via formation of an amide bond between the side chain or branching peptide on amino acid 3 of one stem peptide and the backbone carbonyl of amino acid 4 on another stem peptide, with the loss of the terminal D-ala (Schleifer and Kandler 1972). Crosslinks can also form to the carbonyl of amino acid 3 in some species of Gram-positive organisms (Lavollay *et al.* 2008; Lavollay *et al.* 2011; Mainardi *et al.* 2000; Schleifer and Kandler 1972).

### 3.2 Peptidoglycan biosynthesis

PG biosynthesis takes place in distinct stages, the first of which involves assembly of a UDP-MurNAc pentapeptide in the cytoplasm. This stage is followed by coupling of the phospho-MurNAc pentapeptide to the undecaprenyl phosphate (Und-P) "carrier lipid" embedded in the membrane to form a lipid-linked monosaccharide known as Lipid I, which is glycosylated to form the disaccharide Lipid II. Additional amino acids, if any, are appended to the pentapeptide chain at this point and then Lipid II is translocated across the membrane. In the final stage of PG biosynthesis, Lipid II is polymerized and the resulting glycan strands are cross-linked to give mature PG. The lipid carrier released during glycan chain polymerization is recycled back into the cell to continue synthesis. Most of the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

enzymatic steps for the majority of the biosynthetic pathway are well-conserved across both Gram-negative and Gram-positive bacteria (Fig. 3).

**Assembly of Lipid II—**The first committed step in PG synthesis involves the MurA-catalyzed transfer of enolpyruvate from phosphoenolpyruvate to the C3 hydroxyl of UDP-GlcNAc (Marquardt *et al.* 1992). Some low GC Gram-positive organisms, including *S. aureus, S. pneumoniae* and *B. subtilis* contain two *murA* alleles, which are differently regulated (Blake *et al.* 2009; Du *et al.* 2000; Kock *et al.* 2004). The secondary *murA* allele may allow for increased flux into the PG biosynthetic pathway in response to cell wall stress (Blake *et al.* 2009). MurB reduces the C3 enolate to the lactate, resulting in formation of UDP-MurNAc (Benson *et al.* 1993). The pentapeptide chain is then coupled in a stepwise manner, with MurC, MurD, MurE adding L-alanine, D-glutamic acid and L-lysine (or *m*-DAP), respectively. Using D-Ala produced from L-Ala by D-alanine racemase (Alr), D-Ala-D-Ala ligase (Ddl) makes the dipeptide, which is then added to the UDP-MurNAc-tripeptide by MurF. Since peptide bond formation is thermodynamically unfavorable, the ligases use ATP to activate the amino acids and provide a driving force for coupling (Bouhss *et al.* 1997; Patin *et al.* 2010; Walsh 1989).

The next stage of PG synthesis begins with the transfer of phospho-MurNAc pentapeptide to a lipid carrier in the bacterial membrane, typically Und-P, although *Mycobacterium smegmatis* uses decaprenylphosphate (Mahapatra *et al.* 2005). This step is catalyzed by MraY (Bouhss *et al.* 2004; Chung *et al.* 2013; Pless and Neuhaus 1973) and produces the first lipid-linked intermediate, Lipid I. Finally, MurG catalyzes the addition of GlcNAc to give Lipid II (Hu *et al.* 2003a; Mengin-Lecreulx *et al.* 1991). Amidation of the α-carboxylate of *iso*-glutamic acid at position 2 of the peptide chain, which is observed in many organisms (Vollmer *et al.* 2008), most likely occurs intracellularly after lipid-linked PG precursors are formed. The enzymes involved in this modification were recently identified in *S. aureus* as MurT and GatD (Figueiredo *et al.* 2012, Münch *et al.* 2012).

When a peptide branch is present, the required amino acids are usually added to the completed Lipid II moiety. One exception is *Lactobacillus viridescens* where the first amino acid of the L-Ala-L-Ser bridge is added to the UDP-*N*-acetylmuramylpentapeptide (Rogers *et al.* 1980). In *S. aureus*, the pentaglycine is assembled by FemX, FemA and FemB, which sequentially add one, two and two glycines, respectively. These enzymes utilize glycyl-tRNA donors (Henze *et al.* 1993; Maidhof *et al.* 1991; Rohrer *et al.* 1999; Schneider *et al.* 2004). Serines rather than glycines are incorporated in a similar manner in other staphylococcal strains (Thumm and Götz 1997; Tschierske *et al.* 1997). This incorporation of serine contributes to resistance to lysostaphin, a glycylglycine endopeptidase (Thumm and Götz 1997). The corresponding enzymes in *E. faecalis* and *S. pneumoniae* have also been identified (Bouhss *et al.* 2002; Filipe *et al.* 2000). It is interesting that the Mur ligases use ATP-activated amino acids directly, but the enzymes that assemble the branching peptides use charged tRNAs. When tRNAs were found to be the aminoacyl donors for PG precursors in the 1960s, it caused some excitement because tRNAs were previously known only for their involvement in protein synthesis (Kresge *et al.* 2007). It is now known that phospholipids as well as PG precursors are aminoacylated by acyl-tRNAs (see above).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The final step in the cytoplasmic phase of PG synthesis involves the translocation of Lipid II across the membrane. This is accomplished by a flippase called MurJ, which was identified only recently (Ruiz 2008; Ruiz 2009; Sham *et al.* 2014). In *B. subtilis*, there is also a secondary Lipid II flippase, Amj, that enables survival when MurJ (YtgP) is deleted (Meeske *et al.* 2015). The complete story of the discovery of the Lipid II flippase has been well-described in the chapter by Lam and coworkers in this volume.

**Glycan polymerization and cross-linking—**Once Lipid II is on the outside of the cell, it is polymerized and crosslinked. Glycan polymerization is accomplished by peptidoglycan glycosyltransferases (PGTs; also known as synthetic transglycosylases), while crosslinking is accomplished by transpeptidases. These activities are often found as domains in a single protein, but monofunctional variants of both enzyme classes exist. The nomenclature of PG biosynthetic enzymes is somewhat confusing as many are designated as penicillin-binding proteins, which highlights the fact that they covalently bind β-lactams (Blumberg and Strominger 1974), but obscures their catalytic function, which vary. There are two main categories of PBPs - high molecular mass PBPs that contain a second domain and low-molecular mass PBPs. The high molecular mass PBPs are further divided into Class A and Class B PBPs, with the Class A PBPs distinguished by the presence of an N-terminal PGT domain and the Class B PBPs distinguished by the presence of an N-terminal domain of unknown function. The penicillin-binding domains found in both Class A and Class B PBPs function as transpeptidase domains, serving to crosslink glycan strands. The low molecular mass PBPs, sometimes called Class C PBPs, typically function as D,D-carboxypeptidases, serving to hydrolyze the terminal D-alanine of the stem peptide (Ghuysen 1991; Sauvage *et al.* 2008; Waxman and Strominger 1983). Some organisms including *S. aureus* contain low molecular mass PBPs that function as transpeptidases, rather than carboxypeptidases. Methicillin-sensitive *S. aureus* (MSSA) strains contain four PBPs. PBP1 and PBP3 are Class B PBPs (Pinho *et al.* 2000; Wada and Watanabe 1998), PBP2 is a Class A PBP (Pinho *et al.* 2001a), and PBP4 is a low molecular weight PBP that acts as a transpeptidase to form additional crosslinks in PG (Kozarich and Strominger 1978; Qiao *et al.* 2014; Wyke *et al.* 1981). MRSA strains contain an additional PBP, PBP2A, that is highly resistant to β-lactams. PBP2A serves to crosslink PG when the other PBPs have been inactivated by β-lactams (Hartman and Tomasz 1984; Lim and Strynadka 2002). In addition to these enzymes, *S. aureus* also contains two monofunctional transglycosylases, SgtA and MGT (Heaslet *et al.* 2009; Reed *et al.* 2011; Terrak and Nguyen-Distèche 2006). Under optimal laboratory growth conditions, only PBP1 and PBP2 are essential for viability (Pinho *et al.* 2001b; Reed *et al.* 2015; Wada and Watanabe 1998). It is typical for bacteria to contain multiple PBPs and PGTs, with some essential and others important for survival under stressful conditions. In part, this redundancy reflects the central importance of PG for viability. Rod-shaped organisms such as *B. subtilis* typically have more PBPs than cocci such as *S. aureus* (Zapun *et al.* 2008). In *B. subtilis*, PG synthesis occurs both at the septum during cell division and along the cylindrical walls during cell elongation, and there is considerable evidence that different biosynthetic machines are involved in these different modes of PG synthesis (Claessen *et al.* 2008; Daniel *et al.* 2000; Spratt 1975; Zapun *et al.* 2008). Deconvoluting the cellular functions of PBPs and other cell wall biosynthetic

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

enzymes has been a major challenge due to redundancy and possible interdependency (Reed *et al.* 2015; Scheffers and Pinho 2005).

**Recycling of carrier lipid—**The Und-P carrier lipid is present in limited amounts in bacterial membranes. In addition to serving as a carrier lipid for PG synthesis, Und-P is a carrier for WTA precursors as well as CPS precursors. To ensure an ongoing supply of all these cell wall precursors, the carrier lipid must be rapidly recycled. Hence, once Lipid II has reacted to form the glycan strands of PG, the undecaprenyl pyrophosphate released is converted to Und-P by UppP and other phosphatases (Bouhss *et al.* 2008; El Ghachi *et al.* 2004; El Ghachi *et al.* 2005), and Und-P is flipped back inside the cell by an unknown mechanism to enable another round of precursor synthesis.

### 3.3 Tailoring modifications of peptidoglycan

Tailoring modifications of PG subunits modulate the properties of the cell envelope and may protect bacteria from antimicrobial peptides and proteins (Fig. 3). There are a number of tailoring modifications found in Gram-positive bacteria. These include *N*-deacetylation, the removal of C2-acetyl groups from GlcNAc and/or MurNAc sugars, and *O*-acetylation of the MurNAc C6 hydroxyl (Davis and Weiser 2011; Moynihan *et al.* 2014).

*N*-deacetylation has been shown to protect bacteria from lysozyme, a host muramidase that can cleave the glycosidic bond between GlcNAc and MurNAc residues (Ohno *et al.* 1982). Some Gram-positive organisms including *S. pneumoniae, Bacillus anthracis, B. subtilis* and other *Bacillus* species are naturally lysozyme resistant and contain a high proportion of *N*-deacetylated sugars in their cell wall (Hayashi *et al.* 1973; Vollmer and Tomasz 2000; Zipperle *et al.* 1984). In *S. pneumoniae*, approximately 80% of the glucosamine residues and 10% of the muramic acid residues are *N*-deacetylated (Vollmer and Tomasz 2000). This is comparable to the 88% and 34%, respectively, observed in *B. anthracis* (Zipperle *et al.* 1984). The enzyme responsible for GlcNAc deacteylation, PgdA, was first identified in *S. pneumoniae* (Vollmer and Tomasz 2000). PdaA, a MurNAc deacetylase (Fukushima *et al.* 2005), as well as a second MurNAc deacetylase, PdaC, which also has chitin deacetylase activity (Kobayashi *et al.* 2012), have been identified in *B. subtilis.* The *pgdA* mutant in *S. pneumoniae* was shown to have attenuated virulence (Vollmer and Tomasz 2002) and the *pdaA* mutant in *B. subtilis* is unable to germinate (Fukushima *et al.* 2002), indicating the possibility of other roles of *N*-deacetylation.

*O*-acetylation of the MurNAc moiety has been observed in several Gram-positive and Gram-negative species in variable amounts. In some strains of *S. aureus*, for example, 60% of MurNAc residues are *O*-acetylated (Clarke and Dupont 1992). *O*-acetylation has been shown to be important for lysozyme resistance and the gene responsible was identified as *oatA* in *S. aureus* (Bera *et al.* 2005). Homologs of OatA have also been identified in other Gram-positive organisms, including *S. pneumoniae* (Crisóstomo *et al.* 2006) and *E. faecalis* (Hebert *et al.* 2007). Interestingly, while most Gram-positive organisms use OatA homologs for *O*-acetylation, Gram-negative organisms use proteins of a different family called Pat. *B. anthracis* produces both kinds of acetyltransferases, and the Pat transferases have been implicated in acetylation of secondary cell wall polysaccharide (Laaberki *et al.* 2011;

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Lunderberg *et al.* 2013). In addition to resistance to lysozyme, *O*-acetylation has been shown to play a role in β-lactam resistance in *S. pneumoniae* and *Listeria monocytogenes* (Aubry *et al.* 2011; Crisóstomo *et al.* 2006), and in pathogenesis and immune evasion in *S. aureus* (Bera *et al.* 2006; Shimada *et al.* 2010). *O*-acetylation is critical for infection by *L. monocytogenes* and is reported to decrease cytokine production during early stages of infection of mice (Aubry *et al.* 2011). GlcNAc residues in PG can also be *O*-acetylated but this is more unusual. In *Lactobacillus plantarum*, GlcNAc *O*-acetylation plays a role in inhibiting *L. plantarum*'s major autolysin (Bernard *et al.* 2011).

In addition to these modifications, PG can be modified at the MurNAc C6 position with different glycopolymers including TAs, teichuronic acids and CPS. Proteins are also covalently attached to the pentaglycine branch of stem peptides of PG by sortases (Schneewind and Missiakas 2012). In *S. aureus*, sortase-mediated protein attachment is thought to occur on the outside of the cell before Lipid II is polymerized (Perry *et al.* 2002; Ruzin *et al.* 2002).

## 4 Teichoic Acids

The cell envelopes of Gram-positive bacteria are rich in teichoic acids (TAs). There are two major classes of TAs: lipoteichoic acids (LTAs), which are anchored to a lipid embedded in the cell membrane, and wall teichioic acids (WTAs), which are covalently attached to PG. LTAs are believed to be present in all Gram-positive bacteria with the exception of some *Micrococcus* strains (Powell *et al.* 1975); WTAs are found in many, including *B. subtilis, S. aureus, Staphylococcus epidermidis, S. pneumoniae* and enterococcal species. In organisms where canonical WTAs are not found, other anionic glycopolymers are attached to PG, and may play analogous roles (Neuhaus and Baddiley 2003). Under phosphate-limiting conditions, some *B. subtilis* strains produce teichuronic acids instead of WTAs. Teichuronic acids are described in greater detail in the chapter by Lam and coworkers. It is estimated that WTAs and other polyanionic polymers comprise up to 60% of the cell wall mass (Hancock 1997). Along with LTAs, these polymers play central roles in numerous cellular processes. Some of these functions are covered in detail below.

### 4.1 Wall teichoic acid structure

WTAs typically consist of a disaccharide linkage unit that is connected at the reducing end to PG via a phosphodiester linkage and at the non-reducing end to a main chain polymer. The structure of the main chain can vary considerably across species but always contains phosphodiester linkages that impart anionic charges to the cell wall (Fig 3). In *S. aureus* and *B. subtilis* WTA main chains are composed of glycerol-phosphate or ribitol-phosphate repeats. The WTA main chains are coupled through a disaccharide linkage unit to PG (Armstrong *et al.* 1960; Brown *et al.*, 2013; Kojima *et al.* 1985; Neuhaus and Baddiley 2003).

In *S. pneumoniae*, the main chain repeat is composed of 2-acetamido-4-amino-2,4,6-trideoxygalactose, glucose, ribitol-phosphate and two GalNAc moieties, each decorated with phosphorylcholine. The incorporation of phosphorylcholine in WTAs is extremely rare and appears to be exclusive to *S. pneumoniae* (Denapaite *et al.* 2012; Fischer *et al.* 1993). In *E.*

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

*faecalis* 12030, the repeating unit contains D-glucose, D-galactose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose and ribitol-phosphate (Theilacker *et al.* 2012). In *E. faecium* U0317, the WTA polymer is simpler, consisting of repeating units of two residues of 2-acetamido-2-deoxy-D-galactose and glycerol-phosphate (Bychowska *et al.* 2011).

## 4.2 Wall teichoic acid biosynthesis

The biosynthetic pathways for WTA assembly in *B. subtilis* and *S. aureus* have been well established (Brown *et al.* 2010; Brown *et al.* 2008; Lazarevic *et al.* 2002; Mauël *et al.* 1991), and are covered in the chapter by Lam and coworkers. The assembly begins in a similar manner to PG assembly. Briefly, phosphoGlcNAC is transferred from UDP-GlcNAC to the Und-P lipid carrier and then this "starter unit" is further elaborated by a series of intracellular enzymes to assemble the full polymeric precursor. While the structures of the main chains made in *B. subtilis* and *S. aureus* are similar, particularly in WTAs from *B. subtilis* W23 and *S. aureus*, there are substantial differences in the biosynthetic pathways that were not evident from bioinformatic analysis (Brown *et al*, 2010; Brown *et al.* 2008; Meredith *et al.* 2008; Pereira *et al.* 2008). It is not yet possible to predict the enzymatic functions of putative teichoic acid primases and polymerases accurately. Once the full chain is polymerized inside the cell, it is flipped by a two component ABC transporter to the surface of the bacterial membrane and ligated to the PG. The pathway in *S. pneumoniae* and other species has not been as well elucidated and most of the enzymes, apart from those responsible for choline uptake, have been deduced by bioinformatic analysis and remain to experimentally validated (Denapaite *et al.* 2012).

Unlike PG, WTAs are not essential for survival of *S. aureus in vitro* as the first two genes in the pathway can be deleted. However, the subsequent genes in the pathway were identified as essential (Chaudhuri *et al.* 2009; Kobayashi *et al.* 2003). This apparent paradox was resolved by studies showing that the downstream genes in the WTA pathway can be deleted as long as one of the first two genes has been disrupted (D'Elia *et al.* 2006). This finding implied that the essentiality of the downstream genes was conditional on flux into the pathway, and it was suggested that lethality due to a late block in WTA biosynthesis could arise from accumulation of a toxic metabolite or from sequestration of the Und-P carrier lipid in WTA intermediates, which would lead to inhibition of PG biosynthesis (D'Elia *et al.* 2009). It was recently shown that inhibiting a late step in WTA biosynthesis results in rapid depletion of the PG precursor Lipid II, consistent with lethality arising from inhibition of PG biosynthesis (Qiao *et al.* 2014). Other cell envelope polymers such as CPS are synthesized on the Und-P carrier lipid, and the biosynthetic pathways for some of these also contain a mix of non-essential early genes and conditionally essential late genes (Xayarath and Yother 2007). Conditional essentiality of the late genes depends on whether intermediates can be metabolized through an alternative pathway to release the carrier lipid.

The final step of the WTA pathway involves the ligation of WTAs onto PG. The LytR-CpsA-Psr protein family was recently shown to be involved in this process (Kawai *et al.* 2011; Over *et al.* 2011, Dengler *et al.* 2012). *B. subtilis, S. aureus*, and *S. pneumoniae* strains have three LytR-CpsA-PsR homologs. In the case of *S. aureus*, one of these homologs has been

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

shown to be involved in ligation of CPS to PG (Chan *et al.* 2014). The other two appear to be involved in ligation of WTAs to PG (Chan *et al.* 2013), but their cellular functions have not been clearly delineated. No LytR-CpsA-Psr family member has yet been reconstituted *in vitro*. More details on the discovery of these proteins are provided in the chapter by Lam and coworkers.

### 4.3 Lipoteichoic acid structure

In most organisms, LTAs are synthesized by completely different biosynthetic pathways from WTAs, except in the case of *S. pneumoniae* where the repeating units are structurally identical and are thought to be assembled using the same enzymes (Denapaite *et al.* 2012; Fischer *et al.* 1993). The most common LTA structure comprises a polyglycerol-phosphate chain anchored to a glycolipid in the membrane. This type of LTA is found in *S. aureus, B. subtilis*, and *L. monocytogenes*. In other species of Gram-positive organisms, LTAs contain additional sugar moieties connecting the glycolipid anchor to the polyglycerol-phosphate polymer. The glycolipid anchor is usually diacylglycerol with two glucose moieties ($Glc_2DAG$), as in *S. aureus* and *B. subtilis*, but it can also contain more than two glucose residues (*Clostridium difficile)* as well as other sugar moieties such as galactose (in *L. monocytogenes*) or GlcNAc (in *Clostridium innocuum*) (Fischer 1988; Percy and Gründling 2014).

### 4.4 Lipoteichoic acid synthesis

LTA synthesis begins in the cytoplasm with the assembly of the glycolipid anchor. In *S. aureus* and *B. subtilis*, YpfP (also called UgtP) is responsible for attaching both glucose units to diacylglycerol (DAG) to give the glycolipid anchor, Glc2DAG (Jorasch *et al.* 1998; Kiriukhin *et al.* 2001), which is then flipped across the membrane by LtaA (Gründling and Schneewind 2007a). LtaS then builds the polymer chain by transferring glycerol-phosphate from phosphatidylglycerol to $Glc_2DAG$ (Gründling and Schneewind 2007b). Deleting *ypfP* or *ltaA* does not abolish the synthesis of LTA, but results in polymers with altered structure. Evidently, LTA can be synthesized on DAG, as well as Glc2DAG (Gründling and Schneewind 2007a). LtaS is a polytopic membrane protein with an extracellular domain. The crystal structure of the extracellular domain of LtaS (eLtaS) bound to glycerol-phosphate has been reported and suggests a possible covalent mechanism for LtaS in which an active site threonine reacts with phosphotidylglycerol to form a covalent glycerol-phospho-threonine intermediate. This intermediate is resolved by reaction with the hydroxyl group of the growing LTA chain (Lu *et al.* 2009; Schirner *et al.* 2009). Some organisms such as *L. monocytogenes*, contain a two-enzyme pathway to make LTA main chains (Webb *et al.* 2009). One enzyme, LtaP, functions as a primase to add one unit of glycerol-phosphate to the glycolipid anchor. In the case of *L. monocytogenes*, this glycolipid anchor is Gal-Glc-DAG. A polymerase, LtaS, then extends the chain. LtaP is not essential for LTA synthesis; however LTAs from a *ltaP* null mutant are longer than those from the wild type strain (Webb *et al.* 2009), as in a *ltaA* or *ypfP* deletion in *S. aureus*. The mechanistic basis for length differences between "primed" and "unprimed" glycolipid anchors is not understood. A recent crystal structure of LtaS from *L. monocytoygenes* reveals a glycerol-phosphate binding site that may accommodate part of the growing LTA chain (Campeotto *et al.* 2014). While glycerol-phosphate polymerization activity has not been reconstituted for any LtaS,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

perhaps because some of the transmembrane helices form part of the active site for polymerization, eLtaS from *S. aureus* was shown to be sufficient for cleavage of the phosphodiester bond in phosphatidylglycerol (Karatsa-Dodgson *et al.* 2010). The diacylgylcerol product released in the LtaS reaction with phosphatidylglycerol is recycled back into the cell and the protein responsible for recycling has been identified as diacylglycerol kinase DgkB (Jerga *et al.* 2007).

While *S. aureus* contains only one LtaS and *L. monocytogenes* has LtaP and LtaS, *B. subtilis*, has four LtaS homologs (Gründling and Schneewind 2007b; Schirner *et al.* 2009). It has been reported that while three of these homologs - LtaS, YqgS, and YfnI - have LtaS-like activity, one of them, YvgJ, functions as a primase (Wörmann *et al.* 2011). Unlike in *L. monocytogenes*, the *B. subtilis* primase is not required for normal LTA synthesis, suggesting that the LtaS enzymes are capable of initiating synthesis of LTA polymers efficiently. YfnI has been shown to make LTA polymers that are substantially longer than those produced by LtaS or YqgS (Wörmann *et al.* 2011). The observation that *yfnI* expression is regulated by the alternative sigma factor SigM, which responds to stress conditions (Jervis *et al.* 2007), suggests that certain stresses call for the production of elongated polymers in *B. subtilis* (Wörmann *et al.* 2011). Phenotypically, *ltaS* mutants show increased cell elongation and chain length, reduced cell diameter, cell bending, lysis and abnormally thick septa, whereas single deletions of the other three homologs do not have any obvious defects. The *ltaS-yqgS* double mutant has sporulation defects; all other double mutant combinations with *ltaS* can sporulate. These results implicate LtaS and YqgS in sporulation. The quadruple mutant is viable, although it has a more severe phenotype than the single *ltaS* mutant (Schirner *et al.* 2009). Deletion of *ltaS* in *S. aureus* has also been accomplished, but viable mutants have suppressors that enable growth through a mechanism that involves increased levels of cyclic-di-AMP, which may regulate cell membrane functions (Corrigan *et al* 2011; Corrigan *et al.* 2013). Even with the suppressor, these mutants have severe cell division defects (Corrigan *et al.* 2011; Gründling and Schneewind 2007b; Oku *et al.* 2009). Hence, LTAs are critical even for *in vitro* growth of many Gram-positive organisms.

### 4.5 Tailoring modifications of teichoic acids

Both LTAs and WTAs are often modified with D-alanine esters to modulate the charges of the cell envelope. They can also be modified with sugar moieties. These tailoring modifications have been implicated in numerous functions in cell physiology and infection.

**D-alanylation—**The ribitol (in WTA) or glycerol (as in *S. aureus* LTA) groups in TAs are frequently decorated with D-alanine moieties, which introduce positive charges to neutralize the negatively charged phosphates in the polymer backbone. On ribitol groups, D-alanylation occurs at the C2 position (Neuhaus and Baddiley 2003). D-alanine moieties are added by four proteins, DltABCD, encoded by the *dlt* operon (Fig. 5). DltA activates D-alanine as the AMP ester and then transfers it to the sulfhydryl group on the phosphopantetheinyl arm of the carrier protein DltC (Heaton and Neuhaus 1992, Heaton and Neuhaus 1994; Perego *et al.* 1995; Volkman *et al.* 2001). DltA is similar to carrier protein ligases found in non-ribosomal peptide synthetases (Brown *et al.* 2013; Percy and Gründling 2014; Yonus *et al.* 2008). The next steps are not understood. DltB is a polytopic membrane protein belonging to the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

mBOAT family (for membrane-bound O-acetyl transferases), which is ubiquitous in all kingdoms of life (Hoffman 2000). DltD contains a single membrane spanning helix and an extracellular domain with predicted esterase/thioesterase activity (Brown *et al.* 2013; Reichmann *et al.* 2013). It has been proposed that DltC transfers D-alanine to Und-P to form an acyl-phosphate intermediate, which is then transferred through the membrane by DltB to modify LTAs with the assistance of DltD (Perego *et al.* 1995; Reichmann *et al.* 2013). There is no evidence for the proposed acyl-phosphate intermediate and the reaction to form it from the thioester is thermodynamically unfavorable, although it may conceivably be coupled to hydrolysis of the pyrophosphate released during D-alanine activation by DltA. Pulse-chase experiments have suggested that D-alanines installed on LTAs are subsequently transferred to WTAs (Haas *et al.* 1984; Koch *et al.* 1985), but the mechanistic details of the transfer are unclear. In particular, it is not known whether an enzyme is involved in the process.

**Glycosylation—**The majority of ribitol phosphate groups in WTAs in *S. aureus* are glycosylated with GlcNAc on the ribitol C4 position (Brown *et al.* 2013). Similarly, LTAs can also be glycosylated with GlcNAc or α-galactose in *B. subtilis* (Percy and Gründling 2014). In *S. pneumoniae*, LTA can be glycosylated with GalNAc (Draing *et al.* 2006). In staphylococci, it has been shown that D-alanylation and glycosylation compete for the same position on LTAs. Approximately 70% of the glycerol-phosphates carry D-alanines while 15% carry GlcNAc moieties (Schneewind and Missiakas 2014). WTA precursors are glycosylated intracellularly and the enzymes responsible for glycosylation have been identified in a number of organisms. In *B. subtilis* 168, TagE attaches α-glucosyl units to the polyglycerol-phosphate WTA chains (Allison *et al.* 2011); in *B. subtilis* W23, TarQ attaches β-glucosyl units to the polyribitol-phosphate WTA chains (Brown *et al.* 2012). In *S. aureus*, TarM attaches α-GlcNAc residues while TarS attaches β-GlcNAc residues (Shobanifar *et al.* 2015; Xia *et al.* 2010; Brown *et al.* 2012). No enzymes responsible for LTA glycosylation, which occurs extracellularly, have yet been identified. It is likely that these enzymes use membrane-anchored sugar substrates that cannot diffuse away from the cell, and therefore do not resemble the nucleotide-diphosphate sugar transferases that gylcosylate WTA precursors inside the cell.

### 4.6 Roles of teichoic acids and their tailoring modifications in cell physiology and immune evasion

**Roles in cell division and morphology—**TAs perform several crucial functions for the cell. In *B. subtilis*, WTAs are required to maintain the rod-shaped morphology (Boylan *et al.* 1972; Pollack and Neuhaus 1994; Schirner *et al.* 2015). In the quadruple mutant lacking all four LtaS homologs, there are severe cell division and septation defects that cause filamenting and clumping of cells and the mutant grows very slowly, indicating that LTAs are required for proper cell division (Schirner *et al.* 2009). Disruption of YpfP caused the rod-shaped cells to become bent and distended, and also disrupted the localization of the cytoskeletal protein MreB, important for the rod-shape in *B. subtilis* (Matsuoka *et al.* 2011). Interestingly, YpfP has also been implicated in a metabolic sensing role, localizing to the division site in a nutrient-dependent manner and inhibiting the assembly of FtsZ. It is important that the number of Z-rings to cell length is maintained at a constant ratio so cells do not initiate division before reaching the correct cell mass. Thus, YpfP could play a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

significant role in cell cycle events (Weart *et al.* 2007). In *S. aureus*, both LTAs and WTAs have been implicated in cell division: mutants defective in either LTA or WTA biosynthesis have major septal defects, including placing new septa at angles non-orthogonal to previous septa and forming multiple septa almost simultaneously. These mutants are also impaired in separation after division (Campbell *et al.* 2011; Gründling and Schneewind 2007b; Oku *et al.* 2009). In *S. aureus*, LTAs are more critical to the cell than WTAs *in vitro* as evidenced by the fact that the *ltaS* deletion strain is viable only in the presence of supressors (Corrigan *et al.* 2011), whereas *tarO* mutants grow fairly well. WTAs, however, become very important *in vivo* (Valentino *et al.* 2014; Wang *et al.* 2013; Weidenmaier *et al.* 2005). Simultaneous disruption of WTAs and LTAs is lethal in both *S. aureus* and *B. subtilis* (Oku *et al.* 2009; Santa Maria *et al.* 2014; Schirner *et al.* 2009). In *S. aureus*, cells lacking both polymers are unable to form the essential division ring (Z-ring) (Santa Maria *et al.* 2014). Interestingly, in the absence of WTAs, D-alanyl modifications on LTAs become essential. Both WTAs and D-alanylation have been implicated in autolysin regulation, and when WTAs and D-alanines are both missing, cells lyse rapidly. The evidence suggests that LTAs and WTAs have overlapping but not fully redundant roles in cell division and autolysin regulation (Santa Maria *et al.* 2014).

**Roles in ligand binding and scaffolding—**TAs have been implicated in binding cations, and this correlates inversely with D-alanylation levels (Archibald *et al.* 1973; Neuhaus and Baddiley 2003). Cation homeostasis is thus an important function of TAs that can be regulated through D-alanylation. WTAs also serve as phage receptors in *S. aureus* (Brown *et al.* 2012; Chatterjee 1969; Xia *et al.* 2010; Young 1967). Phage binding is mediated by the GlcNAc modifications added on to WTAs (Brown *et al.* 2012; Xia *et al.* 2010). A requirement for glucose in TAs for phage adsorption has been shown in *B. subtilis* 168 as well (Young 1967, Allison *et al.* 2011). WTAs have also been implicated in other protein scaffolding roles. For instance, in *S. aureus*, FmtA, a protein that plays a role in methicillin-resistance in MRSA strains, was shown to bind to WTAs (Qamar and Golemi-Kotra 2012). In *S. pneumoniae*, several proteins bind specifically to the choline moieties on TAs. These proteins, which include the highly studied virulence protein PspA, have been implicated in numerous functions from adhesion to virulence, and cell wall hydrolysis (Fischer 2000; Giudicelli and Tomasz 1984; Gosink *et al.* 2000; Hakenbeck *et al.* 2009; Rosenow *et al.* 1997). In *L. monocytogenes*, InlB, a protein that promotes entry into mammalian cells is shown to interact with LTAs (Jonquières *et al.* 1999). The domain necessary for interaction with LTAs in this protein contains GW modules (conserved modules of ~80 amino acids which have the dipeptide Gly-Trp). These modules have also been identified in Ami, a *L. monocytogenes* autolysin and the *S. aureus* autolysin Atl (Cabanes *et al.* 2002). Autolysins are hydrolases that degrade PG and thus play an essential role in cell division and separation. In *S. aureus*, WTA plays a role in Atl localization. While Atl is usually localized to the cross-wall, it is mislocalized across the cell surface in WTA deficient strains. Mislocalization of autolysins could be one reason WTA-deficient mutants are prone to autolysis (Schlag *et al.* 2010). It has been suggested that D-alanylation is also involved in autolysin regulation (Peschel *et al.* 2000). Similarly, PBP4 in *S. aureus* is also mislocalized when WTAs are absent (Atilano *et al.* 2010), indicating a role for WTAs in the localization of PG biosynthetic machinery.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

**Roles in antibiotic resistance and virulence—**In MRSA, the lack of WTAs dramatically reduces the organism's resistance to β-lactams, indicating that WTAs play a major role in methicillin-resistance of *S. aureus* (Campbell *et al.* 2011). The influence of WTAs on resistance has been traced specifically to the β-GlcNAc modification on WTAs, which suggests that β-GlcNAcylated WTAs scaffold a factor required for β-lactam resistance (Brown *et al.*, 2012). In *S. aureus*, WTAs also provide resistance to antimicrobial fatty acids on the skin during skin colonization (Kohler *et al.* 2009). D-alanylation plays an important role in modulating resistance to certain antibiotics. It is very important for repelling cationic antimicrobial peptides (CAMPs), a crucial part of host immune response (Collins *et al.* 2002; Kristian *et al.* 2005; Peschel *et al.* 1999). This has been observed in several Gram-positive species including *S. aureus* (Peschel *et al.* 1999)*, S. pneumoniae* (Kovács *et al.* 2006) and *E. faecalis* (Fabretti *et al.* 2006). An increase in D-alanylation is also observed in mutants resistant to daptomycin, an antibiotic used to treat MRSA (Yang *et al.* 2009). Antimicrobial resistance due to D-alanylation has been attributed to its functions in imparting positive charges to the cell surface and its contributions to changes in the biophysical aspects of the cell envelope (Mishra *et al.* 2014; Saar-Dover *et al.* 2012).

TAs in their D-alanylated form play a major role in biofilm formation, adhesion to the surface of cells and medical devices, colonization of host tissue, and virulence, likely due to surface charge effects (Brown *et al.* 2013; Gross *et al.* 2001; Jett *et al.* 1994; Neuhaus and Baddiley 2003; Percy and Gründling 2014). Biofilms, which consist of viable cells held together by an extracellular matrix of DNA and proteins from lysed cells as well as extracellular polysaccharides and other polymers, form on surfaces of medical instruments or in hosts, and enable the organism to evade both natural and synthetic antimicrobials (Hall-Stoodley *et al.* 2004; Sutherland 2001; Abee *et al.* 2011). Thus, adhesion and biofilm formation are key tools in a pathogen's arsenal. The role of TAs in adhesion and effective host colonization has been well established in several Gram-positive organisms (Aly *et al.* 1980; Baur *et al.* 2014; Fabretti *et al.* 2006; Weidenmaier *et al.* 2004). In *S. aureus*, WTA glycosylation has specifically been implicated in adhesion (Winstel *et al.* 2015). For all these reasons, TAs are potent virulence factors and mutants lacking TAs or D-alanylation have highly attenuated virulence (Abachin *et al.* 2002; Collins *et al.* 2002; Fittipaldi *et al.* 2008; Suzuki *et al.* 2011a; Weidenmaier *et al.* 2005; Xu *et al.* 2015). As mentioned above, several choline-binding proteins in *S. pneumoniae* have roles in virulence and mutants made to grow independent of choline have highly attenuated virulence (Kharat and Tomasz 2006).

LTAs contribute to the immune response generated during infection by Gram-positive bacteria (Ginsburg 2002). Although there was some controversy concerning whether the immunomodulation arises from LTAs or from lipoproteins that are often copurified (Hashimoto *et al.* 2006a; Hashimoto *et al.* 2006b), evidence suggests that LTAs likely affect the immune system response on their own as well (Bunk *et al.* 2010; Mohamadzadeh *et al.* 2011; von Aulock *et al.* 2007). LTAs are reported to stimulate the production of cytokines (Bhakdi *et al.* 1991; Draing *et al.* 2008; Ray *et al.* 2013) and those from *S. pneumoniae* and *S. aureus* can activate immune cells via toll-like receptor 2, lipopolysaccharide binding protein and CD14 (Ryu *et al.* 2009; Schröder *et al.* 2003). They also activate the complement system of the immune response (Fiedel and Jackson 1978; Loos *et al.* 1986) and can affect

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

other macrophage parameters, including secretion of tumor necrosis factor α and nitrite (Keller *et al.* 1992). Antibodies have been identified that are directed towards non-D-alanylated LTAs in *E. faecalis* (Theilacker *et al.* 2006). Due to this ability to modify host immunity, efforts are ongoing to develop LTA-conjugated vaccines against gram-positive bacteria (Percy and Gründling 2014). The choline-binding proteins anchored to TAs in *S. pneumoniae* could be used as vaccine candidates as well (Jedrzejas 2001; Rosenow *et al.* 1997).

## 5 Capsular Polysaccharides

Capsular polysaccharides (CPS) are highly variable glycopolymers that are anchored to PG (Chan *et al.* 2014; Sorensen *et al.* 1990; Xayarath and Yother 2007; Yother 2011). They extend above the cell wall and have been implicated in phage resistance and immune evasion (O'Riordan and Lee 2004; Roberts 1996). Although not present in all Gram-positive organisms, encapsulation is observed in most highly-pathogenic strains. The synthesis of CPS is covered in the chapter by Lam and coworkers. Since CPS is best studied in *S. pneumoniae*, we will focus on the structural diversity in CPS in *S. pneumoniae* and their function in immune evasion.

### 5.1 Structural diversity of CPS

A phenomenal 93 different serotypes of pneumococcal capsule have been identified over the years and most of the serotypes can cause infection (Kalin 1998; Yother 2011). Recombinational exchanges at the CPS biosynthetic locus can result in a large amount of variation in capsular type (Coffey *et al.* 1998). Disruption and sequence changes in the genes of the CPS cluster occurring naturally can change the CPS serotype from one to another (Calix *et al.* 2014; Calix and Nahm 2010; van Selm *et al.* 2003) contributing to the diversity of pneumococcal capsules. These differences are usually observed in the gene responsible for modifying sugar moieties in CPS with *O*-acetyl groups. In fact, *in vivo* switching from one capsule type to another has been observed (Venkateswaran *et al.* 1983). This switch has been attributed to a change in the number of short tandem TA nucleotide repeats in the putative *O*-acetyltransferase gene, which could explain reversible switching between serotypes that might occur *in vivo* (van Selm *et al.* 2003).

CPS is made of long chains of repeating oligomeric units and the repeating units vary between serotypes. As an example, the repeat unit of *S. pneumoniae* serotype 2 is made of a backbone with glucose-rhamnose-rhamnose-rhamnose unit and a glucose-glucuronic acid side chain (Kenne *et al.* 1975). Recently, serotypes of *S. pneunomiae* that have CPS containing two different repeat units have been described (Oliver *et al.* 2013a; Oliver *el al.* 2013b). There are multiple different serotypes in *S. aureus* as well. Out of the 11 serotypes described for *S. aureus*, serotpyes 5 and 8 are responsible for the majority of human infections (O'Riordan and Lee 2004).

### 5.2 CPS, host immunity and vaccine development

It has long been known that CPS reduces the ability of bacteriophage to interact with the cell surface (Wilkinson and Holmes 1979). CPS plays a major role in virulence of bacterial

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

pathogens and capsule mutants are avirulent. Capsule has been shown to facilitate abscess formation by activating T-cells in the host immune system (Tzianabos *et al.* 2001). The complement system is important in immune response activation and clearing an infection. Capsule is able to mask the binding of opsonic C3 fragments to the complement receptor, thus decreasing opsonization and phagocytosis by leukocytes (Cunnion *et al.* 2003; Peterson *et al.* 1978). This has also been demonstrated in *E. faecalis*, where capsule masks C3 deposits and LTAs from detection by the host immune system, thereby decreasing tumor necrosis factor α production (Thurlow *et al.* 2009). In Group B *Streptococcus*, the terminal sialic acid groups on capsules have been shown to interact with Siglecs on human leukocytes. They are suggested to mimic the human cell surface glycans, reducing the activation of innate immune response (Carlin *et al.* 2007; Carlin *et al.* 2009).

Due to the high immunomodulatory ability of CPS, it has been explored for vaccine development. It has been known for a long time that immunization with polyvalent pneumococcal polysaccharide is effective as a vaccine (MacLeod *et al.* 1945; Shapiro *et al.* 1991). It was later shown that conjugating the polysaccharides to a carrier protein resulted in a more effective vaccine (De Velasco *et al.* 1995). Today different variations on pneumococcal vaccines are available, incorporating up to 23 polysaccharide variants (PPSV23), or conjugate vaccines incorporating 7 (PCV7) or 13 (PCV13) CPS serotypes (Bogaert *et al.* 2004; Pilishvili and Bennett 2015; Steens *et al.* 2014). PCV13 is used for immunization of infants <2 years of age and has recently also been approved for immunizing adults 50 years or older in series with PPSV23. PPSV23, however, is not effective in infant immunization. This is because PPSV23 generates immune responses that are T-cell independent and therefore, poorly supported by the immature immune systems of children <2 years. In contrast, PCV13 generates immune responses that are mediated by T-cell dependent mechanisms effective in infants (Pilishvili and Bennett 2015). Efforts are being made in improving not only the polysaccharide composition of vaccines but also the carrier protein used to conjugate the polysaccharide. The immunogenic properties of the carrier protein could alter the immune response to the vaccine (Dagan *et al.* 2010; Pobre *et al.* 2014). There is a concern that pneumococcal conjugate vaccines select for non-vaccine serotypes. Pelton *et al.* reported that immunization with PCV7 during 2000–2003 reduced vaccine serotypes from 22% to 2% but increased the incidence of non-vaccine serotypes from 7% to 16% (Pelton *et al.* 2004). With over 90 different serotypes of *S. pneumoniae*, this is an important concern, and studies are ongoing to resolve this issue (Jefferies *et al.* 2011; Nurhonen *et al.* 2014).

Capsular conjugate vaccines against serotypes 5 and 8 of *S. aureus* have also been explored (Creech *et al.* 2009; Fattom *et al.* 2004; Robbins *et al.* 2004). However, these vaccines have so far not passed clinical trials (Bagnoli *et al.* 2012; Cook *et al.* 2009), and evidence has emerged that this reduced efficacy could be due to interference from natural non-opsonic antibodies to PNAG, the *S. aureus* exopolysaccharide, present in human serum (Skurnik *et al.* 2012).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## 6 Exopolysaccharides and biofilm formation

Apart from these major cell envelope structures, other glycopolymers called exopolysachharides are secreted by cells as well. These exopolysaccharides are long chains that associate with each other to form the biofilm matrix (Sutherland 2001; Otto 2008; Vlamakis *et al.* 2013). Polysaccharide intercellular adhesin (PIA) in *S. epidermidis* is a well-studied component of biofilms (Mack *et al.* 1996; Itoh *et al.* 2005). It is a linear polymer of β-1,6-linked GlcNAc moieties, although some residues can be *N*-deacetylated. PIA/PNAG is suggested to be held to the cell surface by ionic interactions of the positively charged, un-acetylated moieties of the polymer, so *N*-deacetylation is important for surface localization of PIA (Vuong *et al.* 2004). PIA is synthesized by the *icaADBC* operon in *S. epidermidis*, and homologs have been identified in other species including *S. aureus* (Gerke *et al.* 1998; Heilmann *et al.* 1996; Mack *et al.* 1996; Rohde *et al.* 2010). In *S. aureus*, this high molecular mass exopolysaccharide termed PNAG is produced by biofilm forming strains. Due to its role in modulating immune responses, vaccines using conjugated PNAG are also being explored (Maira-Litrán *et al.* 2012). Its role in biofilm formation has created interest in the study of the role of each enzyme in the *icaADBC* operon and how it is regulated (Arciola *et al.* 2015; O'Gara 2007). There are also *ica*-independent methods for biofilm formation which include roles by TAs and cell-surface associated proteins. The mechanism for biofilm formation in MRSA appears to be *ica*-independent; whereas it is *ica*-dependent in the sensitive strains (O'Gara 2007). Biofilm formation is thus a complex and highly regulated system.

## 7 Antibiotics targeting the cell envelope

Due to the crucial importance of the cell envelope to cell survival, many antibiotics that target cell envelope synthesis have been developed over the years (Fig. 3) (Walsh 2003). There are some antibiotics that target the intracellular steps of PG synthesis, including fosfomycin, which inhibits MurA, the first committed step of PG synthesis (Kahan *et al.* 1974). However, the greatest clinical successes have been achieved by those antibiotics that target the extracellular steps of cell wall synthesis. These include the unusual substrate-binding antibiotics, which form complexes with cell wall precursors instead of the enzymes that process them. Binding to these precursors prevents their use and results in inhibition of cell wall synthesis. Vancomycin, a glycopeptide antibiotic used to treat MRSA, belongs to the substrate-binding class of antibiotics. It binds to the D-Ala-D-Ala motif of the stem peptide in Lipid II and nascent PG, thereby interfering with both Lipid II polymerization to form PG strands and with subsequent crosslinking of the strands (Anderson *et al.* 1967; Perkins and Nieto 1974; Perkins 1969; Reynolds 1989). Binding to and sequestering Lipid II has been established as the mechanism of action of some other antibiotics including ramoplanin, a cyclic lipoglycodepsipeptide antibiotic (Lo *et al.* 2000; Hu *et al.* 2003b), nisin and other lantibiotics (Brötz *et al.* 2002, Hsu *et al.* 2004; Oman *et al.* 2011; Patton and van der Donk 2005), and the recently discovered teixobactin (Ling *et al.* 2015). All these compounds recognize the pyrophosphate-sugar moiety of Lipid II. Plectasin, a fungal defensin, also acts by binding to Lipid II (Schneider *et al.* 2010). Human defensins have also been shown to interact with Lipid II (Sass *et al.* 2010; De Leeuw *et al.* 2010). It is interesting that antimicrobial peptides produced by the host as part of the innate immune response use

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



Lipid II binding to counteract bacterial threats. The structural diversity of the compounds that bind Lipid II is truly astonishing and indicates that this cell wall precursor is an exceptional target.

Development of resistance to compounds which bind to essential substrates is particularly slow for several reasons. They typically act on the extracellular surface of the membrane and are not subject to efflux pump-mediated resistance mechanisms. Moreover, because they do not bind to a protein target, a single mutation in the gene encoding the target cannot confer high level resistance (Wright 2011). In the case of vancomycin, intermediate resistance can arise through multiple mutations that modify the envelope, but high level resistance only arises due to modification of the structure of the target substrate (Gardete and Tomasz 2014; Walsh and Howe 2002; Healy *et al.* 2000). The modification, which involves replacing D-Ala-D-Ala with a dipeptide to which vancomycin cannot bind, requires several enzymes as well as a two component sensing system, and the genes encoding these enzymes are encoded on a cassette that is transferred between organisms (Arthur and Courvalin 1993; Palmer *et al.* 2010). Glycopeptide resistance genes originated in a glycopeptide producer as a means of self-immunity, but have now spread widely, particularly in enterococcal strains (Marshall *et al.* 1998). D-Ala-D-Lac, synthesized by the *vanA* cassette, is the most common replacement for D-Ala-D-Ala in vancomycin resistant strains. Vancomycin has a thousand fold lower affinity for D-Ala-D-Lac because a crucial hydrogen bond between the drug and the target can no longer be formed (Arthur and Courvalin 1993; Handwerger *et al.* 1992; Bugg *et al.* 1991). A change from D-Ala-D-Ala to D-Ala-D-Ser in Lipid II can also cause moderate resistance to vancomycin (Depardieu *et al.* 2007; Lebreton *et al.* 2011). Although high level vancomycin resistance is common in enterococci (VRE), it has not yet emerged as a major problem in *S. aureus*, likely due to reduced frequency of transfer of the resistance cassette between enterococci and staphylococci (Palmer *et al.* 2010; Périchon and Courvalin 2009). The several cases where vancomycin-resistant S. aureus (VRSA) have been identified have involved co-infection with VRE (Weigel *et al.* 2003; Zhu *et al.* 2008; Sievert *et al.* 2008; Chang *et al.* 2003; Whitener *et al.* 2004). The barriers that prevent facile transfer of *vanA* resistance into *S. aureus* are not well understood, and there is concern that these barriers may be overcome with continued evolution. While there is interest in substrate binders as a class, none of the ones that recognize the sugar pyrophosphate portion of Lipid II have been developed for clinical use, although ramoplanin is in clinical trials (Paknikar and Narayana 2012). As with vancomycin, high level resistance to ramoplanin does not develop spontaneously. Moderate ramoplanin resistance develops after multiple passaging and involves cell envelope modifications that may impede access to the Lipid II target on the cell surface (Schimdt *et al.* 2010). If any Lipid II binders come to be used clinically, resistance genes from the producing organisms may eventually find their way into relevant pathogens, like in the case of vancomycin.

β-lactams, a remarkably successful class of antibiotics, are also among the extracellular PG synthesis inhibitors. β-lactams are proposed structural mimics of D-Ala-D-Ala and inhibit the transpeptidase activity of PBPs by acylating the active site, preventing the cross-linking of stem peptides (Yocum *et al.* 1980; Yocum *et al.* 1979). Widespread resistance to β-lactams first emerged in the form of β-lactamases, which degrade β-lactams (Gutkind *et al.* 2013). Combination antibiotics of β-lactams with β-lactamase inhibitors are used to treat many β-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

lactam resistant infections. One example is Augmentin, a combination of amoxicillin and clavulanic acid (Drawz *et al.* 2014; Reading and Cole 1977, White *et al.* 2004). While β-lactamases continue to be a major concern in Gram-negative organisms such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Hong *et al.* 2015; Pitout *et al.* 2015), some Gram-positive organisms have acquired a different mechanism of resistance. Methicillin-resistant *S. aureus* (MRSA) expresses a penicillin-binding protein (PBP2A) that has reduced affinity for β-lactams (Hartman and Tomasz 1984; Lim and Strynadka 2002; Fuda *et al.* 2004). When native PBPs are inhibited by β-lactams, PBP2A can continue to crosslink PG. Due to the growing concern about the spread of MRSA, a significant amount of time has been invested in designing next generation β-lactams that can target the resistant PBP, including ceftobiprole (Davies *et al.* 2007) and ceftaroline (Moisan *et al.* 2010). In addition, other classes of antibiotics have been developed to treat MRSA, including daptomycin, tedizolid, linezolid, and the glycopeptide analog oritavancin (Hall and Michaels 2015; Holmes and Howden 2014; Leach *et al.* 2011; McDaneld *et al.* 2013; Mitra *et al.* 2015).

## 8. The quest for novel antibiotic targets

Resistance to antibiotics of all classes is a serious concern for the future of human health, and efforts should be made to identify novel pathways that can be targeted by new antibiotics or whose inhibition can potentiate the effects of existing antibiotics in resistant strains. Efforts are ongoing to identify and target the multiple other steps involved in the PG biosynthetic pathway. For instance, inhibitors of the Lipid II flippase in *S. aureus*, DMPI and CDFI, have been identified (Huber *et al.* 2009). Targeting pathways that contribute to resistance to current antibiotics is also being explored as a viable option. Apart from the β-lactamases described above, the potential for targeting such auxiliary proteins and pathways is immense, particularly in the case of MRSA, where many cellular factors contribute to β-lactam resistance (Berger-bächi and Rohrer 2002). For instance, changes to the stem peptide and interpeptide bridge re-sensitize MRSA to β-lactams (Ludovice *et al.* 1998; De Jonge *et al.* 1993; Maidhof *et al.* 1991; Tschierske *et al.* 1997). This has also been observed in *S. pneumoniae* (Weber *et al.* 2000). In *S. aureus*, inactivation of one of the PBPs involved in cross-linking of stem peptides, PBP4, is shown to play a role in resistance to β-lactams (Memmi *et al.* 2008). This has also been shown for the inhibition of PG amidation (Figueirdo *et al.* 2012). Inactivation of *tarO*, encoding the first step in WTA biosynthesis, also sensitizes MRSA to β-lactams (Campbell *et al.* 2011). Finally, factors affecting methicillin-resistance also include proteins of hitherto unknown functions. FmtA is an example of one such protein factor (Komatsuzawa *et al.* 1997). Further understanding of the roles and identification of compounds that target these auxiliary factors could be useful in designing effective combination therapies with β-lactams to treat MRSA.

Since TAs and their modifications perform such important functions in cell survival, virulence, and β-lactam resistance, they are being investigated for their potential in combination therapies and as anti-virulence targets (Fig. 5). Tunicamycin, a well-known natural product inhibitor of the first step for WTA synthesis (Hancock *et al.* 1976), has been shown to restore β-lactam susceptibility in MRSA (Campbell *et al.* 2011). Although tunicamycin is toxic to eukaryotes, potent, non-toxic TarO inhibitors could have great potential (Farha *et al.* 2014). In addition, the conditionally essential nature of the WTA

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

pathway has been exploited in a pathway-specific screen to identify downstream inhibitors with antibiotic activity (Swoboda *et al.* 2009). Targocil and several other downstream inhibitors of the ABC transporter (TarGH) that exports WTA polymers have been reported (Lee *et al.* 2010; Campbell *et al.* 2012; Suzuki *et al.* 2011b; Wang *et al.* 2013). An inhibitor of LTA polymerization (compound 1771,[2-oxo-2-(5-phenyl-1,3,4-oxodiazol-2ylamino-ethyl-2-naphtho[2,1-b]furan-1-ylacetate]) was also described recently (Richter *et al.* 2013). Finally, due to its numerous roles in adhesion, virulence, and biofilm formation, the D-alanylation pathway is a potential candidate for anti-virulence therapy. A compound that inhibits the first enzyme in the pathway has been reported (May *et al.* 2005), but has not been shown to inhibit D-alanylation in cells. Agents that inhibit biofilm formation and adhesion mediated by other factors are being actively investigated as well (Chen *et al.* 2013). Inhibitors of TAs and their modifications are yet to make it to the clinic (Silver 2013), although late stage WTA inhibitors have shown some efficacy in combination with MRSA in animal models (Wang *et al.* 2013).

## Acknowledgments

Suzanne Walker is supported by funds from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01AI099144 and P01AI083214.

## Abbreviations

| | |
|---|---|
| **PG** | Peptidoglycan |
| **MRSA** | Methcillin-resistant *Staphylococcus aureus* |
| **GlcNAc** | *N*-acetylglucosamine |
| **GalNAc** | *N*-acetylgalactosamine |
| **MurNAc** | *N*-acetlymuramic acid |
| **PBP** | Pencillin-binding protein |
| **PGT** | Peptidoglycan glycosyltransferase |
| **Und-P** | Undecaprenyl phosphate |
| **TA** | Teichoic acid |
| **WTA** | Wall teichoic acid |
| **LTA** | Lipoteichoic acid |
| **CPS** | Capsular polysaccharides |
| **PIA** | Polysaccharide intercellular adhesin |

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## References


Abachin E, Poyart C, Pellegrini E, Milohanic E, Fiedler F, Berche P, Trieu-Cuot P. Formation of D-alanyl-lipoteichoic acid is required for adhesion and virulene of *Listeria monocytogenes*. Mol Microbiol. 2002; 43:1–14. [PubMed: 11849532]

Abee T, Kovács AT, Kuipers, van der Veen A. Biofilm formation and dispersal in Gram-positive bacteria. Curr Opin Biotechnol. 2011; 22:172–179. [PubMed: 21109420]

Allison SE, D'Elia MA, Arar S, Monteiro MA, Brown ED. Studies of the genetics, function, and kinetic mechanism of TagE, the wall teichoic acid glycosyltransferase in *Bacillus subtilis* 168. J Biol Chem. 2011; 286(27):23708–23716. http://doi.org/10.1074/jbc.M111.241265. [PubMed: 21558268]

Aly R, Shinefield HR, Litz C, Maibach HI. Role of teichoic acid in the binding of *Staphylococcus aureus* to nasal epithelial cells. J Infect Dis. 1980; 141(4):463–465. [PubMed: 7373081]

Anderson JS, Matsuhashi M, Haskin MA, Strominger JL. Biosynthesis of the peptidoglycan of bacterial cell walls. II. Phospholipid carriers in the reaction sequence. J Biol Chem. 1967; 242(13): 3180–3190. [PubMed: 6027793]

Archibald AR, Baddiley J, Heptinstall S. The alanine ester content and magneisum binding capacity of walls of *Staphylococcus aurues* H grown at different pH values. Biochim Biophys Acta. 1973; 291(3):629–634. [PubMed: 4696410]

Arciola CR, Campoccia D, Ravaioli S, Montanaro L. Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. Front Cell Infect Microbiol. 2015; 5(7):1–10. [PubMed: 25674541]

Armstrong JJ, Baddiley J, Buchanan JG. Structure of the ribitol teichoic acid from the walls of *Bacillus subtilis*. Biochem J. 1960; 76(3):610–621. [PubMed: 13684313]

Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob Agents Chemother. 1993; 37(8):1563–1571. [PubMed: 8215264]

Atilano ML, Pereira PM, Yates J, Reed P, Veiga H, Pinho MG, Filipe SR. Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *Staphylococcus aureus*. Proc Natl Acad Sci U S A. 2010; 107(44):18991–18996. [PubMed: 20944066]

Aubry C, Goulard C, Nahori MA, Cayet N, Decalf J, Sachse M, Boneca IG, Cossart P, Dussurget O. OatA, a peptidoglycan *O*-acetyltransferase involved in *Listeria monocytognes* immune escape, is critical for virulence. J Infect Dis. 2011; 204(5):731–740. [PubMed: 21844299]

Bagnoli F, Bertholet S, Grandi G. Inferring reasons for the failure of *Staphylococcus aureus* vaccines in clinical trials. Front Cell Infect Microbiol. 2012; 2(16):1–4. [PubMed: 22919593]

Baur S, Rautenberg M, Faulstich M, Grau T, Severin Y, Unger C, Hoffmann WH, Rudel T, Autenrieth IB, Weidenmaier C. A nasal epithelial receptor for *Staphylococcus aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization. PLoS Pathog. 2014; 10(5):e1004089. [PubMed: 24788600]

Bayer AS, Schneider T, Sahl HG. Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. Ann N Y Acad Sci. 2013; 1277:139–158. [PubMed: 23215859]

Benson TE, Marquardt JL, Marquardt AC, Etzkorn FA, Walsh CT. Overexpresson, purification, and mechanistic study of UDP-*N*-acetylenolpyruvylglucosamine reductase. Biochemistry. 1993; 32(8): 2024–2030. [PubMed: 8448160]

Bera A, Biswas R, Herbert S, Götz F. The presence of peptidogylcan *O*-acetyltransferase in various staphylococcal species correlates with lysozyme resistance and pathogenicity. Infect Immun. 2006; 74(8):4598–4604. [PubMed: 16861647]

Bera A, Herbert S, Jakob A, Vollmer W, Götz F. Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan *O*-acetyltransferase OatA is the major determinant for lysozyme resistance of *Staphylococcus aureus*. Mol Microbiol. 2005; 55(3):778–787. [PubMed: 15661003]

Berger-Bächi B, Rohrer S. Factors influencing methicillin resistance in staphylococci. Arch Microbiol. 2002; 178(3):165–171. [PubMed: 12189417]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


Bernard E, Rolain T, Courtin P, Guillot A, Langella P, Hols P, Chapot-Chartier M. Characterization of *O*-acetylation of *N*-acetylglucosamine: a novel structural variation of bacterial peptidoglycan. J Biol Chem. 2011; 286(27):23950–23958. [PubMed: 21586574]

Bhakdi S, Klonisch T, Nuber P, Fischer W. Stimulation of monokine production by lipoteichoic acids. Infect Immun. 1991; 59(12):4614–4620. [PubMed: 1937822]

Blake KL, O'Neill AJ, Mengin-Lecreulx D, Henderson PJ, Bostock JM, Dunsmore CJ, Simmons KJ, Fishwick CW, Leeds JA, Chopra I. The nature of *Staphylococcus aureus* MurA and MurZ and approaches for detection of peptidoglycan biosynthesis inhibitors. Mol Microbiol. 2009; 72(2): 335–343. [PubMed: 19298367]

Blumberg PM, Strominger JL. Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. Bacteriol Rev. 1974; 38(3):291–335. [PubMed: 4608953]

Bogaert D, Hermans PW, Adrian PV, Rümke HC, de Groot R. Pneumococcal vaccines: an update on current strategies. Vaccine. 2004; 22(17–18):2209–2220. [PubMed: 15149779]

Boneca IG, Huang ZH, Gage DA, Tomasz A. Characterization of *Staphylococcus aureus* cell wall glycan strands, evidence for a new β-*N*-acetylglucosaminidase activity. J Biol Chem. 2000; 275(14):9910–9918. [PubMed: 10744664]

Bouhss A, Crouvoisier M, Blanot D, Mengin-Lecreulx D. Purification and characteriztaion of the bacterial MraY translocase catalyzing the first membrane step of peptidoglycan synthesis. J Biol Chem. 2004; 279:29974–29980. [PubMed: 15131133]

Bouhss A, Josseaume N, Severin A, Tabei K, Hugonnet JE, Shlaes D, Mengin-Lecreulx D, van Heijenoort J, Arthur M. Synthesis of the L-alanyl-L-alanyl cross-bridge of *Enterococcus faecalis* peptidoglycan. J Biol Chem. 2002; 277(48):45935–45941. [PubMed: 12324463]

Bouhss A, Mengin-Lecreulx D, Blanot D, van Heijenoort J, Parquet C. Invariant amino acids in the Mur peptide synthetases of bacterial peptidoglycan synthesis and their modification by site-directed mutagenesis in the UDP-MurNAc:L-alanine ligase from *Escherichia coli*. Biochemistry. 1997; 36(39):11556–11563. [PubMed: 9305945]

Bouhss A, Trunkfield AE, Bugg TD, Mengin-Lecreulx D. The biosynthesis of peptidoglycan lipid-linked intermediates. FEMS Microbiol Rev. 2008; 32(2):208–233. [PubMed: 18081839]

Boylan RJ, Mendelson NH, Brooks D, Young FE. Regulation of the bacterial cell wall: analysis of a mutant of *Bacillus subtilis* defective in biosynthesis of teichoic acid. J Bacteriol. 1972; 110(1): 281–290. [PubMed: 4622900]

Brötz H, Josten M, Wiedemann I, Schneider U, Götz F, Bierbaum G, Sahl HB. Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin nd other lantibiotics. Mol Microbiol. 2002; 30(2):317–327. [PubMed: 9791177]

Brown S, Meredith T, Swoboda J, Walker S. *Staphylococcus aureus* and *Bacillus subtilis* W23 make polyribitol wall teichoic acids using different enzymatic pathways. Chem Biol. 2010; 17(10): 1101–1110. [PubMed: 21035733]

Brown S, Santa Maria JP Jr, Walker S. Wall teichoic acids of Gram-positive bacteria. Annu Rev Microbiol. 2013; 67:313–336. [PubMed: 24024634]

Brown S, Xia G, Luhachack LG, Campbell J, Meredith TC, Chen C, Winstel V, Gekeler C, Irazoqui JE, Peschel A, Walker S. Methicillin resistance in *Staphylococcus aureus* requires glycosylated wall teichoic acids. Proc Natl Acad SciU S A. 2012; 109(46):18909–18914.

Brown S, Zhang YH, Walker S. A revised pathway proposed for *Staphylococcus aureus* wall teichoic acid biosynthesis based on *in vitro* reconstitution of the intracellular steps. Chem Biol. 2008; 346(17):2816–2819.

Bugg TD, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Ente* rococcus faecium BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. Biochem. 1991; 30:10408–10415. [PubMed: 1931965]

Buist G, Steen A, Kok J, Kuipers OP. LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol Microbiol. 2008; 68(4):838–847. [PubMed: 18430080]

Bunk S, Sigel S, Metzdorf D, Sharif O, Triantafilou K, Triantafilou M, Hartung T, Knapp S, von Aulock S. Internalization and coreceptor expression are critical for TLR2-mediated recognition of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

lipoteichoic acid in human peripheral blood. J Immunol. 2010; 185(6):3708–3717. [PubMed: 20713893]

Bychowska A, Theilacker C, Czerwicka M, Marszewska K, Huebner J, Holst O, Stepnowski P, Kaczy ski Z. Chemical structure of wall teichoic acid isolated from *Enterococcus faecium* strain U0317. Carbohydr Res. 2011; 346(17):2816–2819. [PubMed: 22024569]

Cabanes D, Dehoux P, Dussurget O, Frangeul L, Cossart P. Surface proteins and the pathogenic potential of *Listeria monocytogenes*. Trends Microbiol. 2002; 10(5):238–245. [PubMed: 11973158]

Calix JJ, Brady AM, Du VY, Saad JS, Nahm MH. Spectrum of pneumococcal serotype 11A variants results from incomplete loss of capsule *O*-acetylation. J Clin Microbiol. 2014; 52(3):758–765. [PubMed: 24352997]

Calix JJ, Nahm MH. A new pneumococcal serotype, 11E, has variably inactivated *wcjE* gene. J Infect Dis. 2010; 202(1):29–38. [PubMed: 20507232]

Campbell J, Singh AK, Swoboda JG, Gilmore MS, Wilkinson BJ, Walker S. An antibiotic that inhibits a late step in wall teichoic acid biosynthesis induces the cell wall stress stimulon in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2012; 56(4):1810–1820. [PubMed: 22290958]

Campbell J, Singh AK, Santa Maria JP, Kim Y, Brown S, Swoboda JG, Mylonakis E, Wilkinson BJ, Walker S. Synthetic lethal compound combinations reveal a fundamental connection between wall teichoic acid and peptidoglycan biosyntheses in *Staphylococcus aureus*. ACS Chem Biol. 2011; 6(1):106–116. [PubMed: 20961110]

Campeotto I, Percy MG, MacDonald JT, Förster A, Freemont PS, Gründling A. Structural and mechanistic insight into the *Listeria monocytogenes* two-enzyme lipoteichoic acid synthesis system. J Biol Chem. 2014; 289(41):28054–28069. [PubMed: 25128528]

Carlin AF, Lewis AL, Varki A, Nizet V. Group B Streptococcal capsular sialic acids interact with siglecs (immunoglobulin-like lectins) on human leukocytes. J Bacteriol. 2007; 189(4):1231–1237. [PubMed: 16997964]

Carlin AF, Uchiyama S, Chang YC, Lewis AL, Nizet V, Varki A. Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response. Blood. 2009; 113(14):3333–3336. [PubMed: 19196661]

Chan YG, Frankel MB, Dengler V, Schneewind O, Missiakas D. *Staphylococcus aureus* mutants lacking the LytR-CpsA-Psr family of enzymes release cell wall teichoic acids into the extracellular medium. J Bacteriol. 2013; 195(20):4650–4659. [PubMed: 23935043]

Chan YG, Kim HK, Schneewind O, Missiakas D. The capsular polysaccharide of *Staphyloccocus aureus* is attached to peptidoglycan by the LytR-CpsA-Psr (LCP) family of enzymes. J Biol Chem. 2014; 289(22):15680–15690. [PubMed: 24753256]

Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, et al. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. N Engl J Med. 2003; 348:1342–1347. [PubMed: 12672861]

Chatterjee AN. Use of bacteriophage-resistant mutants to study the nature of the bacteriophage receptor site of *Staphylococcus aureus*. J Bacteriol. 1969; 98(2):519–527. [PubMed: 4239385]

Chaudhuri RR, Allen AG, Owen PJ, Shalom G SK, et al. Comprehensive dentification of essential *Staphylococcus aureus* genes using transpson-mediated differential hybridisation (TMDH). BMC Genomics. 2009; 10(291)

Chen M, Yu Q, Sun H. Novel strategies for the prevention and treatment of biofilm related infections. Int J Mol Sci. 2013; 14(9):18488–18501. [PubMed: 24018891]

Chung BC, Zhao J, Gillespie RA, Kwon DY, Guan Z, Hong J, Zhou P, Lee SY. Crystal structure of MraY, an essential membrane enzyme for bacterial cell wall synthesis. Science. 2013; 341(6149): 1012–1016. [PubMed: 23990562]

Claessen D, Emmins R, Hamoen LW, Daniel RA, Errington J, Edwards DH. Control of the cell elongation-division cycle by shuttling of PBP1 protein in *Bacillus subtilis*. Mol Microbiol. 2008; 68(4):1029–1046. [PubMed: 18363795]

Clarke AJ, Dupont C. *O*-acetylated peptidoglycan: its occurrence, pathobiological significance, and biosynthesis. Can J Microbiol. 1992; 38(2):85–91. [PubMed: 1521192]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Clejan S, Krulwich TA, Mondrus KR, Seto-Young D. Membrane lipid composition of obligatively and facultatively alkalophilic strains of *Bacillus* spp. J Bacteriol. 1986; 168(1):334–340. [PubMed: 3093462]

Coffey TJ, Enright MC, Daniels M, Morona JK, Morona R, Hryniewicz W, Paton JC, Spratt BG. Recombinational exchanges at the capsular polysaccharide biosynthetic locus lead to frequent serotype changes among natural isolates of *Streptococcus pneumoniae*. Mol Microbiol. 1998; 27(1):73–83. [PubMed: 9466257]

Collins LV, Kristian SA, Weidenmaier C, Faigle M, van Kessel KP, van Strijp JA, Götz F, Neumeister B, Peschel A. *Staphylococcus aureus* strains lacking D-alanine modifications of teichoic acids are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J Infect Dis. 2002; 186(2):214–219. [PubMed: 12134257]

Cook J, Hepler R, Pancari G, Kuklin N, Fan H, Wang XM, Cope L, Tan C, Joyce J, Onishi J, Montogomery D, Anderson A, McNeely T. *Staphylcoccus aurues* capsule type 8 antibodies provide inconsistent efficacy in murine models of staphylococcal infection. Hum Vaccin. 2009; 5(4):254–263. [PubMed: 18787395]

Corrigan RM, Abbott JC, Burhenne H, Kaever V, Gründling A. c-di-AMP is a new second messenger in *Staphylococcus aureus* with a role in controlling cell size and envelope stress. PLoS Pathog. 2011; 7(9):e1002217. [PubMed: 21909268]

Corrigan RM, Campeotto I, Jeganathan T, Roelofs KG, Lee VT, Gründling A. Systematic identification of conserved bacterial c-di-AMP receptor proteins. Proc Natl Acad Sci U S A. 2013; 110(22):9084–9089. [PubMed: 23671116]

Creech CB, Johnson BG, Alsentzer AR, Hohenboken M, Edwards KM, Talbot TR 3rd. Vaccination as infection control: a pilot study to determine the impact of *Staphylococcus aureus* vaccination on nasal carriage. Vaccine. 2009; 28(1):256–260. [PubMed: 19799842]

Crisóstomo MI, Vollmer W, Kharat AS, Inhülsen S, Gehre F, Buckenmaier S, Tomasz A. Attenuation of penicillin resistance in a peptidoglycan *O*-acetyltransferase mutant of *Streptococcus pneumoniae*. Mol Microbiol. 2006; 61(6):1497–1509. [PubMed: 16968223]

Cunnion KM, Zhang HM, Frank MM. Availability of complement bound to *Staphylococcus aureus* to interact with membrane complement receptors influences efficiency of phagocytosis. Infect Immun. 2003; 71(2):656–662. [PubMed: 12540542]

D'Elia MA, Pereira MP, Chung YS, Zhao W, Chau A, Kenney TJ, Sulavik MC, Black TA, Brown ED. Lesions in teichoic acid biosynthesis in *Staphylococcus aureus* lead to a lethal gain of function in the otherwise dispensable pathway. J Bacteriol. 2006; 188(12):4183–4189. [PubMed: 16740924]

D'Elia MA, Millar KE, Bhavsar AP, Tomljenovic AM, Hutter B, Schaab C, Moreno-Hagelsieb G, Brown ED. Probing teichoic acid genetics with bioactive molecules reveals new interactions among diverse processes in bacterial cell wall biogenesis. Chem Biol. 2009; 16(5):548–556. [PubMed: 19477419]

Dagan R, Poolman J, Siegrist CA. Glycoconjugate vaccines and immune interference: A review. Vaccine. 2010; 28(34):5513–5523. [PubMed: 20600514]

Daniel RA, Harry EJ, Errington J. Role of penicillin-binding protein PBP 2B in assembly and functioning of the division machinery of *Bacillus subtilis*. Mol Microbiol. 2000; 35(2):299–311. [PubMed: 10652091]

Davies TA, Page MG, Shang W, Andrew T, Kania M, Bush K. Binding of ceftobiprole and comparators to the penicillin-binding proteins of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Antimicrob Agents Chemother. 2007; 51(7):2621–2624. [PubMed: 17470659]

Davis KM, Weiser JN. Modifications to the peptidoglycan backbone help bacteria to establish infection. Infect Immun. 2011; 79(2):562–570. [PubMed: 21041496]

De Jonge BL, Sidow T, Chang Y, Labischinski H, Berger-Bächi B, Gage DA, Tomasz A. Altered muropeptide composition in *Staphylococcus aureus* strains with an inactivated *femA* Locus. J Bacteriol. 1993; 175(9):2779–2782. [PubMed: 8478340]

De Jonge BL, Handwerger S, Gage D. Altered peptidoglycan composition in vancomycin-resistant *Enterococcus faecalis*. Antimicrob Agents Chemother. 1996; 40(4):863–869. [PubMed: 8849241]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


De Leeuw E, Li C, Zeng P, Li C, Diepeveen-de Buin M, Lu WY, Breukink E, Lu W. Functional interaction of human neutrophil peptide-1 with the cell wall precursor Lipid II. FEBS Lett. 2010; 584(8):1543–1548. [PubMed: 20214904]

De Mendoza D. Temperature sensing by membranes. Annu Rev Microbiol. 2014; 68:101–116. [PubMed: 24819366]

De Velasco EA, Merkus D, Anderton S, Verheul AF, Lizzio EF, Van der Zee R, Van Eden W, Hoffman T, Verhoef J, Snippe H. Synthetic peptides representing T-cell epitopes act as carriers in pneumococcal polysacchride conjugate vaccines. Infect Immun. 1995; 63(3):961–968. [PubMed: 7532630]

Denapaite D, Brückner R, Hakenbeck R, Vollmer W. Biosynthesis of teichoic acids in *Streptococcus pneumoniae* and closely related species: lessons from genomes. Microb Drug Resist. 2012; 18(3): 344–358. [PubMed: 22432701]

Dengler V, Meier PS, Heusser R, Kupferschmied P, Fazekas J, Friebe S, Staufer SB, Majcherczyk PA, Moreillon P, Berger-Bächi, McCallum N. Deletion of hypothetical wall teichoic acid ligases in *Staphylococcus aureus* activates the cell wall stress response. FEMS Microbiol Lett. 2012; 333(2): 09–120.

Depardieu F, Podglajen I, Leclercq R, Collatz E, Courvalin P. Modes and modulations of antibiotic resistance gene expression. Clin Microbiol Rev. 2007; 20(1):79–114. [PubMed: 17223624]

Draing C, Pfitzemaier M, Zummo S, Mancuso G, Geyer A, Hartung T, von Aulock S. Comparison of lipoteichoic acid from different serotypes of *Streptococcus pneumoniae*. J Biol Chem. 2006; 281(45):33849–33859. [PubMed: 16943191]

Draing C, Sigel S, Deininger S, Traub S, Munke R, Mayer C, Hareng L, Hartung T, von Aulock S, Hermann C. Cytokine induction by Gram-positive bacteria. Immunobiology. 2008; 213(3–4):285–296. [PubMed: 18406374]

Drawz SM, Papp-Wallace KM, Bonomo RA. New β-lactamase inhibitors: a therapeutic renaissance in an MDR world. Antimicrob Agents Chemother. 2014; 58(4):1835–1846. [PubMed: 24379206]

Du W, Brown JR, Sylvester DR, Huang J, Chalker AF, So CY, Holmes DJ, Payne DJ, Wallis NG. Two active forms of UDP-*N*-acetylglucosamine enolpyruvyl transferase in Gram-positive bacteria. J Bacteriol. 2000; 182(15):4146–4152. [PubMed: 10894720]

El Ghachi M, Bouhss A, Blanot D, Mengin-Lecreulx D. The *bacA* gene of *Escherichia coli* encodes an undecaprenyl pyrophosphate phosphatase activity. J Biol Chem. 2004; 279(29):30106–30113. [PubMed: 15138271]

El Ghachi M, Derbise A, Bouhss A, Mengin-Lecreulx D. Identification of multiple genes encoding membrane proteins with undecaprenyl pyrophosphate phosphatase (UppP) activity in *Escherichia coli*. J Biol Chem. 2005; 280(19):18689–18695. [PubMed: 15778224]

Epand RF, Savage PB, Epand RM. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). Biochim Biophys Acta. 2007; 1768(10):2500–2509. [PubMed: 17599802]

Ernst CM, Peschel A. Broad-spectrum antimicrobial peptide resistance by MprF-mediated aminoacylation and flipping of phospholipids. Mol Microbiol. 2011; 80(2):290–299. [PubMed: 21306448]

Ernst CM, Staubitz P, Mishra NN, Yang SJ, Hornig G, Kalbacher H, Bayer AS, Kraus D, Peschel A. The bacterial defensin resistance protein MprF consists of separable domains for lipid lysinylation and antimicrobial peptide repulsion. PLoS Pathogens. 2009; 5(11):e1000660. [PubMed: 19915718]

Fabretti F, Theilacker C, Baldassarri L, Kaczynski Z, Kropec A, Holst O, Huebner J. Alanine esters of enterococcal lipoteichoic acid play a role in biofilm formation and resistance to antimicrobial peptides. Infect Immun. 2006; 74(7):4164–4171. [PubMed: 16790791]

Falord M, Mäder U, Hiron A, Débarbouillé M, Msadek T. Investigation of the *Staphylococcus aureus* GraSR regulon reveals novel links to virulence, stress response and cell wall signal transduction pathways. PLoS One. 2011; 6(7):e21323. [PubMed: 21765893]

Farha MA, Koteva K, Gale RT, Sewell EW, Wright GD, Brown ED. Designing analogs of ticlopidine, a wall teichoic acid inhibitor, to avoid formation of its oxidative metabolites. Bioorg Med Chem Lett. 2014; 24(3):905–910. [PubMed: 24393581]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Fattom AI, Horwith G, Fuller S, Propst M, Naso R. Development of StaphVAX, a polysaccharide conjugate vaccine against *S. aureus* infection: from lab bench to phase III clinical trials. Vaccine. 2004; 22(7):880–887. [PubMed: 15040941]

Fiedel BA, Jackson RW. Activation of the alternative complement pathway by a streptococcal lipoteichoi acid. Infect Immun. 1978; 22(1):286–287. [PubMed: 365748]

Figueiredo TA, Sobral RG, Ludovice AM, Almeida JM, Bui NK, Vollmer W, de Lancastre H, Tomasz A. Identification of genetic determinants and enzymes involved with the amidation of glutamic acid residues in the peptidoglycan of *Staphylococcus aureus*. PLoS Pathog. 2012; 8(1):e1002508. [PubMed: 22303291]

Filipe SR, Pinho MG, Tomasz A. Characterization of the *murMN* operon involved in the synthesis of branched peptidoglycan peptides in *Streptococcus pneumoniae*. J Biol Chem. 2000; 275(36): 22768–27774.

Fischer W. Physiology of lipoteichoic acids in bacteria. Adv Microb Phsyiol. 1988; 29:233–302.

Fischer W. Phosphocholine of pneumococcal teichoic acids: role in bacterial physiology and pneumococcal infection. Res Microbiol. 2000; 151(6):421–427. [PubMed: 10961454]

Fischer W, Behr T, Hartmann R, Peter-Katalini J, Egge H. Teichoic acids and lipoteichoic acid of Streptococcus pneumoniae possess identical chain structures. Eur J Biochem. 1993; 215(3):851–857. [PubMed: 8354290]

Fittipaldi N, Sekizaki T, Takamatsu D, Harel J, Domínguez-Punaro MdeL, von Aulock SV, Draing C, Marois C, Kobisch M, Gottschalk M. D-alanylation of lipoteichoic acid contributes to the virulence of *Streptococcus suis*. Infect Immun. 2008; 76(8):3587–3594. [PubMed: 18474639]

Friedman L, Alder JD, Silverman JA. Genetic changes that correlate with reduced susceptibility to Daptomycin in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2006; 50(6):2137–2145. [PubMed: 16723576]

Fuda C, Suvorov M, Vakulenko SB, Mobashery S. The basis for resistance to β-lactam antibiotics by penicillin-binding protein 2A of Methicillin-resistant *Staphylococcus aureus*. J Biol Chem. 2004; 279:40802–40806. [PubMed: 15226303]

Fukushima T, Kitajima T, Sekiguchi J. A polysaccharide deacetylase homologue, PdaA, in *Bacillus subtilis* acts as an *N*-acetylmuramic acid deacetylase *in vitro*. J Bacteriol. 2005; 187(4):1287–1292. [PubMed: 15687192]

Fukushima T, Yamamoto H, Atrih A, Foster SJ, Sekiguchi J. A polysaccharide deacetylase gene (*pdaA*) is required for germination and for production of muramic δ-lactam residues in the spore cortex of *Bacillus subtilis*. J Bacteriol. 2002; 184(21):6007–6015. [PubMed: 12374835]

Garcia-Bustos JF, Chait BT, Tomasz A. Structure of the peptide network of pneumococcal peptidoglycan. J Biol Chem. 1987; 262(32):15400–15405. [PubMed: 2890629]

Gardete S, Tomasz A. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. J Clin Invest. 2014; 124(7):2936–2840.

Gerke C, Kraft A, Süssmuth R, Schweitzer O, Götz F. Characterization of the *N*-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. Journal of Biological Chemistry. 1998; 273(29):18586–18593. [PubMed: 9660830]

Ghuysen JM. Serine β-lactamases and penicillin-binding proteins. Annu Rev Microbiol. 1991; 45:37–67. [PubMed: 1741619]

Ginsburg I. Role of lipoteichoic acid in infection and inflammation. Lancet Infect Dis. 2002; 2(3):171–179. [PubMed: 11944187]

Giudicelli S, Tomasz A. Attachment of pneumococcal autolysin to wall teichoic acids, an essential step in enzymatic wall degradation. J Bacteriol. 1984; 158(3):1188–1190. [PubMed: 6144667]

Gosink KK, Mann ER, Guglielmo C, Tuomanen EI, Masure HR. Role of novel choline binding proteins in virulence of *Streptococcus pneumoniae*. Infect Immun. 2000; 68(10):5690–5695. [PubMed: 10992472]

Gross M, Cramton SE, Götz F, Peschel A. Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect Immun. 2001; 69(5):3423–3426. [PubMed: 11292767]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


Gründling A, Schneewind O. Genes required for glycolipid synthesis and lipoteichoic acid anchoring in *Staphylococcus aureus*. J Bacteriol. 2007a; 189(6):2521–2530. [PubMed: 17209021]

Gründling A, Schneewind O. Synthesis of glycerol phosphate lipoteichoic acid in *Staphylococcus aureus*. Proc Natl Acad Sci U S A. 2007b; 104(20):8478–8483. [PubMed: 17483484]

Gutkind GO, Di Conza J, Power P, Radice M. β-lactamase-mediated resistance: a biochemical, epidemiological and genetic overview. Curr Pharm Des. 2013; 19(2):164–208. [PubMed: 22894615]

Haas R, Koch HU, Fischer W. Alanyl turnover from lipoteichoic acid to teichoic acid in *Staphylococcus aureus*. FEMS Microbiol Lett. 1984; 21(1):27–31.

Hakenbeck R, Madhour A, Denapaite D, Brückner R. Versatality of choline metabolism and choline-binding proteins in *Streptococcus pneumoniae* and commensal streptococci. FEMS Microbiol Rev. 2009; 33(3):572–586. [PubMed: 19396958]

Hall RG, Michaels HN. Profile of tedizolid phosphate and its potential in the treatment of acute bacterial skin and skin structure infections. Infect Drug Resist. 2015; 8:75–82. [PubMed: 25960671]

Hall-Stoodley L, Costerton W, Stoodley P. Bacterial biofilms: from the natural enviroment to infectious diseases. Nat Rev Microbiol. 2004; 2:95–108. [PubMed: 15040259]

Hancock IC. Bacterial cell surface carbohydrates: structure and assembly. Biochem Soc Trans. 1997; 25(1):183–187. [PubMed: 9056868]

Hancock IC, Wiseman G, Baddiley J. Biosynthesis of the unit that links teichoic acid to the bacterial wall: inhibition by tunicamycin. FEBS Lett. 1976; 69(1):75–80. [PubMed: 825388]

Handwerger S, Pucci MJ, Volk KJ, Liu J, Lee MS. The cytoplasmic peptidoglycan precursor of vancomycin-resistant *Enterococcus faecalis* terminates in lactate. J Bacteriol. 1992; 174(18): 5982–5984. [PubMed: 1522072]

Haque MA, Russell NJ. Strains of *Bacillus cereus* vary in the phenotypic adaptation of their membrane lipid composition in response to low water activity, reduced temperature and growth in rice starch. Microbiol. 2004; 150(Pt 5):1397–1404.

Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aurues*. J Bacteriol. 1984; 158(2):513–516. [PubMed: 6563036]

Hashimoto M, Tawaratsmida K, Kariya H, Aoyama K, Tamura T, Suda Y. Lipoprotein is a dominant Toll-like receptor 2 ligand in *Staphylococcus aureus* cell wall components. Int Immunol. 2006a; 18(2):355–362. [PubMed: 16373361]

Hashimoto M, Tawaratsmida K, Kariya H, Kiyohara A, Suda Y, Krikae F, Kirikae T, Götz F. Not lipoteichoic acid but lipoprotein appear to be the dominant immunobiologically active compounds in *Staphylococcus aureus*. J Immunol. 2006b; 177(5):3162–3169. [PubMed: 16920954]

Hayashi H, Araki Y, Ito E. Occurence of glucosamine residues with free amino groups in cell wall peptidoglycan from bacilli as a factor responsible for resistance to lysozyme. J Bacteriol. 1973; 113(2):592–598. [PubMed: 4632317]

Hayhurst EJ, Kailas L, Hobbs JK, Foster SJ. Cell wall peptidoglycan architecture in *Bacillus subtilis*. Proc Natl Acad Sci U S A. 2008; 105(38):14603–14608. [PubMed: 18784364]

Healy VL, Lessard IA, Roper DI, Knox JR, Walsh CT. Vancomycin resistance in enterococci: reprogramming of the D-ala-D-ala ligases in bacterial peptidoglycan biosynthesis. Chem Biol. 2000; 7(5):109–119.

Heaslet H, Shaw B, Mistry A, Miller A. Characterization of the active site of *S. aureus* monofunctional transglycosylase (Mtg) by site-directed mutation and structural analysis of the protein complexed with moenomycin. J Struct Biol. 2009; 167(2):129–135. [PubMed: 19416756]

Heaton MP, Neuhaus FC. Biosynthesis of D-alanyl-lipoteichoic acid: cloning, nucleotide sequence, and expression of the *Lactobacillus casei* gene for the D-alanine-activating enzyme. J Bacteriol. 1992; 174(14):4707–4717. [PubMed: 1385594]

Heaton MP, Neuhaus FC. Roles of the D-alanyl carrier protein in the biosynthesis of D-alanyl-lipoteichoic acid. J Bacteriol. 1994; 176(3):681–690. [PubMed: 8300523]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Hebert L, Courtin P, Torelli R, Sanguinetti M, Chapot-Chartier MP, Auffray Y, Benachour A. *Enterococcus faecalis* constitutes an unusual bacterial model in lysozyme resistance. Infect Immun. 2007; 75(11):5390–5398. [PubMed: 17785473]

Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Götz F. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol Microbiol. 1996; 20(5):1083–1091. [PubMed: 8809760]

Henze U, Sidow T, Wecke J, Labischinski H, Berger-Bächi B. Influence of *femB* on methicillin resistance and peptidoglycan metabolism in *Staphylococcus aureus*. J Bacteriol. 1993; 175(6): 1612–1620. [PubMed: 8383661]

Hoffman K. A superfamily of membrane-bound *O*-acetyltansferases with implications for wnt signalling. Trends Biochem Sci. 2000; 25(3):111–112. [PubMed: 10694878]

Holmes NE, Howden BP. What's new in the treatment of serious MRSA infection? Curr Opin Infect Dis. 2014; 27(6):471–478. [PubMed: 25211361]

Hong DJ, Bae IK, Jang IH, Jeong SH, Kang HK, Lee K. Epidemiology and characteristics of metall-β-lactamase-producing *Pseudomonas aeruginosa*. Infect Chemother. 2015; 47(2):81–97. [PubMed: 26157586]

Hsu ST, Breukink E, Tischenko E, Lutters MA, de Kruijiff B, Kaptein R, Bonvin AM, van Nuland NA. Nat Struct Mol Biol. 2004; 11(10):963–967. [PubMed: 15361862]

Hu Y, Chen L, Ha S, Gross B, Falcone B, Walker D, Mokhtarzadeh M, Walker S. Crystal structure of the MurG:UDP-GlcNAc complex reveals common structural principles of a superfamily of glycosyltransferases. Proc Natl Acad Sci U S A. 2003a; 100(3):845–849. [PubMed: 12538870]

Hu Y, Helm JS, Chen L, Ya XY, Walker S. Ramoplanin inhibits bacterial transglycosylases by binding as a dimer to Lipid II. J Am Chem Soc. 2003b; 125(29):8736–8737. [PubMed: 12862463]

Huber J, Donald RG, Lee SH, Jarantow LW, Salvatore MJ, Meng X, Painter R, Onishi RH, Occi J, Dorso K, Young K, Park YW, Skwish S, Szymonifka MJ, Waddell TS, Miesel L, Phillips JW, Roemer T. Chemical genetic identification of peptidoglycan inhibtors potentiating carbapenem activity against methicillin-resistant *Staphylococcus aureus*. Chem Biol. 2009; 16:837–848. [PubMed: 19716474]

Itoh Y, Wang X, Hinnebusch BJ, Preston JF, Romeo T. Depolymerization of β-1,6-*N*-acetyl-D-glucosamine disrupts the integrity of diverse bacterial biofilms. J Bacteriol. 2005; 187(1):382–387. [PubMed: 15601723]

Jedrzejas MJ. Pneumococcal virulence factors: structure and function. Microbiol Mol Biol Rev. 2001; 65(2):187–207. [PubMed: 11381099]

Jefferies JM, Clarke SC, Webb JS, Kraaijeveld AR. Risk of red queen dynamics in pneumococcal vaccine strategy. Trends Microbiol. 2011; 19(8):377–381. [PubMed: 21763141]

Jerga A, Lu YJ, Schujman GE, de Mendoza D, Rock CO. Identification of a soluble diacylglycerol kinase required for lipoteichoic acid production in *Bacillus subtilis*. J Biol Chem. 2007; 282(30): 21738–21745. [PubMed: 17535816]

Jervis AJ, Thackray PD, Houston CW, Horsburgh MJ, Moir A. SigM-responsive genes of *Bacillus subtilis* and their promoters. J Bacteriol. 2007; 189(12):4534–4538. [PubMed: 17434969]

Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. Clin Microbiol Rev. 1994; 7(4):462–478. [PubMed: 7834601]

Jonquiéres R, Bierne H, Fiedler F, Gounon P, Cossart P. Interaction between the protein InlB of *Listeria monocytogenes* and lipoteichoic acid: a novel mechanism of protein association at the surface of Gram-positive bacteria. Mol Microbiol. 1999; 34(5):902–914. [PubMed: 10594817]

Jorasch P, Wolter FP, Zähringer U, Heinz E. A UDP glucosyltransferase from *Bacillus subtilis* successively transfer up to four glucose residues to 1,2-diacylglycerol: expression of *ypfP* in *Escherichia coli* and structural analysis of its reaction product. *Molecular Microbiology*. 1998; 29(2):419–430. [PubMed: 9720862]

Julian K, Kosowska-Shick K, Whitener C, Roos M, Labischinski H, Rubio A, Parent L, Ednie L, Koeth L, Bogdanovich T, Appelbaum PC. Characterization of a daptomycin-nonsusceptible vancomycin-intermediate *Staphylococcus aureus* strain in a patient with endocarditis. Antimicrob Agents Chemother. 2007; 51(9):3445–3448. [PubMed: 17620372]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Kahan FM, Kahan JS, Cassidy PJ, Kropp H. The mechanism of action of fosfomycin (phosphonomycin). Ann N Y Acad Sci. 1974; 235:364–386. [PubMed: 4605290]

Kalin M. Pneumococcal serotypes and their clinical relevance. Thorax. 1998; 53(3):159–162. [PubMed: 9659348]

Karatsa-Dodgson M, Wörmann ME, Gründling A. *In vitro* anaylsis of the *Staphylococcus aureus* lipoteichoic acid synthase enzyme using fluorescently labeled lipids. J Bacteriol. 2010; 192(20): 5341–5349. [PubMed: 20709894]

Kawai Y, Marles-Wright J, Cleverley RM, Emmins R, Ishikawa S, Kuwano M, Heinz N, Bui NK, Hoyland CN, Ogasawara N, Lewis RJ, Vollmer W, Daniel RA, Errington J. A widespread family of bacterial cell wall assembly proteins. EMBO J. 2011; 30(24):4931–4941. [PubMed: 21964069]

Keller R, Fischer W, Keist R, Bassetti S. Macrophage response to bacteria: induction of marked secretory and cellular activities by lipoteichoic acids. Infect Immun. 1992; 60(9):3664–3672. [PubMed: 1500175]

Kenne L, Lindberg B, Svensson S. The structure of capsular polysaccharide of the pneumococcus type II. Carbohydr Res. 1975; 40:69–75. [PubMed: 236092]

Kharat AS, Tomasz A. Drastic reduction in the virulence of *Streptococcus pneumoniae* expressing type 2 capsular polysaccharide but lacking choline residues in the cell wall. Mol Microbiol. 2006; 60(1):93–107. [PubMed: 16556223]

Kiriukhin MY, Debabov DV, Shinabarger DL, Neuhaus FC. Biosynthesis of the glycolipid anchor in lipoteichoic acid of *Staphylococcus aureus* RN4220: role of YpfP, the diglucosyldiacylglycerol synthase. J Bacteriol. 2001; 183(11):3506–3514. [PubMed: 11344159]

Kobayashi K, Ehrlich SD, Albertini A, Amati G, Andersen KK, Arnaud M, et al. Essential *Bacillus subtilis* genes. Proc Natl Acad Sci U S A. 2003; 100(8):4678–4683. [PubMed: 12682299]

Kobayashi K, Sudiarta P, Kodoma T, Fukushima T, Ara K, Ozaki K, Sekiguchi J. Identification and characterization of a novel polysaccharide deacetylase C (PdaC) from *Bacillus subtilis*. J Biol Chem. 2012; 287(13):9765–9776. [PubMed: 22277649]

Koch HU, Döker R, Fischer W. Maintenance of D-alanine ester substitution of lipoteichoic acid by reesterification in *Staphylococcus aureus*. J Bacteriol. 1985; 164(3):1211–1217. [PubMed: 4066613]

Kock H, Gerth U, Hecker M. MurAA, catalyzing the first committed step in peptidoglycan biosynthesis, is a target for Clp-dependent proteolysis in *Bacillus subtilis*. Mol Microbiol. 2004; 51(4):1087–1102. [PubMed: 14763982]

Kohler T, Weidenmaier C, Peschel A. Wall teichoic acid protects *Staphylococcus aureus* against antimicrobial fatty acids from human skin. J Bacteriol. 2009; 191(13):4482–4484. [PubMed: 19429623]

Kojima N, Araki Y, Ito E. Structure of the linkage units between ribitol teichoic acids and peptidoglycan. J Bacteriol. 1985; 161(1):299–306. [PubMed: 3918002]

Komatsuzawa H, Ohta K, Fujiwara T, Choi GH, Labischinski H, Sugai M. Cloning and sequencing of the gene, *fmtC*, which affects oxacillin resistance in methicillin-resistant *Staphylococcus aureus*. FEMS Microbiol Lett. 2001; 203(1):49–54. [PubMed: 11557139]

Komatsuzawa H, Sugai M, Ohta K, Fujiwara T, Nakashima S, Lee CY, Suginaka H. Cloning and characterization of the *fmt* gene which affects the methicillin-resistance level and autolysis in the presence of triton X-100 in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 1997; 41(11):2355–2361. [PubMed: 9371333]

Kovács M, Halfmann A, Fedtke I, Heintz M, Peschel A, Vollmer W, Hakenbeck R, Brückner R. A functional *dlt* operon, encoding proteins required for incorporation of D-alanine in teichoic acids in Gram-positive bacteria, confers resistance to cationic antimicrobial peptides in *Streptococcus pneumoniae*. J Bacteriol. 2006; 188(16):5797–5805. [PubMed: 16885447]

Kovacs-Simon A, Titball RW, Michell SL. Lipoproteins of bacterial pathogens. Infect Immun. 2011; 79(2):548–561. [PubMed: 20974828]

Kozarich JW, Strominger JL. A membrane enzyme from *Staphylococcus aureus* which catalyzes transpeptidase, carboxypeptidase, and penicillinase activities. J Biol Chem. 1978; 253(4):1272–1278. [PubMed: 624730]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Kresge N, Simoni RD, Hill RL. t-RNA involvement in peptidoglycan synthesis: the work of Dieter Söll. J Biol Chem. 2007; 282(26):e20–e21.

Kristian SA, Dürr M, Van Strijp JA, Neumeister B, Peschel A. MprF-mediated lysinylation of phospholipids in *Staphylococcus aureus* leads to protection against oxgen-independent neutrophil killing. Infect Immun. 2003; 71(1):546–549. [PubMed: 12496209]

Kristian SA, Vivekanand D, Wiedenmaier C, Kansal R, Fedtke I, Peschel A, Gallo RL, Nizet V. D-alanylation of teichoic acids promotes Group A *Streptococcus* antimicrobial peptide resistance, neutrophil survival, and epithelial cell invasion. J Bacteriol. 2005; 187(19):6719–6725. [PubMed: 16166534]

Laaberki MH, Pfeffer J, Clarke AJ, Dworkin J. *O*-acetylation of peptidoglycan is required for proper cell separation and S-layer anchoring in *Bacillus anthracis*. J Biol Chem. 2011; 286(7):5278–5288. [PubMed: 21135105]

Lavollay M, Arthur M, Fourgeaud M, Dubost L, Marie A, Veziris N, Blanot D, Gutmann L, Mainardi J. The peptidoglycan of stationary-phase *Mycobacterium tuberculosis* predominantly contains cross-links generated by L,D-transpeptidation. J Bacteriol. 2008; 190(12):4360–4366. [PubMed: 18408028]

Lavollay M, Fourgeaud M, Hermann JL, Dubost L, Marie A, Gutmann L, Arthur M, Mainardi J. The peptidoglycan of *Mycobacterium abscessus* is predominantly cross-linked by L,D-transpeptidases. J Bacteriol. 2011; 193(3):778–782. [PubMed: 21097619]

Lazarevic V, Abellan FX, Möller SB, Karamata D, Mauël C. Comparison of ribitol and glycerol teichoic acid genes in *Bacillus subtilis* W23 and 168: identical function, similar divergent organization, but different regulation. Microbiology. 2002; 148(Pt 3):815–824. [PubMed: 11882717]

Leach KL, Brickner SJ, Noe MC, Miller PF. Linezolid, the first ozazolidinone antibacterial agent. Ann N Y Acad Sci. 2011; 1222:49–54. [PubMed: 21434942]

Lee K, Campbell J, Swoboda JG, Cuny GD, Walker S. Development of improved inhibitors of wall teichoic acid biosynthesis with potent activity against *Staphylococcus aureus*. Bioorg Med Chem Lett. 2010; 20:1767–1770. [PubMed: 20138521]

Lebreton F, Depardieu F, Bourdon N, Fines-Guyon M, Berger P, Camiade S, Leclerq R, Courvalin P, Cattoir V. D-ala-D-ser VanN-type transferable vancomycin resistance in *Enterococcus faecium*. Antimicrob Agents Chemother. 2011; 55(10):4606–4612. [PubMed: 21807981]

Li M, Cha DJ, Lai Y, Villaruz AE, Sturdevant DE, Otto M. The antimicrobial peptide-sensing system *aps* of *Staphylococcus aureus*. Mol Microbiol. 2007a; 66(5):1136–1147. [PubMed: 17961141]

Li M, Lai Y, Villaruz AE, Cha DJ, Sturdevant DE, Otto M. Gram-positive three-component antimicrobial peptide-sensing system. Proc Natl Acad of Sci U SA. 2007b; 104(22):9469–9474.

Lim D, Strynadka NC. Structural basis for the β-lactam resistance of PBP2a from methicillin-resistant *Staphylocccus aureus*. Nat Struct Biol. 2002; 9(11):870–876. [PubMed: 12389036]

Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon P, Mueller A, et al. A new antibiotic kills pathogens without detectable resistance. Nature. 2015; 517(7535):455–459. [PubMed: 25561178]

Lo MC, Men H, Branstrom A, Helm J, Yao N, Goldman R, Walker S. A new mechanism of action proposed for ramoplanin. J Am Chem Soc. 2000; 122:3540–3541.

Loos M, Clas F, Fisher W. Interaction of purified lipoteichoic acid with classical complement pathway. Infect Immun. 1986; 53(3):595–599. [PubMed: 3488963]

Lu D, Wörmann ME, Zhang X, Schneewind O, Gründling A, Freemont PS. Structure-based mechanism of lipoteichoic acid synthesis of *Staphylococcus aureus* LtaS. Proc Natl Acad SciU S A. 2009; 106(5):1584–1589.

Ludovice AM, Wu SW, De Lencastre H. Molecular cloning and DNA sequencing of the *Staphylococcus aureus* UDP-*N*-acetylmuramyl tripeptide synthetase (*murE*) gene, essential for the optimal expression of methicillin resistance. Microb Drug Resist. 1998; 4(2):85–90. [PubMed: 9650993]

Lunderberg JM, Nguyen-Mau SM, Richter GS, Wang YT, Dworkin J, Missiakas DM, Schneewind O. *Bacillus anthracis* acetyltransferases PatA1 and PatA2 modify the secondary cell wall

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

polysaccharide and affect the assembly of S-layer proteins. J Bacteriol. 2013; 195(5):977–989. [PubMed: 23243307]

Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H, Laufs R. The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear β-1,6-linked glucosaminoglycan: purification and structural analysis. J Bacteriol. 1996; 178(1):175–183. [PubMed: 8550413]

MacLeod CM, Hodges RG, Heidelbrger M, Bernhard WG. Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. J Exp Med. 1945; 82(6):445–465.

Mahapatra S, Yagi T, Belisle JT, Espinosa BJ, Hill PJ, McNeil MR, Brennan PJ, Crick D. Mycobaterial Lipid II is composed of a complex mixture of muramyl and peptide moieties linked to a decaprenly phosphate. J Bacteriol. 2005; 187(8):2747–2757. [PubMed: 15805521]

Maidhof H, Reinicke B, Blümel P, Berger-Bächi B, Labischinski H. *femA* which encodes a factor essential for methicillin resistance, affects glycine content of peptidoglycan in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. J Bacteriol. 1991; 173(11): 3507–3513. [PubMed: 2045371]

Mainardi JL, Legrand R, Arthur M, Schoot B, van Heijenoort J, Gutmann L. Novel mechanism of beta-lactam resistance due to bypass of DD-transpeptidation in *Enterococcus faecium*. J Biol Chem. 2000; 275(22):16490–16496. [PubMed: 10748168]

Maira-Litrán T, Bentancor LV, Bozkurt-Guzel C, O'Malley JM, Cywes-Bentley C, Pier GB. Synthesis and evaluation of a conjugate vaccine composed of *Staphylococcus aureus* poly-*N*-acetylglucosamine and clumping factor A. PLoS One. 2012; 7(9):e43813. [PubMed: 22970144]

Marquardt JL, Siegele DA, Kolter R, Walsh CT. Cloning and sequencing of *Escherichia coli murZ* and purification of its product, a UDP-*N*-acetylglucosamine enolpyruvyl transferase. J Bacteriol. 1992; 174(17):5748–5752. [PubMed: 1512209]

Marshall CG, Lessard IA, Park I, Wright GD. Glycopeptide antibiotic resistance genes in glycopeptides-producing organisms. Antimicrob Agents Chemother. 1998; 42(9):2215–2220. [PubMed: 9736537]

Matsuoka S, Chiba M, Tanimura Y, Hashimoto M, Hara H, Matsumoto K. Abnormal morphology of *Bacillus subtilis ugtP* mutant cells lacking glucolipids. Genes Genet Syst. 2011; 86(5):295–304. [PubMed: 22362028]

Mauël C, Young M, Karamata D. Genes concerned with synthesis of poly(glycerol phosphate), the essential teichoic acid in *Bacillus subtilis* strain 168, are organized in two divergent transcription units. J Gen Microbiol. 1991; 137(4):929–941. [PubMed: 1906926]

May JJ, Finking R, Wiegshoff F, Weber TT, Bandur N, Koert U, Marahiel MA. Inhibition of the D-alanine:D-alanyl carrier protein ligase from *Bacillus subtilis* increases the bacterium's susceptibility to antibiotics that target the cell wall. FEBS J. 2005; 272(12):2993–3003. [PubMed: 15955059]

McDaneld PM, Spooner LM, Mohr JF, Belliveau PP. Use of daptomycin to treat infections with methicillin-resistant *Staphylococcus aureus* having vancomycin minimum inhibitory concentrations of 1.5 to 2μg/mL. Ann Pharmacother. 2013; 47(12):1654–1665. [PubMed: 24259618]

Meeske AJ, Sham LT, Kimsey H, Koo BM, Gross CA, Bernhardt TG, Rudner DZ. MurJ and a novel lipid II flippase are required for cell wall biogenesis in *Bacillus subtilis*. Proc Natl Acad Sci U S A. 2015; 112(20):6437–6442. [PubMed: 25918422]

Memmi G, Filipe SR, Pinho MG, Fu Z, Cheung A. *Staphylococcus aureus* PBP4 is essential for β-lactam resistance in community-acquired methicillin-resistant strains. Antimicrob Agents Chemother. 2008; 52(11):3955–3966. [PubMed: 18725435]

Mengin-Lecreulx D, Texier L, Rousseau M, van Heijenoort J. The *murG* gene of *Escherichia coli* codes for the UDP-*N*-acetylglucosamine: *N*-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol *N*-acetylglucosamine transferase involved in the membrane steps of peptidoglycan synthesis. J Bacteriol. 1991; 173(15):4625–4636. [PubMed: 1649817]

Meredith TC, Swoboda JG, Walker S. Late-stage polyribitol phosphate wall teichoic acid biosynthesis in *Staphylococcus aureus*. J Bacteriol. 2008; 190(8):3046–3056. [PubMed: 18281399]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Minnikin DE, Abdolraimzadeh H. Effect of pH on the proportions of polar lipds, in chemostat cultures of *Bacillus subtilis*. J Bacteriol. 1974; 120(3):999–10003. [PubMed: 4215800]

Mishra NN, Bayer AS, Weidenmaier C, Grau T, Wanner S, Stefani S, Cafiso V, Bertuccio T, Yeaman MR, Nast CC, Yang SJ. Phenotypic and genotypic characterization of daptomycin-resistant methicillin-resistant *Staphylococcus aureus* strains: relative roles of mprF and dlt operons. PLoS One. 2014; 9(9):e107426. [PubMed: 25226591]

Mitchell P, Moyle J. Osmotic structure and function in bacteria. Symp Soc Gen Microbiol. 1956; 6:150–180.

Mitra S, Saeed U, Havlichek DH, Stein GE. Profile of oritavancin and its potential in the treatment of acute bacterial skin structure infections. Infect Drug Resist. 2015; 8:189–197. [PubMed: 26185459]

Mohamadzadeh M, Pfeiler EA, Brown JB, Zadeh M, Gramarossa M, Managlia E, Bere P, Sarraj B, Khan MW, Pakanati KC, Ansari MJ, O'Flaherty S, Barrett T, Klaenhammer TR. Regulation of induced colonic inflammation by *Lactobacillus acidophilus* deficient in lipoteichoic acid. Proc Natl Acad Sci U S A. 2011; 108(Supplement 1):4623–4630. [PubMed: 21282652]

Moisan H, Pruneau M, Malouin F. Binding of ceftaroline to penicillin-binding proteins of *Staphylococcus aureus* and *Streptococcus pneumoniae*. J Antimicrob Chemother. 2010; 5(4): 713–716. [PubMed: 20097788]

Moynihan PJ, Sychantha D, Clarke AJ. Chemical biology of peptidoglycan acetylation and deacetylation. Bioorg Chem. 2014; 54:44–50. [PubMed: 24769153]

Münch D, Roemer T, Lee SH, Engeser M, Sahl HG, Schneider T. Identification and *in vitro* analysis of the GatD/MurT enzyme-complex catalyzing Lipid II amdiation in *Staphylococcus aureus*. PLoS Pathog. 2012; 8(1):e1002509. [PubMed: 22291598]

Navarre WW, Schneewind O. Surface proteins of Gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol Mol Biol Rev. 1999; 63(1):174–229. [PubMed: 10066836]

Neuhaus FC, Baddiley J. A continuum of anionic charge: structures and functions of D -alanyl-teichoic acids in Gram-positive bacteria. Microbiol Mol Biol Rev. 2003; 67(4):686–723. [PubMed: 14665680]

Nishi H, Komatsuzawa H, Fujiwara T, McCallum N, Sugai M. Reduced content of lysyl-phosphatidylglycerol in the cytoplasmic membrane affects susceptibility to moenomycin, as well as vancomycin, gentamicin, and antimicrobial peptides, in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2004; 48(12):4800–4807. [PubMed: 15561859]

Norris, V., Sweeney, S. Deformations in the cytoplasmic membrane of Escherichia coli direct the repair of peptidoglycan. In: de Pedro, MA.Höltje, JV., Löffelhardt, W., editors. Bacterial growth and lysis: metabolism and structure of the bacterial sacculus. Springer US: 1993. p. 375-385.

Nurhonen M, Auranen K. Optimal serotype compositions for pneumococcal conjugate vaccination under serotype replacement. PloS Comput Biol. 2014; 10(2):e1003477. [PubMed: 24550722]

O'Gara JP. *ica* and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. FEMS Microbiol Lett. 2007; 270(2):179–188. [PubMed: 17419768]

O'Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol Rev. 2004; 17(1):218–234. [PubMed: 14726462]

Ohno N, Yadomae T, Miyazaki T. Identification of 2-amino-2-deoxyglucose residues in the peptidoglucan of *Streptococcus pneumoniae*. Carbohydr Res. 1982; 107(1):152–155. [PubMed: 7139657]

Oku Y, Kurokawa K, Matsuo M, Yamada S, Lee BL, Sekimizu K. Pleiotropic roles of polyglycerolphosphate synthase of lipoteichoic acid in growth of *Staphylococcus aureus* cells. J Bacteriol. 2009; 191(1):141–151. [PubMed: 18952789]

Oliver MB, Jones C, Larson TR, Calix JJ, Zartler ER, Yother J, Nahm MH. *Streptococcus pneumoniae* serotype 11D has a bi-specific glycosyltransferase and expresses two different capsular polysaccharide repeating units. J Biol Chem. 2013a; 288(30):21945–21954. [PubMed: 23737526]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Oliver MB, van der Linden MP, Küntzel SA, Saad JS, Nahm MH. Discovery of *S* reptococcus pneumoniae serotype 6 variants with glycosyltransferases synthesizing two different repeating units. J Biol Chem. 2013b; 288(36):25976–25985. [PubMed: 23897812]

Oman TJ, Lupoli TJ, Wang TSA, Kahne D, Walker S, van der Donk WA. Haloduracin α binds the peptidoglycan precursor Lipid II with 2:1 stoichiometry. J Am Chem Soc. 2011; 133(44):17544–17547. [PubMed: 22003874]

Otto M. Staphylococcal biofilms. Curr Top Microbiol Immunol. 2008; 322:207–228. [PubMed: 18453278]

Over B, Heusser R, McCallum N, Schulthess B, Kupferschmied P, Gaiani JM, Sifri CD, Berger-Bächi B, Meier PS. LytR-CpsA-Psr proteins in *Staphylococcus aureus* display partial functional redundancy and the deletion of all three severely impairs spetum placement and cell separation. FEMS Microbiol Lett. 2011; 320(2):142–151. [PubMed: 21554381]

Paknikar SS, Narayana S. Newer antibacterials in therapy and clinical trials. N Am J Med Sci. 2012; 4(11):537–547. [PubMed: 23181224]

Palmer KL, Kos VN, Gilmore MS. Horizontal Gene transfer and the genomics of enterococcal antibiotic resistance. Curr Opin Microbiol. 2010; 13(5):632–639. [PubMed: 20837397]

Parsons JB, Rock CO. Bacterial lipids: metabolism and membrane homeostasis. Prog Lipid Res. 2014; 52(3):249–276. [PubMed: 23500459]

Patin D, Boniface A, Kova A, Hervé M, Dementin S, Barreteau H, Mengin-Lecreulx D, Blanot D. Purification and biochemical characterization of Mur ligases from *Staphylococcus aureus*. Biochimie. 2010; 92(12):1793–1800. [PubMed: 20659527]

Patti GJ, Chen J, Schaefer J, Gross ML. Characterization of structural variation in the peptidoglycan of vancomyin susceptible *Enterococcus faecium*: undertanding glycopeptide-antibiotic binding sites using mass spectrometry. J Am Soc Mass Spectrom. 2008; 19(10):1467–1475. [PubMed: 18692403]

Patton GC, van der Donk WA. New developments in lantibiotic biosynthesis and mode of action. Curr Opin Microbiol. 2005; 8:543–551. [PubMed: 16118063]

Pelton SI, Loughlin AM, Marchant CD. Seven valent pneumococcal conjugate vaccine immunization in two Boston communities: changes in serotypes and antimicrobial susceptibility among *Streptococcus pneumoniae* isolates. Pediatr Infect Dis J. 2004; 23(11):1015–1022. [PubMed: 15545856]

Percy MG, Gründling A. Lipoteichoic acid synthesis and function in Gram-positive bacteria. Annu Rev Microbiol. 2014; 68:81–100. [PubMed: 24819367]

Perego M, Glaser P, Minutello A, Srauch MA, Leopold K, Fischer W. Incorporation of D-alanine into lipoteichoic acid and wall teichoic acid in *Bacillus subtilis*: identification of genes and regulation. J Biol Chem. 1995; 270(26):15598–15606. [PubMed: 7797557]

Pereira MP, D'Elia MA, Troczynska J, Brown ED. Duplication of teichoic acid biosynthetic genes in Staphylococcus aureus leads to functionally redundant poly(ribitol phosphate) polymerases. J Bacteriol. 2008; 190(16):5642–5649. [PubMed: 18556787]

Périchon B, Courvalin P. VanA-type vancomycin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2009; 53(11):4580–4587. [PubMed: 19506057]

Perkins HR. Specificity of combination between mucopeptide precursors and vancomycin or ristocetin. Biochem J. 1969; 111(2):195–205. [PubMed: 5763787]

Perkins HR, Nieto M. The chemical basis for the action of the vancomycin group of antibiotics. Ann N Y Acad Sci. 1974; 235:348–363. [PubMed: 4369274]

Perry AM, Ton-That H, Mazmanian SK, Schneewind O. Anchoring of surface proteins to the cell wall of *Staphylococcus aureus* III. Lipid II is an *in vivo* peptidoglycan substrate for sortase-catalyzed surface protein anchoring. J Biol Chem. 2002; 277(18):16241–16248. [PubMed: 11856734]

Peschel A, Jack RW, Otto M, Collins LV, Staubitz P, Nicholson G, Kalbacher H, Nieuwenhuizen WF, Jung G, Tarkowski A, van Kessel KP, van Strijp JA. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor *mprF* is based on modification of membrane lipids with L-lysine. J Exp Med. 2001; 193(1):1067–1076. [PubMed: 11342591]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Götz F. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J Biol Chem. 1999; 274(13):8405–8410. [PubMed: 10085071]

Peschel A, Vuong C, Otto M, Götz F. The D-alanine residues of *Staphylococcus aureus* teichoic acids alter the susceptibility to vancomycin and the activity of autolytic enzymes. Antimicrob Agents Chemother. 2000; 44(10):2845–2847. [PubMed: 10991869]

Peterson PK, Wilkinson BJ, Kim Y, Schmeling D, Quie PG. Influence of encapsulation on staphylococcal opsonization and phagocytosis by human polymorphonuclear leukocytes. Infect Immun. 1978; 19(3):943–949. [PubMed: 640738]

Pilishvili T, Bennett NM. Pneumococcal disease prevention among adults: strategies for the use of pneumococcal vaccines. Vaccine. 2015

Pinho MG, de Lancastre H, Tomasz A. Cloning, characterization, and inactivation of the gene *pbpC* encoding pencillin-binding protein 3 of *Staphylococcus aureus*. J Bacteriol. 2000; 182(4):1074–1079. [PubMed: 10648534]

Pinho MG, de Lancastre H, Tomasz A. An aquired and a native pencillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. Proc Natl Acad Sci U S A. 2001a; 98(19): 10886–10891. [PubMed: 11517340]

Pinho MG, Filipe SR, de Lancastre H, Tomasz A. Complementation of the essential peptidoglycan transpeptidase function of Penicillin-binding protein 2 (PBP2) by the drug resistance PBP2A in *Staphylococcus aureus*. J Bacteriol. 2001b; 183(22):6525–6531. [PubMed: 11673420]

Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*: a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother. 2015

Pless D, Neuhaus FC. Initial membrane reaction in peptidoglycan synthesis. Lipid dependence of phospho-*N*-acetylmuramylpentapeptide translocase (exchange reaction). J Biol Chem. 1973; 248(5):1568–1576. [PubMed: 4694725]

Pobre K, Tashani M, Ridda I, Rashid H, Wong M, Booy R. Carrier priming or suppression: understanding carrier priming enhancement of anti-polysaccharide antibody response to conjugate vaccines. Vaccine. 2014; 32(13):1423–1430. [PubMed: 24492014]

Pollack JH, Neuhaus FC. Changes in wall teichoic acid during the rod-sphere transition of *Bacillus subtilis* 168. J Bacteriol. 1994; 176(23):7252–7259. [PubMed: 7961496]

Powell DA, Duckworth M, Baddiley J. A membrane-associated lipomannan in micrococci. Biochemistry J. 1975; 151(2):387–397.

Qamar A, Golemi-Kotra D. Dual roles of FmtA in *Staphylococcus aureus* cell wall biosynthesis and autolysis. Antimicrob Agents Chemother. 2012; 56(7):3797–3805. [PubMed: 22564846]

Qiao Y, Lebar MD, Schirner K, Schaefer K, Tsukamoto H, Kahne D, Walker S. Detection of lipid-linked peptidoglycan precursors by exploiting an unexpected transpeptidase reaction. J Am Chem Soc. 2014; 136(42):14678–14681. [PubMed: 25291014]

Ray A, Cot M, Puzo G, Gilleron M, Nigou J. Bacterial cell wall macroamphiphiles: pathogen-/microbe-associated molecular patterns detected by mammalian innate immune system. Biochimie. 2013; 95(1):33–42. [PubMed: 22706280]

Reading C, Cole M. Clavulanic acid: a β-lactamase-inhibiting β-lactam from *Streptomyces clavuligerus*. Antimicrob Agents Chemother. 1977; 11(5):852–857. [PubMed: 879738]

Reed P, Atilano ML, Alves R, Hoiczyk E, Sher X, Reichmann NT, Pereira PM, Roemer T, Filipe SR, Periera-Leal JB, Ligoxygakis P, Pinho MG. *Staphylococcus aureus* survives with a minimal peptidoglycan synthesis machine but sacrifices virulence and antibiotic resistance. PLoS Pathog. 2015; 11(5):e1004891. [PubMed: 25951442]

Reed P, Veiga H, Jorge AM, Terrak M, Pinho MG. Monofunctional transglycosylases are not essential for *Staphylococcus aureus* cell wall synthesis. J Bacteriol. 2011; 193(10):2549–2556. [PubMed: 21441517]

Reichmann NT, Cassona CP, Gründling A. Revised mechanism of D-alanine incorporation into cell wall polymers in Gram-positive bacteria. Microbiology. 2013; 159(Pt 9):1868–1877. [PubMed: 23858088]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Revilla-Guarinos A, Gebhard S, Mascher T, Zuniga M. Defence against antimicrobial peptides: different strategies in Firmicutes. Environ Microbiol. 2014; 16(5):1225–1237. [PubMed: 24548478]

Reynolds PE. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur J Clin Microbiol Infect Dis. 1989; 8(11):943–950. [PubMed: 2532132]

Richter SG, Eli D, Kim HK, Hendrickx APA, Sorg JA, Schneewind O, Missiakas D. Small molecule inhibitor of lipoteichoic acid synthesis is an antibiotic for Gram-positive bacteria. Proc Natl Acad Sci U S A. 2013; 110(9):3531–3536. [PubMed: 23401520]

Robbins JB, Schneerson R, Horwith G, Naso R, Fattom A. *Staphylococcus aureus* type 5 and 8 capsular polysaccharide-protein conjugate vaccines. Am Heart J. 2004; 147(4):593–598. [PubMed: 15077073]

Roberts IS. The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu Rev Microbiol. 1996; 50:285–315. [PubMed: 8905082]

Rogers, HJ., Perkins, HR., Ward, JB. Microbial Cell Walls and Membranes. London: Chapman and Hall; 1980. Biosynthesis of peptidoglycan; p. 239-290.

Rohde H, Frankenberger S, Zähringer U, Mack D. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to *Staphylococcus epidermidis* biofilm formation and pathogenesis of biomaterial-associated infections. Eur J Cell Biol. 2010; 89(1):103–111. [PubMed: 19913940]

Rohrer S, Ehlert K, Tschierske M, Labischinski H, Berger-Bächi B. The essential *Staphylococcus aureus* gene *fmhB* is involved in the first step of peptidoglycan pentaglycine interpeptide formation. Proc Natl Acad Sci U S A. 1999; 96(16):9351–9356. [PubMed: 10430946]

Rosenow C, Ryan P, Weiser JN, Johnson S, Fontan P, Ortqvist A, Masure HR. Contribution of novel choline-binding proteins to adherence, colonization and immunogenicity of *Streptococcus pneumoniae*. Mol Microbiol. 1997; 25(5):819–829. [PubMed: 9364908]

Ruiz N. Bioinformatics identification of MurJ (MviN) as the peptidoglyan lipidII flippase in *Escherichia coli*. Proc Natl Acad Sci U S A. 2008; 105(40):15553–15557. [PubMed: 18832143]

Ruiz N. *Streptococcus pyogenes* YtgP (Spy_0390) complements *Escherichia coli* strains depleted of the putative peptidoglycan flippase MurJ. Antimicrob Agents Chemother. 2009; 53(8):3604–3605. [PubMed: 19528283]

Ruzin A, Severin A, Ritacco F, Tabei K, Singh G, Bradford PA, Siegel MM, Projan SJ, Shales DM. Further evidence that a cell wall precursor [C(55)-MurNAc-(peptide)-GlcNAc] serves as an acceptor in a sorting reaction. J Bacteriol. 2002; 184(8):2141–2147. [PubMed: 11914345]

Ryu YH, Baik JE, Yang JS, Kang SS, Im J, Yun CH, Kim DW, Lee K, Chung DK, Ju HR, Han SH. Differential immunostimulatory effects of Gram-positive bacteria due to their lipoteichoic acids. Int Immunopharmacol. 2009; 9(1):127–133. [PubMed: 19013542]

Saar-Dover R, Bitler A, Nezer R, Shmuel-Galia L, Firon A, Shimoni E, Trieu-Cuot P, Shai Y. D-alanylation of lipoteichoic acids confer resistance to cationic peptides in Group B *Streptococcus* by increasing the cell wall density. PLoS Pathog. 2012; 8(9):e1002891. [PubMed: 22969424]

Saas V, Schneider T, Wilmes M, Körner C, Tossi A, Novikova N, Shamova O, Sahl HG. Human β-defensin 3 inhibits cell wall biosynthesis in staphylococci. Infect Immun. 2010; 78(6):2793–2800. [PubMed: 20385753]

Santa Maria JP, Sadaka A, Moussa SH, Brown S, Zhang YJ, Rubin EJ, Gilmore MS, Walker S. Compound-gene interaction mapping reveals distinct roles for Staphylococcus aureus teichoic acids. Proc Natl Acad Sci U S A. 2014; 111(34):12510–12515. [PubMed: 25104751]

Sauvage E, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev. 2008; 32(2):234–258. [PubMed: 18266856]

Scheffers DJ, Pinho MG. Bacterial cell wall synthesis: new insights from localization studies. Microbiol Mol Biol Rev. 2005; 69(4):585–607. [PubMed: 16339737]

Schirner K, Eun YJ, Dion M, Luo Y, Helmann JD, Garner EC, Walker S. Lipid-linked cell wall precursors regulate membrane association of bacterial actin MreB. Nat Chem Biol. 2015; 11(1):38–45. [PubMed: 25402772]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Schirner K, Marles-Wright J, Lewis RJ, Errington J. Distinc and essential morphogenic functions for wall- and lipo-teichoic acids in *Bacillus subtilis*. EMBO J. 2009; 28(7):830–842. [PubMed: 19229300]

Schlag M, Biswas R, Krismer B, Kohler T, Zoll S, Yu W, Schwarz H, Peschel A, Götz F. Role of staphylococcal wall teichoic acid in targeting the major autolysin Atl. Mol Microbiol. 2010; 75(4):864–873. [PubMed: 20105277]

Schleifer KH, Kandler O. Peptidoglycan. Types of Bacterial Cell Walls and their Taxonomic Implications. Bacterial Rev. 1972; 36(4):407–477.

Schneewind O, Missiakas D. Lipoteichoic acids, phosphate-containing polymers in the envelope of Gram-positive bacteria. J Bacteriol. 2014; 196(6):1133–1142. [PubMed: 24415723]

Schneewind O, Missiakas DM. Protein secretion and surface display in Gram-positive bacteria. Philos Trans R Soc Lond B Biol Sci. 2012; 367(1592):1123–1139. [PubMed: 22411983]

Schneider T, Kruse T, Wimmer R, Wiedemann I, Saas V, Pag U, Jansen A, Nielsen AK, Mygind PH, Raventós DS, Soren N, Ravn B, Bonvin AM, De Maria L, Andersen AS, Gammelgaard LK, Sahl HG, Kristensen HH. Plectasin, a fungal defensin targets the bacterial cell wall precursor Lipid II. Science. 2010; 328:1168–1172. [PubMed: 20508130]

Schneider T, Senn MM, Berger-Bächi B, Tossi A, Sahl HG, Wiedermann I. *In vitro* assembly of a complete interpeptide bridge containing cell wall precursor (lipid-II-Gly5) of *Staphylococcus aureus*. Mol Microbiol. 2004; 53(2):675–685. [PubMed: 15228543]

Schmidt JW, Greenough A, Burns M, Luteran AE, McCafferty DG. Generation of ramoplanin-resistant *Staphylococcus aureus*. FEMS Microbiol Lett. 2010; 310(2):104–111. [PubMed: 20659164]

Schröder NW, Morath S, Alexander C, Hamann L, Hartung T, Zähringer U, Göbel UB, Weber JR, Schumann RR. Lipoteichoic acid (LTA) of *Streptococcus pneumoniae* and *Staphylococcus aureus* activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide binding protein (LBP) and CD14, whereas TLR-4 and MD-2 are not involved. J Biol Chem. 2003; 278(18):15587–15594. [PubMed: 12594207]

Severin A, Tomasz A. Naturally Occurring Peptidoglycan Variants of *Streptococcus pneumoniae*. J Bacteriol. 1996; 178(1):168–174. [PubMed: 8550412]

Sham LT, Butler EK, Lebar MD, Kahne D, Bernhardt TG, Ruiz N. Bacterial cell wall. MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. Science. 2014; 345(6193):220–222. [PubMed: 25013077]

Shapiro ED, Berg AT, Austrian R, Shroeder D, Parcells V, Margolis M, Adair RK, Clemens JD. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N Engl J Med. 1991; 325(21):1453–1460. [PubMed: 1944423]

Shimada T, Park BG, Wolf AJ, Brikos C, Goodridge HS, Becker CA, Reyes CN, Miao EA, Aderem A, Götz F, Liu GY, Underhill DM. *Staphylococcus aureus* evades lysozyme-based peptidoglycan digestion that links phagocytosis, inflammasome activation, and IL-1beta secretion. Cell Host Microbe. 2010; 7(1):38–49. [PubMed: 20114027]

Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC. Vancomycin-resistant *Staphylococcus aureus* in the United States, 2002–2006. Clin Infect Dis. 2008; 46(5):668–674. [PubMed: 18257700]

Silhavy TJ, Kahne D, Walker S. The Bacterial Cell Envelope. Cold Spring Harb Perspect Biol. 2010; 2:a000414. [PubMed: 20452953]

Silver L. Viable screening targets related to the bacterial cell wall. Ann N Y Acad Sci. 2013; 1277:29–53. [PubMed: 23278681]

Skurnik D, Kropec A, Roux D, Theilacker C, Huebner J, Pier GB. Natural antibodies in normal human serum inhibit *Staphylococcus aureus* capsular polysaccharide vaccine efficacy. Clin Infect Dis. 2012; 55(9):1188–1197. [PubMed: 22806596]

Sobhanifar S, Worrall LJ, Gruninger RJ, Wasney GA, Blaukopf M, Baumann L, Lameignere E, Solomonson M, Brown ED, Withers SG, Strynadka NC. Structure and mechanism of *Staphylococcus aureus* TarM, the wall teichoic acid α-glycosyltransferase. Proc Natl Acad Sci U S A. 2015; 112(6):E576–E585. [PubMed: 25624472]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



Sorensen UB, Henrichsen H, Chen HC, Szu SC. Covalent linkage between the capsular polysaccharide and the cell wall peptidoglycan of *Streptococcus pneumoniae* revealed by immunological methods. Microb Pathog. 1990; 8(5):325–334. [PubMed: 2215183]

Spratt BG. Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. Proc Natl Acad Sci U S A. 1975; 72(8):2999–3003. [PubMed: 1103132]

Steens A, Vestrheim DF, Aaberge IS, Wiklund BS, Storsaeter J, Riise Bergsaker MA, Ronning K, Furuseth E. A review of the evidence to inform pneumococcal vaccine recommendations for risk groups aged 2 years and older. Epidemeol Infect. 2014; 142(12):2471–2482.

Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem. 2000; 69:183–215. [PubMed: 10966457]

Sutherland IW. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology. 2001; 147(Pt 1):3–9. [PubMed: 11160795]

Suzuki T, Campbell J, Swoboda JG, Walker S, Gilmore MS. Role of wall teichoic acids in *Staphylococcus aureus* endophthalmitis. Invest Ophthalmol Vis Sci. 2011a; 52(6):3187–3192. [PubMed: 21345983]

Suzuki T, Swoboda JG, Campbell J, Walker S, Gilmore MS. *In vitro* antimicrobial activity of wall teichoic acid biosynthesis inhibitors against *Staphylococcus aureus* isolates. Antimicrob Agents Chemother. 2011b; 55(2):767–774. [PubMed: 21098254]

Swoboda JG, Meredith TC, Campbell J, Brown S, Suzuki T, Bollenbach T, Malhowski AJ, Kishony R, Gilmore MS, Walker S. Discovery of a small molecule that blocks wall teichoic acid biosynthesis in *Staphylococcus aureus*. ACS Chem Biol. 2009; 4(10):875–883. [PubMed: 19689117]

Terrak M, Nguyen-Distéche M. Kinetic characterization of the monofunctional glycosyltransferase from *Staphylococcus aureus*. J Bacteriol. 2006; 188(7):2528–2532. [PubMed: 16547040]

Theilacker C, Holst O, Lindner B, Huebner J, Kacyoski Z. The structure of the wall teichoic acid isolated from *Enterococcus faecalis* strain 12030. Carbohydr Res. 2012; 354:106–109. [PubMed: 22551470]

Theilacker C, Kaczy ski Z, Kropec A, Fabretti F, Sange T, Holst O, Huebner J. Opsonic antibiodies to *Enterococcus faecalis* strain 12030 are directed against lipoteichoic acid. Infect Immun. 2006; 74(10):5703–5712. [PubMed: 16988246]

Thumm G, Götz F. Studies on prolysostaphin processing and characterization of the lysostaphin immunity factor (Lif) of *Staphylococcus simulans* biovar staphylolyticus. Mol Microbiol. 1997; 23(6):1251–1255. [PubMed: 9106216]

Thurlow LR, Thomas VC, Fleming SD, Hancock LE. *Enterococcus faecalis* capsular polysaccharide serotypes C and D and their contributions to host innate immune evasion. Infect Immun. 2009; 77(12):5551–5557. [PubMed: 19805541]

Tschierske M, Ehlert K, Strandén AM, Berger-Bächi B. Lif, the lysostaphin immunity factor, complements FemB in staphylococcal peptidoglycan interpeptide bridge formation. FEMS Microbiol Lett. 1997; 153(2):261–264. [PubMed: 9271851]

Tzianabos AO, Wang JY, Lee JC. Structural rationale for the modulation of abscess formation by *Staphylococcus aureus* capsular polysaccharides. Proc Natl Acad Sci U S A. 2001; 98(16):9365–9370. [PubMed: 11470905]

Valentino MD, Foulston L, Sadaka A, Kos VN, Villet RA, Santa Maria J Jr, Lazinski DW, Camili A, Walker S, Hooper DC, Gilmore M. Genes contributing to *Staphylococcus aureus* fitness in abscess- and infection-related ecologies. mBio. 2014; 5(5):e01729–e01714. [PubMed: 25182329]

Van Selm S, van Cann LM, Kolkman MA, van der Zeijst BA, van Putten JP. Genetic basis for the structural difference between *Streptococcus pneumoniae* serotype 15B and 15C capsular polysaccharides. Infect Immun. 2003; 71(11):6192–6198. [PubMed: 14573636]

Venkateswaran PS, Stanton N, Austrian R. Type variation of strains of *Streptococcus pneumoniae* in capsular serogroup 15. J Infect Dis. 1983; 147(6):1041–1054. [PubMed: 6854063]

Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R. Sticking together: building a biofilm the *Bacillus subtilis* way. Nat Rev Microbiol. 2013; 11(3):157–168. [PubMed: 23353768]

Volkman BF, Zhang Q, Debabov DV, Rivera E, Kresheck GC, Neuhaus FC. Biosynthesis of D-alanyl-lipoteichoic acid: the tertiary structure of apo-D-alanyl carrier protein. Biochemistry. 2001; 40(27):7964–7972. [PubMed: 11434765]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Vollmer W, Blanot D, de Pedro MA. Peptidoglycan structure and architecture. FEMS Microbiol Rev. 2008; 32(2):149–167. [PubMed: 18194336]

Vollmer W, Tomasz A. The *pgdA* gene encodes for a peptidoglycan *N*-acetylglucosamine deacetylase in *Streptococcus pneunomiae*. J Biol Chem. 2000; 275(27):20496–20501. [PubMed: 10781617]

Vollmer W, Tomasz A. Peptidoglycan *N*-acetylglucosamine deacetylase, a putative virulence factor in *Streptoccus pneumoniae*. Infect Immun. 2002; 70(12):7176–7178. [PubMed: 12438406]

Von Aulock S, Hartung T, Hermann C. Comment on "Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compound in *Staphylococcus aureus*". J Immunol. 2007; 178(5):2610–2611. [PubMed: 17312096]

Vuong C, Kocianova S, Voyich JM, Yao Y, Fischer ER, DeLeo FR, Otto M. A critical role for exopolysaccharide modification in bacterial biofilm formation, immune evasion and virulence. J Biol Chem. 2004; 279(52):54881–54886. [PubMed: 15501828]

Wada A, Watanabe H. Pencillin-binding protein 1 of *Staphylococcus aureus* is essential for growth. J Bacteriol. 1998; 180(10):2759–2765. [PubMed: 9573165]

Walsh, C. Antibiotics: actions, origins, resistance. Washington D.C.: ASM Press; 2003. Antibiotics that act on cell wall biosynthesis; p. 24-49.

Walsh CT. Enzymes in the D-alanine branch of bacterial cell wall peptidogylcan assembly. J Biol Chem. 1989; 264(5):2393–2396. [PubMed: 2644260]

Walsh CT, Howe RA. The prevalence and mechanisms of vancomycn resistance in *Staphylococcus aureus*. Ann Rev Microbiol. 2002; 56:657–675. [PubMed: 12142482]

Wang H, Gill CJ, Lee SH, Mann P, Zuck P, Meredith TC, Murgolo N, She X, Kales S, Liang L, Liu J, Wu J, Santa Maria JS, Su J, Pan J, Hailey J, Mcguinness D, Tan CM, Flattery A, Walker S, Black T, Roemer T. Discovery of novel wall teichoic acid inhibitors as effective anti-MRSA β-lactam combination agents. Chem Biol. 2013; 20(2):272–284. [PubMed: 23438756]

Ward JB. The Chain Length of the Glycans in Bacterial Cell Walls. J Biochem. 1973; 133(2):395–398.

Waxman D, Strominger JL. Penicillin-binding proteins and the mechanism of action of β-lactam antibiotics. Annu Rev Microbiol. 1983; 52:825–829.

Weart RB, Lee AH, Chien AC, Haeusser DP, Hills NS, Levin PA. A metabolic sensor governing cell size in bacteria. Cell. 2007; 130(2):335–347. [PubMed: 17662947]

Webb AJ, Karatsa-Dodgson M, Gründling A. Two-enzyme systems for glycolipid and polyglycerophosphate lipoteichoic acid synthesis in *Listeria monocytogenes*. Mol Microbiol. 2009; 74(2):299–314. [PubMed: 19682249]

Weber B, Ehlert K, Diehl A, Reichmann P, Labischinski H, Hakenbeck R. The fib locus in *Streptococcus pneumoniae* is required for peptidoglycan crosslinking and PBP-mediated β-lactam resistance. FEMS Microbiol Lett. 2000; 188(1):81–85. [PubMed: 10867238]

Weidenmaier C, Kokai-Kun JF, Kristian SA, Chanturiya T, Kalbacher H, Gross M, Nicholson G, Neumeister B, Mond JJ, Peschel A. Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med. 2004; 10(3):243–245. [PubMed: 14758355]

Weidenmaier C, Peschel A, Xiong YQ, Kristian SA, Dietz K, Yeaman MR, Bayer AS. Lack of wall teichoic acids in *Staphylococcus aureus* leads to reduced interactions with endothelial cells and to attenuated virulence in rabbit model endocarditis. J Infect Dis. 2005; 191(10):1771–1777. [PubMed: 15838806]

Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, Kolonay JF, Shetty J, Killgore GE, Tenover FC. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. Science. 2003; 302(5650):1569–1571. [PubMed: 14645850]

White AR, Kaye C, Poupard J, Pypstra R, Woodnutt G, Wynne B. Augmentin® (amoxicillin/clavulanate) in the treatment of community-acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent. J Antimicrob Chemother. 2004; 53(Suppl 1):i3–i20. [PubMed: 14726431]

Whitener CJ, Park SY, Browne FA, Parent LJ, Julian K, Bozdogan B, et al. Vancomycin-resistant *Staphylococcus aureus* in the absense of vancomycin exposure. Clin Infect Dis. 2004; 38(8):1049–1055. [PubMed: 15095205]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Wilkinson BJ, Holmes KM. *Staphylococcus aureus* cell surface: capsule as a barrier to bacteriophage adsorption. Infect Immun. 1979; 23(2):549–552. [PubMed: 154475]

Winstel V, Kühner P, Salomon F, Larsen J, Skov R, Hoffmann W, Peschel A, Weidenmaier C. Wall teichoic acid glycosylation governs *Staphylococcus aureus* nasal colonization. mBio. 2015; 6(4):e00632–e00615. [PubMed: 26126851]

Wörmann ME, Corrigan RM, Simpson PJ, Matthews SJ, Gründling A. Enzymatic activities and functional interdependencies of *Bacillus subtilis* lipoteichoic acid synthesis enzymes. Mol Microbiol. 2011; 79(3):566–583. [PubMed: 21255105]

Wright G. Molecular mechanism of antibiotic resistance. Chem Comm. 2011; 47:4055–4061. [PubMed: 21286630]

Wyke AW, Ward JB, Hayes MV, Curtis NA. A role *in vivo* for penicillin-binding protein-4 of *Staphylococcus aureus*. Eur J Biochem. 1981; 119(2):389–393. [PubMed: 7308191]

Xayarath B, Yother J. Mutations blocking side chain assembly, polymerization, or transport of a Wzy-dependent *Streptococcus pneumoniae* capsule are lethal in the absence of suppressor mutations and can affect polymer transfer to the cell wall. J Bacteriol. 2007; 189(9):3369–3381. [PubMed: 17322316]

Xia G, Maier L, Sanchez-Carballo P, Li M, Otto M, Holst O, Peschel A. Glycosylation of wall teichoic acid in *Staphylococcus aureus* by TarM. J Biol Chem. 2010; 285(18):13405–13415. [PubMed: 20185825]

Xu H, Wang L, Huang J, Zhang Y, Ma F, Wang J, Xu W, Zhang X, Yin Y, Wu K. Pneumococcal wall teichoic acid is required for pathogenesis of *Streptococcus pneumoniae* in murine models. J Microbiol. 2015; 53(2):147–154. [PubMed: 25626371]

Yang SJ, Kreiswirth BN, Sakoulas G, Yeaman MR, Xiong YQ, Sawa A, Bayer AS. Enhanced expression of *dltABCD* is associated with the development of daptomycin nonsusceptibility in a clinical endocarditis isolate of *Staphylococcus aureus*. J Infect Dis. 2009; 200(12):1916–1920. [PubMed: 19919306]

Yang SJ, Bayer AS, Mishra NN, Meehl M, Ledala N, Yeaman MR, Xiong YQ, Cheung AL. The *Staphylococcus aureus* two-component sensing system, GraRS, senses and confers resistance to selected cationic antimicrobial peptides. Infect Immun. 2012; 80(1):74–81. [PubMed: 21986630]

Yocum RR, Rasmussen JR, Strominger JL. The mechanism of action of penicillin. Penicillin acylates the active site of *Bacillus stearothermophilus* D-alanine carboxypeptidase. J Biol Chem. 1980; 255(9):3977–3986. [PubMed: 7372662]

Yocum RR, Waxman DJ, Rasmussen JR, Strominger JL. Mechanism of penicillin action: penicillin and substrate bind covalently to the same active site serine in two bacterial D-alanine carboxypeptidases. Proc Natl Acad Sci U S A. 1979; 76(6):2730–2734. [PubMed: 111240]

Yonus H, Neumann P, Zimmermann S, May JJ, Marahiel MA, Stubbs MT. Crystal structure of DltA. Implications for the reaction mechanism of non-ribosomal peptide synthetase adenylation domains. J Biol Chem. 2008; 283(47):32484–32491. [PubMed: 18784082]

Yother J. Capsules of *Streptococcus pneumoniae* and other bacteria: paradigms for polysaccharide bioynthesis and regulation. Annu Rev Microbiol. 2011; 65:563–581. [PubMed: 21721938]

Young FE. Requirement of glucosylated teichoic acid for adsorption of phage in *Bacillus subtilis* 168. Proc Natl Acad Sci U S A. 1967; 58(6):2377–2384. [PubMed: 4969329]

Zapun A, Vernet T, Pinho MG. The different shapes of cocci. FEMS Microbiol Rev. 2008; 32(2):345–360. [PubMed: 18266741]

Zhang YM, Rock CO. Membrane lipid homeostasis in bacteria. Nat Rev Microbiol. 2008; 6(3):222–233. [PubMed: 18264115]

Zhen M, Jacobsen FE, Giedroc DP. Metal transporters and metal sensors: how coordination chemistry controls bacterial metal homeostasis. Chem Rev. 2009; 109(10):4644–4681. [PubMed: 19788177]

Zhu W, Clark NC, McDougal LK, Hageman J, McDonald LC, Patel JB. Vancomycin-resistant *Staphylococcus aureus* isolates associated with Inc18-like *vanA* plasmids in Michigan. Antimicrob Agents Chemother. 2008; 52(2):452–457. [PubMed: 18056272]

Zipperle GF Jr, Ezzell JW Jr, Doyle RJ. Glucosamine substitution and muramidase susceptibility in *Bacillus anthracis*. Can J Microbiol. 1984; 30(5):553–559. [PubMed: 6430537]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



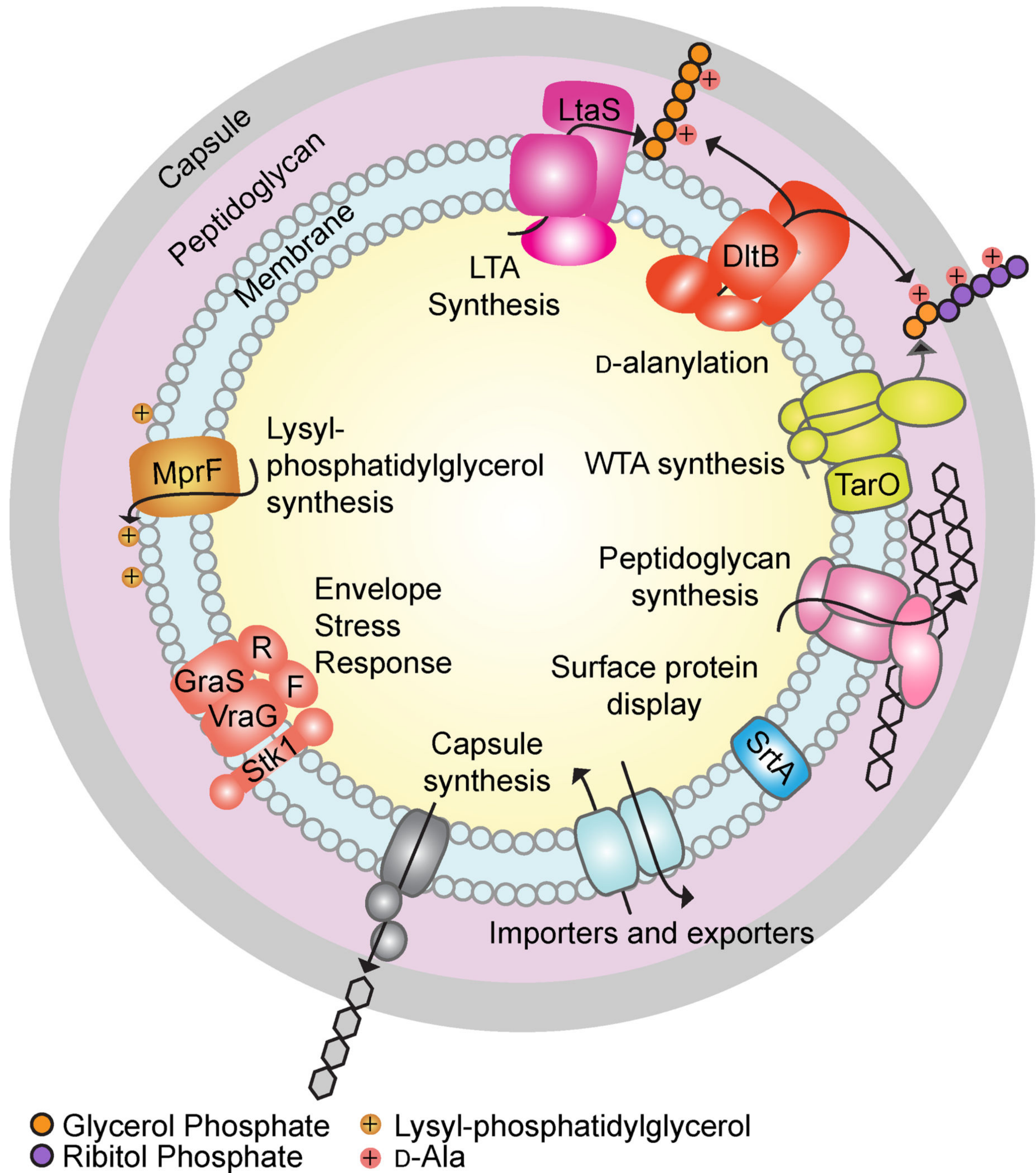
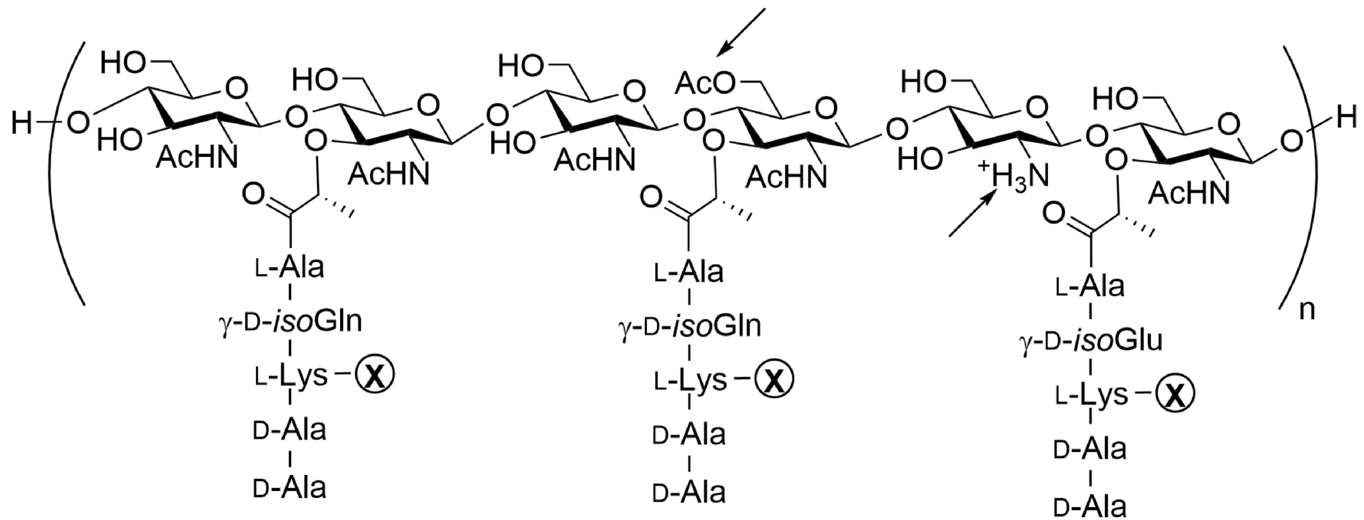



**Fig 1.**

The Gram-positive cell envelope. The complex Gram-positive cell envelope is the first line of defense for the organism. Here, the *S. aureus* envelope is shown as an example. Major pathways involved in the synthesis of the cell envelope include capsule, PG and TA synthesis. TAs can be modified by D-alanlyation. D-alanylation and lysylphosphatidylglycerol synthesis are known factors for antibiotic resistance. Envelope stress response regulators modulate the organism's response to toxic molecules or conditions that perturb the cell envelope. Importers and exporters, ubiquitously present among bacteria,

serve the necessary role of channeling in nutrients and pumping out toxic molecules. Finally, surface protein display systems function to tether proteins to the cell membrane or cell wall, which perform important roles in adhesion and interaction with the environment.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript




X = $(Gly)_5$ in *S. aureus*
L-Ala-L-Ser in *S. pneumoniae*
L-Ala-L-Ala in *E. faecalis*
D-Asp in *E. faecium*

*O*-Acetylation

*N*-Deacetylation

**Fig 2.**

PG structure, and common variations. PG consists of chains of alternating GlcNAc and MurNAc residues. The MurNAc residues are functionalized with pentapeptide units which are cross-linked via the substituents on L-Lys to generate the mature PG. The linear glycan chain is highly conserved across both Gram-positives and Gram-negatives. The stem pentapeptide is well conserved across Gram-positives, aside from *B. subtilis* which contains *meso*-diaminopimmelic acid instead of L-Lysine at position 3 of the stem pentapeptide. There is considerable variation in the substituents on the L-Lys across Gram-positive species as indicated. PG can be modified by *O*-acetylation of MurNAc or *N*-deacetylation of GlcNAc moieties in response to challenge from antimicrobials such as lysozyme.



Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

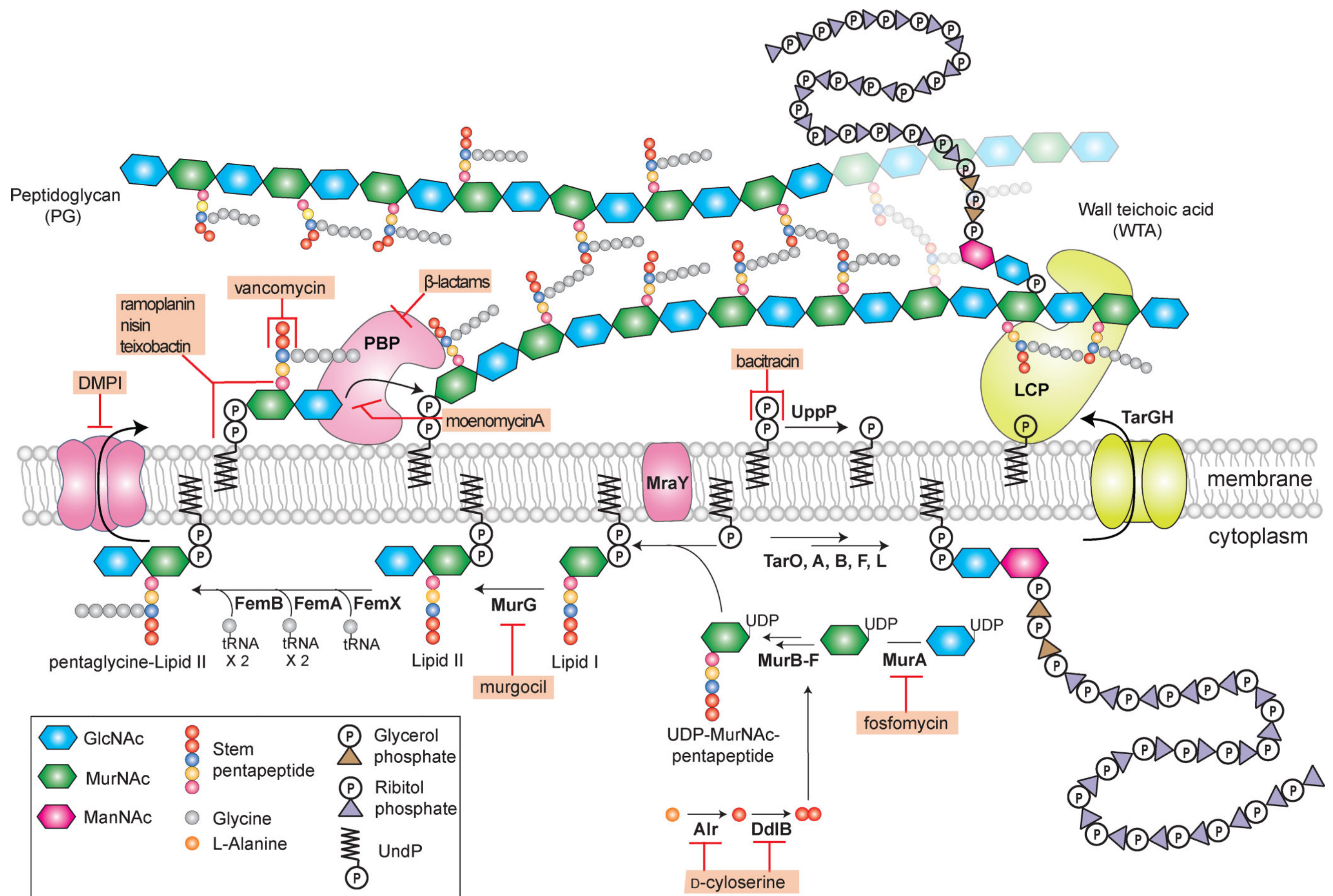



**Fig 3.**

Synthesis of PG and antibiotics that target PG synthesis. The enzymatic steps for PG synthesis are well conserved across species. Here, the biosynthesis of *S. aureus* PG is shown as an example. The synthesis begins with the assembly of the GlcNAc-MurNAc-pentapeptide and its attachment to carrier lipid Und-P in the cell membrane. After this point, the L-Lysine at position 3 is substituted with additional amino acids and then flipped to the outside of the cell where it is cross-linked by PBPs. The same lipid carrier is also utilized for WTA (shown here) and capsule synthesis. The synthesis of PG is crucial to the cell and over time, several antibiotics have been discovered that target various steps in PG biosynthesis.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

| Strain | WTA structure | Reference |
| --- | --- | --- |
| *S. aureus*<br>*B. subtilis* W23 | —GlcNAc−ManNAc(GroP)$_{1\text{-}3}$(RboP)$_n$— | Brown et al. 2010<br>Brown et al. 2013<br>Pereria and Brown, 2009 |
| *B. subtilis* 168 | —GlcNAc−ManNAc(GroP)$_n$— | |
| *S. pneumoniae* | (2-acetamido-4-amino-2,4,6-trideoxyGal—Gluc—RboP−GalNAc−GalNAc—)$_n$<br>choline-P choline-P | Denapite et al. 2012 |
| *E. faecalis* 12030 | Gluc<br>(Gal—GalNAc—GlcNAc—Gal—RboP)$_n$ | Theilacker et al. 2012 |
| *E. faecium* U0317 | (GalNAc−GalNAc−GroP)$_n$ | Bychowska et al. 2012 |

**Fig 4.**

WTA structure, and common variations. WTAs are anionic polymers with a sugar-phosphate backbone attached to the C6 position of MurNAc in PG. The structure of WTAs is highly variable across Gram-positive species. WTA polymer structures for specific strains are indicated here with the following abbreviations: Glycerol-phosphate (GroP), Ribitol Phosphate (RboP), *N*-acetylmannosamine (ManNAc), Galactose (Gal), Glucose (Gluc), *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine (GlcNAc), phosphorylcholine (choline-P).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

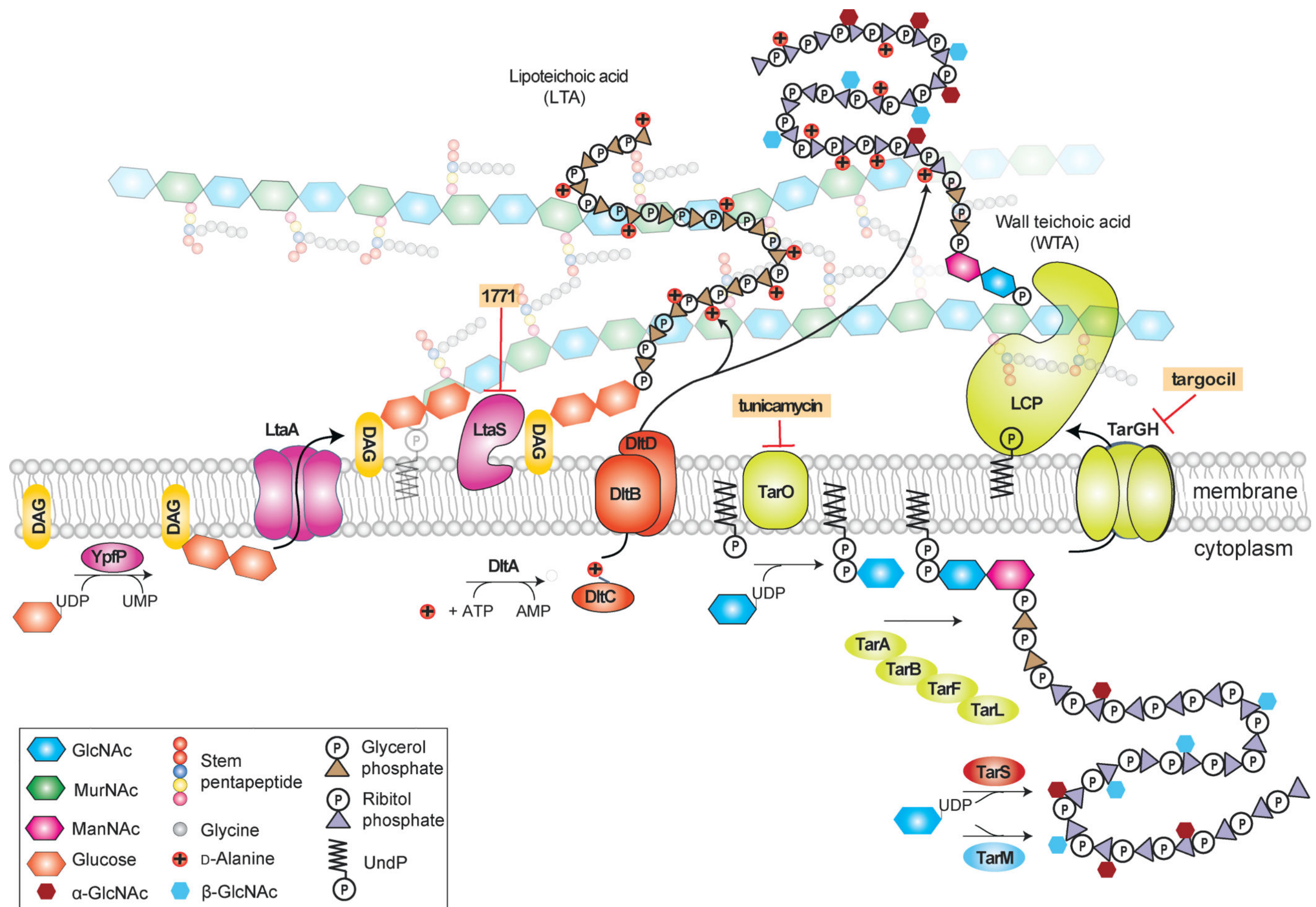



**Fig 5.**

TA biosynthesis and modification pathways. LTA and WTA biosynthetic pathways in *S. aureus* are shown here. Although both are anionic sugar-phosphate backbones, they are assembled differently by separate biosynthetic pathways in *S. aureus*. TAs are further modified with D-alanine residues by the *dlt* pathway and with α- or β- GlcNAC residues installed by glycosyltransferases TarM and TarS, respectively. TAs perform several functions for the cell including playing roles in biofilm formation, adhesion, phage attachment, virulence and antibiotic resistance, most notably resistance to β-lactams. D-alanylation has been shown to play an important role in these functions as well. Specifically, the absence of D-alanine modifications sensitizes to cationic antimicrobial peptides, including host defensins. The only known roles for α- and β- GlcNAC modifications are in phage attachment, and for β-GlcNAcs, in β-lactam resistance. Due to its roles in adhesion, virulence and antibiotic resistance, attempts are being made to target TA biosynthesis and modification pathways. The known compounds targeting these pathways are shown here.