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Glycopeptide resistance: links with inorganic phosphate metabolism and cell envelope stress

Fernando Santos-Beneit¹, María Ordóñez-Robles², Juan F. Martín³

1) Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Medical
5 School, Newcastle University, NE2 4AX, Newcastle upon Tyne, UK

2) Department of Biotechnology, Faculty of Natural Sciences and Technology, Norwegian
University of Science and Technology, NO-7491, Trondheim, Norway

3) Microbiology Area, Department of Molecular Biology, University of León, 24071, León,
Spain

10 Abstract

Antimicrobial resistance is a critical health issue today. Many pathogens have become resistant to many or all available antibiotics and limited new antibiotics are in the pipeline. Glycopeptides are used as a ‘last resort’ antibiotic treatment for many bacterial infections, but worryingly, glycopeptide resistance has spread to very important
15 pathogens such as *Enterococcus faecium* and *Staphylococcus aureus*. Bacteria confront multiple stresses in their natural environments, including nutritional starvation and the action of cell-wall stressing agents. These stresses impact bacterial susceptibility to different antimicrobials. This article aims to review the links between glycopeptide resistance and different stresses, especially those related with cell-wall biosynthesis and
20 inorganic phosphate metabolism, and to discuss promising alternatives to classical antibiotics to avoid the problem of antimicrobial resistance.

Corresponding author: Juan F. Martín.

E-mail: jf.martin@unileon.es

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- **Antibiotic resistance**
- 30 • **Cell-wall stress**

1. Introduction: the problem of antibiotic resistance

In recent years the world has been facing two important and linked problems: i) the emergence and spread of antibiotic resistance in pathogenic bacteria and ii) failure to discover new chemotherapeutic agents. Antibiotic resistance in pathogenic bacteria does not only generate an important economic burden for the healthcare system, but also reduces the efficacy of antibiotics and increases mortality. Thus, antibiotic resistance is considered a worldwide threat for human health, since once treatable infections may be fatal again. There was a time in which clinics were revolutionized by the introduction of antibiotics, which enabled the treatment of infections and the performance of invasive surgeries without fear. Those times have come to an end, and we have already entered into what is called the “resistance era” (1).

The “antibiotic era” started with the discovery of penicillin and sulfonamides in the late 1920s and 1930s. A “Golden Age” for antibiotic discovery then started, which continued until the 1960s. In that period, most of the antibiotic classes used today were discovered. Penicillin, the first antibiotic of natural origin commercialized, was discovered in 1928 by Alexander Fleming (2). After the introduction of penicillin into clinics in the 1940s, its extensive use against *Streptococcus pneumoniae* led to the first report on a penicillin-resistant strain in 1967 (3). But the development of resistance had been observed before, as Fleming advertised in his Nobel Prize speech in 1945. He had noted that penicillin resistant microbes were obtained easily in the laboratory, and warned that the same thing might occur in the human body.

Antibiotic resistance is a result of Darwinian selection, and there are no examples of antibiotics to which bacteria have not developed resistance within a few years of their use; being the emergence of resistance to the last-resort antibiotic, colistin, one of the

latest examples (4-6). Resistance to antibiotics can be gained by mutations or acquisition of antibiotic resistance genes. In the latter, horizontal gene transfer by conjugation is considered the most important mechanism, although others processes (such as transformation or phage-mediated transduction) might play a larger part than previously expected; recently reviewed by von Wintersdorff and co-workers (7). The spontaneous generation of resistant strains by mutations, even before any exposure to the antibiotic, is a phenomenon known since 1955 (8).

A large number of resistance genes of clinical relevance reportedly have their origin in non-pathogenic bacteria (7). In fact, antibiotic resistance genes are as ancient as the biosynthetic equivalents, and have been detected in the DNA of 30,000-year-old permafrost samples, as well as in the microbiome of caves that have been isolated for millions of years (9, 10). In spite of being a natural process, the threat of antibiotic resistance is increasingly alarming because of the acceleration of its rate of appearance. The main factor for this acceleration is the misuse of antibiotics through excessive use in human and veterinary clinics, and as growth promoters in farming. This excessive use has led to an increased transmission of resistance elements that has facilitated the selection of resistant bacteria.

According to the Center for Disease Control and Prevention (CDC), it is estimated that more than 2 million people are infected with antibiotic resistant microbes in one year in the USA and, of them, more than 23,000 die due to the infection. The prognostics for the future are also alarming; in 2050, 10 million deaths per year are predicted worldwide due to antibiotic resistance (11). Thus, it is clear that the correct use of antibiotics is necessary to slow down the rate of the emergence of drug resistance, and that a new arsenal of antibiotics is urgently required. However, the introduction of new chemotherapeutics into clinical practice has been very slow and only one antibiotic from

a new family, named teixobactin, has been discovered recently (see below). To overtake this problem, several drugs that were discarded in the past are currently gaining attention. For example fidaxomicin, originally discovered in the 1970s, was approved for use in 2011 by the US Food and Drug Administration (FDA), and has been successfully applied to the treatment of *Clostridium difficile* in recent years (12, 13). Ancient remedies and natural medicine are also gaining attention for the treatment of infections. For example, an “ancientbiotic” from the 10th century has been proven to be effective against *Staphylococcus aureus* (14). Similarly, a natural clay from Canada has been shown to act as a potent antibacterial against multi drug-resistant ESKAPE strains, an acronym referring to *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species, which are all important pathogens responsible for nosocomial infections around the world (15, 16).

2. Cell-wall as an antibiotic target

The majority of antimicrobials used currently target the bacterial DNA, RNA, protein or cell-wall biosynthesis. In this section we will focus on drugs acting on the biosynthesis of the main component of the cell-wall, peptidoglycan (PG). PG is a polymer composed of glycan strands crosslinked by peptides. These glycan strands are formed by alternating units of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). MurNAc is associated with peptides responsible for crosslinking the PG strands. The crosslink between peptide chains can be direct (in most Gram-negative bacteria) or indirect, through an interpeptide bridge (in most Gram-positive bacteria). It is important to note that variations in PG composition exist depending on the species, for example regarding glycan chain length, the nature of the residues in the peptide stem or the length and nature of the residues in the cross-linking bridge (17, 18). In all cases,

PG confers rigidity to the cell, defining shape and increasing mechanical strength. It also serves as a scaffold for other components such as proteins or teichoic acids (17).

PG biosynthesis involves two different phases: the first takes place in the cytoplasm (19) and the second is associated with the cell membrane (20). The first step of PG

110 synthesis is the formation of an UDP-N-acetylmuramic acid pentapeptide in the cytoplasm (see Fig 1). In this phase, MurA and MurB generate UDP-MurNAc from phosphoenol pyruvate and UDP-GlcNAc. Subsequently, the ATP-dependent ligases MurC, MurD, MurE and MurF link five amino acid residues to form UDP-N-acetylmuramic acid pentapeptide. MurF then catalyzes the incorporation of the

115 dipeptide D-Ala-D-Ala, and also involves the alanine racemase (Alr) and the alanine ligase (Ddl). The UDP-N-acetylmuramic acid pentapeptide is then anchored to the membrane-embedded undecaprenyl phosphate by the action of the phospho-MurNAc-pentapeptide translocase MraY, generating Lipid I. Addition of GlcNAc to Lipid I by the glycosyltransferase MurG yields Lipid II, which is then translocated through the

120 membrane to the periplasm. This step requires the activity of proteins named flippases, which remain unidentified. In fact, controversy exists about which of the potential candidates proposed are involved; recently reviewed by Ruiz (21). In the periplasm, polymerization and other processing reactions result in the formation of the PG mesh.

Polymerization of glycan strands and cross-linking between glycan chains takes place 125 through transglycosylation and transpeptidation reactions, respectively, which are catalyzed by penicillin-binding proteins (PBPs). These enzymes receive this acronym because they are able to bind penicillin. Please refer to the work by Sauvage and co-workers (22) for an in-depth review about PBPs structure and role on PG biosynthesis.

The majority of chemotherapeutic agents used in the clinic inhibit the lipid-linked steps 130 of the PG synthetic pathway (see Figure 1), although the cytoplasmic steps are also

considered good targets for new antibiotics (23). **Fosfomycin**, useful against both Gram-positive and Gram-negative bacteria, is a unique Mur inhibitor currently used in the clinic. This compound acts by alkylating a conserved cysteine residue in MurA, which results in its irreversible inhibition (24). In addition, several natural products that act as MurB or other Mur inhibitors have been discovered; although their specificities *in vivo* have not been demonstrated yet (25-27). Most of the known Mur ligase inhibitors show little or no antibacterial activity, which might be related with the arrangement of the Mur enzymes in multiprotein complexes *in vivo* (28). **D-cycloserine**, an analog of D-Alanine, targets both Alr and Ddl and is a cornerstone among the second-line chemotherapeutic agents in the treatment of multi drug-resistant *Mycobacterium tuberculosis* infections (29). The limited use of D-cycloserine is due to the prevalence of neurological side effects associated with its application (30, 31). Although MraY is an essential enzyme and is the target of several antibiotics (tunicamycin, mureidomycin, liposidomycin, capuramycin and michellamine B), no chemotherapeutic drugs directed against this enzyme are currently in clinical use (32-36). No specific ligands have been developed to target proteins involved in flipping Lipid II across the membrane. Regarding the membrane-linked steps of PG biosynthesis, the complex **moenomycin** (also known as flavomycin) and the antibiotic **ramoplanin** inhibit the transglycosylation steps (37, 38). Moenomycin is used for animal feeding and has been reported to be effective against *Helicobacter pylori* (39). Ramoplanin shows potent activity against *C. difficile* and is being developed as an alternative treatment for this disease (40, 41). The main group of antibiotics that act during the membrane phase of PG biosynthesis is the **β -lactams**, which share a common core (the β -lactam ring) and display a broad spectrum of antibacterial activity. β -lactams are analogs of the D-Ala-D-Ala dipeptides and thus are recognized by transpeptidases, resulting in the irreversible

inhibition of these enzymes (42-44). **Glycopeptide** antibiotics, which will be further described in the next section, target the D-Ala-D-Ala termini on PG peptides and its precursor Lipid II. Whilst β -lactams can be used against Gram-positive and Gram-negative bacteria, glycopeptides are only effective against Gram-positive bacteria; mainly due to the low permeability of the outer membrane in Gram-negatives (hampering the diffusion of the glycopeptide molecule). Other antibiotics used in the clinic that target PG biosynthesis are: **bacitracin**, which inhibits the recycling of undecaprenyl phosphate (45) and lipopeptides, such as **daptomycin**, which induces depolarization of the cell membrane (46). The targets of all the antibiotics mentioned above are depicted in Figure 1.

Recently, an 11-aminoacid peptide (named **teixobactin**) from a new genus related to *Aquabacteria* (provisionally named *Eleftheria terrae*) has been found to inhibit cell-wall biosynthesis by binding to Lipid II and Lipid III, which are precursors of teichoic acids (47). This compound is effective against methicillin-resistant *S. aureus* (MRSA) in mice and other Gram-positive bacteria in cell culture. Nevertheless, researchers should be cautious since it is still in early-stage development (48, 49).

3. Glycopeptides: a very important class of antibiotics

Glycopeptide antibiotics are highly important for the treatment of infections caused by Gram-positive bacteria, and usually represent the only treatment for infections caused by multi drug-resistant strains of enterococci, streptococci and staphylococci. In fact, the two main glycopeptides, vancomycin and teicoplanin, are widely used in the treatment of MRSA and penicillin-resistant *S. pneumoniae*.

Antibiotics from this family share a common heptapeptide structure and are synthesised by non-ribosomal peptide synthetases (NRPS). They are divided into five structural

180 subclasses according to the residues at positions 1 and 3 of the heptapeptide (50). These groups include not only the natural products vancomycin (Type I) and teicoplanin (Type IV), but also the semisynthetic glycopeptides, such as telavancin, oritavancin and dalbavancin. Please refer to the work by Nicolau and co-workers (50) for an extensive review on natural glycopeptides and semisynthetic derivatives. Ristocetin is a naturally
185 occurring glycopeptide that is not used as an antibiotic treatment but it has a clinical application. This compound is used in the detection of the platelet type-von Willebrand disease, since it triggers platelet aggregation in patients who lack the platelet factor receptor (51).

In Gram-positive bacteria, glycopeptides can diffuse through the cell-wall to reach the
190 particular target(s). As mentioned in the previous section, this family of drugs acts by binding to the D-Ala-D-Ala dipeptide termini of PG and its precursor Lipid II. As a result of this binding, there is reduced activity of transglycosylases and transpeptidases that catalyze the incorporation of new units into the nascent PG chains and the cross-linking events, respectively (52), leading to the eventual weakening of the cell-wall and
195 a blockage in cell division, resulting in cell death.

Some glycopeptides, such as vancomycin, are able to dimerize in solution, which enhances their antibacterial activity (53-55). On the other hand, teicoplanin increases its antibiotic activity with a long hydrocarbon chain in its structure that allows drug anchoring to the cell membrane. This enhances the concentration of antibiotic on the
200 membrane surface and the interaction with Lipid II. In addition, this lipophilic group can affect the membrane potential leading to its disruption (55-57).

4. Main glycopeptide antibiotics

Vancomycin was discovered in 1955 at Eli Lilly (Indianapolis, USA), constituting the first member of the glycopeptides family (58). It was approved for the use of penicillin-resistant staphylococcal infections by the FDA in 1958. The use of vancomycin started to increase in the early 1980s, rising rapidly during the 1980s and early 1990s (59). The first attempts to reduce its use in response to the spread of vancomycin-resistant bacteria resulted in a slight decline in use after 1994. Currently, vancomycin is used to treat complicated infections with MRSA (60) and *C. difficile* (61).

Teicoplanin is a lipoglycopeptide complex composed of 6 closely related compounds (62, 63). The ratio of each component in the mixture varies depending on fermentation conditions and this also affects its protein binding characteristics. Thus, the therapeutic implications for critical patients require a stronger standardization of the final product (64-66). Although teicoplanin was approved in Europe in 1988 and is also available in other countries, this antibiotic is not used in the USA.

Oritavancin is a derivative of the Type I glycopeptide chloroemomycin approved by the FDA in 2014. It shows a unique mechanism of action as it maintains the binding affinity for the modified peptide termini of vancomycin-resistant bacteria (see below). Moreover, it inhibits transpeptidation by steric hindrance of the transpeptidases, reducing cell-wall integrity (67). In addition, oritavancin generates depolarization and permeabilization of the membrane leading to cell death (68).

Telavancin was approved by the FDA in 2009. Derived from vancomycin, telavancin adds an additional property with respect to vancomycin: in being able to disrupt the cell-membrane potential of Gram-positive bacteria. It is more potent than vancomycin against a number of Gram-positive bacteria. In addition, it shows stronger binding to D-Ala-D-Ala termini and cell membranes, increasing its effectiveness against MRSA and

VanB vancomycin-resistant enterococci strains (see below). It is like oritavancin, in that it disrupts membrane potential and permeabilizes the membrane (69, 70).

Dalbavancin is a derivative of a teicoplanin-like glycopeptide that was developed in the 1990s but not approved by the FDA until 2014. Like teicoplanin, this antibiotic is a mixture of 5 related compounds. It also shows improved D-Ala-D-Ala binding properties, although it does not appear to destabilize cell membranes (71).

The structures of the main compounds cited in this work are depicted in Figure 2.

5. Mechanisms of glycopeptide resistance and transcriptional regulation

The unique mode of action of glycopeptide antibiotics was promising and therefore a slow development of resistances in pathogenic bacteria was expected. In fact, it took over 30 years after the introduction into the clinic to find the first vancomycin-resistant enterococci (VRE) strains (72, 73). This is a very long period in comparison to the emergence of other cases of antibiotic resistance. Nowadays, VRE have become significant nosocomial pathogens worldwide, mainly due to their adaptability in hospital environments and the limited treatment options.

The main mechanism of glycopeptide resistance relies on two main factors: i) the substitution of the target D-Ala-D-Ala by other low affinity termini (D-Ala-D-Lac or D-Ala-D-Ser) and ii) the removal of D-Ala-D-Ala precursors. The second factor is especially important since the production of both high and low affinity precursors does not confer resistance (74, 75). Substitution of D-Ala-D-Ala by D-Ala-D-Lac decreases binding affinity 1000-fold (76, 77) whilst substitution with D-Ala-D-Ser reduces binding affinity to a lower extent (78). Ten types of glycopeptide resistance determinants (VanA, VanB, VanC, VanD, VanE, VanF, VanG, VanL, VanM, and VanN) have been reported and well characterized on the basis of phenotypic and

genotypic criteria (79, 80). VanA, VanB, VanD, VanF and VanM types are characterized by the formation of D-Ala-D-Lac whilst VanC, VanE, VanG, VanL and VanN types rely on the formation of D-Ala-D-Ser. Among all of the known types, VanA and VanB genotypes predominate worldwide (80).

255 Other mechanisms contributing to vancomycin resistance are found in the so-called vancomycin-intermediate *S. aureus* (VISA). These strains normally have thicker cell-walls and an increased number of free D-Ala-D-Ala groups which act as false targets for vancomycin binding. Consequently, the amount of antibiotic available to reach the cell-wall synthesis machinery is significantly reduced (81). A higher risk is the vancomycin
260 resistant *S. aureus* (VSRA) strains. The transfer of vancomycin resistance genes (*van*) from VRE to *S. aureus* has been demonstrated *in vitro* and in animal models (82). The first described case of a fully vancomycin-resistant MRSA strain in humans was reported in 2002 and, worryingly, there have been an increasing number of cases in the subsequent years (83, 84). Several national healthcare systems recommend that
265 glycopeptide treatments should be limited to those patients with known MRSA infections in order to limit appearance of new glycopeptide-resistant strains (85).

Teicoplanin, telavancin and dalbavancin are effective against VanB strains, but not against VanA strains, since these three glycopeptides induce expression of the *vanA* operon but not that of *vanB* operon (79, 86). On the other hand, oritavancin is effective
270 against both VanB and VanA strains (87). Oritavancin interacts with both Lipid II and the pentaglycine bridge segment of the nascent PG (67, 88). Despite being approved recently as a promising alternative to older counterparts, resistance to oritavancin is likely to emerge in the future since it has already been observed *in vitro* (89). To extend the lifetime of glycopeptides, a similar approach to that used for β -lactam antibiotics by

275 using β -lactamase inhibitors, is being developed. Inhibitors of the VanX dipeptidase, involved in hydrolyzing D-Ala-D-Ala, have been designed against VRE (90).

The *vanX* gene forms part of the core of the main *van* clusters, which are normally composed of five genes (*vanSRHAX*). Depending on the *van* cluster there are different accessory genes, such as *vanY* and *vanZ* in VanA types or *vanY* and *vanW* in VanB
280 types. The *vanSR* genes encode a two-component system that regulates the transcriptional activation of all the *van* genes of the cluster (91). *vanHAX* encodes the enzymes that collectively reconstruct the bacterial peptidoglycan to terminate in D-Ala-D-Lac, in place of the canonical D-Ala-D-Ala, which is required for vancomycin binding and subsequent antibiotic action. *vanH* encodes a dehydrogenase that converts
285 pyruvate to D-Lac (92), *vanA* codes for a D-Ala-D-Lac ligase (93), and *vanX* encodes a D,D-dipeptidase that cleaves D-Ala-D-Ala dipeptides to ensure that only altered peptidoglycan precursors terminated in D-Ala-D-Lac are accumulated (94).

In VanA and VanB types, exposure of cells to vancomycin triggers autophosphorylation of VanS on a histidine residue (VanS-P). Phosphorylation of VanS results in the switch
290 from phosphatase to kinase activity. VanS-P phosphorylates VanR, resulting in the accumulation of VanR-P, which, in turn activates transcription from *van* promoters. In the absence of vancomycin, the constitutive phosphorylation of the response regulator VanR is suppressed by the phosphatase activity of VanS (see Figure 3). Although the sensor kinase is presumed to detect the signal, a direct interaction between vancomycin
295 and VanS has only been demonstrated in a few bacteria, with *Streptomyces coelicolor* being one of the first reported (95). VanS is not essential for the full activation of the *van* promoters, since VanR can be phosphorylated independently by acetylphosphate or other kinases encoded by the host chromosome (89). To date, VanR is the only transcriptional activator that has been shown to activate the transcription of the

vancomycin-resistance genes. However, in light of recent studies, it has been noted that transcriptional regulation of vancomycin regulation is not as simple as several *van* promoters regulated by a single transcriptional regulator. For instance, induction of vancomycin resistance is highly influenced by the inorganic phosphate concentration of the culture media in some actinomycetes such as *S. coelicolor* and *Streptomyces lividans* (see below).

6. Pho regulon: a phosphate dependent global response

Inorganic phosphate (Pi) is an essential nutrient that forms part of many of the key components within the cell, such as nucleic acids or phospholipids. Pi is involved in many important cellular processes, including energy metabolism and intracellular signalling. Despite the essentiality of Pi for life, this nutrient is normally found at very low concentrations in its free form in the environment, especially in soils. To adapt and survive in Pi scarcity, bacteria have evolved an array of biochemical and physiological responses aimed to secure this nutrient. To date, the main known mechanism responding to Pi starvation is the Pho regulon, which constitutes a very efficient and sensible regulatory mechanism in bacteria.

The Pho regulon was first characterised in *E. coli*, and later in many other bacterial species (96). It is controlled by a two-component regulatory system comprising an inner-membrane histidine kinase sensor protein (PhoR) and a cytoplasmic transcriptional response regulator, named PhoB in *E. coli* and PhoP in *Bacillus subtilis* (97, 98). In *E. coli*, under Pi limitation, PhoB is activated by PhoR acting as a kinase, but under Pi-replete conditions, PhoB activation is interrupted by PhoR acting as a phosphatase (99). Another protein, PhoU, is required for PhoB dephosphorylation under Pi-rich conditions in an unknown manner. In *E. faecium*, PhoB can be phosphorylated

by the sensor kinase VanS, suggesting cross-talk between the response to Pi starvation
325 and vancomycin resistance in this bacterium (100). The Pi signalling pathway in *B.*
subtilis includes a positive feedback loop between the PhoP-PhoR and ResD-ResE two-
component systems (101). Alternative players are also involved in this network, such
as: i) the Spo0B-Spo0A two-component system (102), ii) the AbrB and ScoC transition
state regulators (103, 104) and iii) an intermediate of the teichoic acid synthesis
330 pathway (105).

In all cases, upon Pi scarcity the response regulator is phosphorylated on an aspartic
acid residue by the sensor kinase. The phosphorylated response regulator is able to bind
to specific sequences on the DNA and activate or repress the transcription of the target
genes. These specific sequences, known as PHO boxes, were first characterized in *E.*
335 *coli* (106). In most bacteria the PHO boxes are defined as 11-nucleotide sequences
composed of seven well conserved and four less conserved nucleotides (107). The PHO
boxes are normally found in genes encoding extracellular enzymes capable of obtaining
Pi from organic phosphates, Pi-specific transporters and enzymes involved in storage
and saving of the nutrient. However, these sequences have also been found in a wide
340 range of other genes that are not directly related to Pi metabolism, revealing a
surprisingly diverse role for the Pho regulon, not only as a regulator of Pi assimilation,
but also as a master regulator of the cell (108). Indeed, the Pho regulon is involved in
regulation of nutritional cross-talk, secondary metabolite production and pathogenesis,
among other important cell processes (109).

345 7. Links between the Pho regulon and pathogenesis

Bacterial pathogens of humans, animals and plants normally have to adapt to Pi-limiting
and/or Pi-rich environments in the host depending on the site of infection. There are

numerous studies that report links between Pi regulation and pathogenesis; see the following references (110-112) for details.

350 The Pho regulon can influence virulence by increasing toxin production, biofilm formation, acid tolerance and resistance to antimicrobial compounds, among other processes. In some bacteria, the Pho system is able to respond to external acidity by controlling the transcription of genes involved in the protection against the low pH environments of the stomach or the gut (113). In *Vibrio cholera*, the regulatory cascade
355 leading to toxin production is repressed by PhoB under Pi-limiting conditions, while under the normally high Pi conditions of the intestine, the regulatory cascade is active, leading to the production of toxins (114). A link between Pi regulation and biofilm formation has been reported in many different bacteria, including *Agrobacterium tumefaciens*, *Proteus mirabilis*, *P. aeruginosa*, *E. coli* and *V. cholerae* (115-118).
360 Biofilms are formed by a matrix of extracellular polymers in which a number of mixed microbial populations are embedded. The advantages of these communities for the bacteria are numerous, and include: tolerance to environmental stresses, resistance to antimicrobial agents, accumulation of virulence factors, nutrition, adhesion, etc (119, 120). However, involvement of Pi regulation in antibiotic resistance by distinct
365 mechanisms to those depending on the protection of biofilms has also been reported (see next section).

8. Glycopeptide resistance is highly influenced by Pi.

A considerable number of studies have reported that the biosynthesis of most antibiotics is regulated by Pi; reviewed by Martín (121). There are also studies that establish links
370 between Pi regulation and antibiotic resistance. For instance, oxytetracycline production in *Streptomyces rimosus* and its corresponding resistance events seem to have a simple

mechanism of Pi regulation, which ensures that resistance to the produced antibiotic increases in proportion to its biosynthesis (122). In addition, inactivation of the high-affinity Pi transporter gene (*pstS*) led to increased penicillin susceptibility in 12 independent clinical isolates of *S. pneumoniae* (123), as well as in several pathogenic *E. coli* strains (110). Similarly, a point mutation of another Pi transporter (*pitA*) in *S. aureus* caused enhanced tolerance towards daptomycin and various combinations of other drugs. This increased tolerance was accompanied by elevated intracellular concentrations of Pi and polyphosphate, pointing to a correlation between intracellular Pi and drug tolerance (124). Furthermore, increased sensitivity to vancomycin and different cationic antimicrobial peptides has been described in *M. tuberculosis* upon inactivation of the *phoU* homolog gene (*phoY2*), which is involved in the activation of the Pho regulon (125). In *E. coli*, it has been shown that accumulation of phosphatidic acid (an intermediate of phospholipid biosynthesis) increases resistance to vancomycin (126). Changes in phospholipid composition had previously been shown to increase resistance to moenomycin and vancomycin in *S. aureus* (127). In addition, inactivation of the stringent response in *P. aeruginosa* and *E. coli* enhances their susceptibility to several antimicrobials in antibiotic-treated animals (128). Although the stringent response is normally activated by amino acid deprivation, a variety of other nutritional stresses, such as Pi depletion, can also activate it (129). This culminates in an increased production of the alarmone guanosine 5'-(tri)diphosphate 3'-diphosphate [(p)ppGpp], growth arrest and the expression of resistance genes. The synthesis of ppGpp has been linked to vancomycin resistance in *E. faecalis* since mutants that were unable to synthesize ppGpp showed an increased susceptibility to vancomycin (130).

Recently, the complexities of vancomycin resistance regulation in *Streptomyces* have been reviewed (131). *S. coelicolor* and *S. lividans* display a very strong Pi regulation of

vancomycin resistance. In these bacteria, vancomycin resistance is almost blocked with Pi concentrations above 0.25%, suggesting a key role of this nutrient in the repression of vancomycin resistance (132). *S. coelicolor* and *S. lividans* do not produce glycopeptide antibiotics but they have a VanB-type cluster, which confers inducible resistance to vancomycin, but not to teicoplanin (133). The Pi regulation of vancomycin resistance is not PhoP-dependent since similar minimum inhibitory concentrations were obtained in *S. coelicolor* parental and $\Delta phoP$ mutant strains under distinct Pi concentrations (132). This Pi regulation was not observed in the vancomycin-producer *Amycolatopsis orientalis* strain (132). Santos-Beneit and co-workers (134) were able to generate a *S. coelicolor* mutant strain (SCO2594::Tn5062) that lacked the Pi control over vancomycin resistance. The function of the SCO2594 gene product is unknown but appeared to be related with cell-wall metabolism since the mutant displayed cell-wall synthesis defects. Furthermore, the mutant also showed an increased transcription of genes putatively involved in teichoic acid synthesis and export as well as of regulators associated with the response to cell-wall-perturbing stresses. The role of SCO2594 in cell-wall metabolism and its downstream consequences on vancomycin resistance has not been yet elucidated, but there is a clear connection between this protein, Pi regulation, cell-wall metabolism and vancomycin resistance. Interestingly, the SCO2594::Tn5062 mutant (in the presence of vancomycin) not only up-regulates transcription of the *van* genes, but also that of three putative small non-coding RNAs (sRNAs) within the *van* cluster; one of them being located in the promoter region of the *vanRS* regulatory genes (134). sRNAs have been postulated to play crucial roles in the control of gene expression in response to environmental changes, stress, and in virulence (135, 136). The role of these sRNAs on vancomycin resistance control is currently under investigation.

9. D-alanylation of cell-wall components and Pi control: teichoic acids.

The peptidoglycan layers protect Gram-positive bacteria from osmotic lysis and constitute a barrier against toxic compounds (137). As described before, peptidoglycan
425 also functions as a scaffold for the attachment of proteins, capsular polysaccharides and wall teichoic acids (WTAs). In order to save Pi, some bacteria are able to replace the Pi-rich WTAs with Pi-free teichuronic acids. This process is regulated by PhoP, which in *B. subtilis* initiates teichuronic acid synthesis under Pi starvation through activation of the *tuaA-H* operon and reduces WTAs synthesis through repression of *tagAB* (138). It
430 has been demonstrated that the Pi-dependent PhoR-PhoP activation signal may emanate from WTA metabolism in *B. subtilis* (105). Consequently, the PhoR kinase domain appears to monitor the cellular level of a WTA synthetic intermediate through an interaction with the protein. This intermediate could inhibit PhoR autokinase activity during growth under Pi-replete conditions and, consequently, the Pho response.

435 Changes in the structure and metabolism of WTAs have been noted to influence vancomycin resistance, cell-wall turnover and autolysis in different bacteria. In addition, some biochemical modifications of these compounds, such as D-alanylation, also impact antibiotic resistance in bacteria. D-alanylation of cell-wall components (such as WTAs and lipoteichoic acids) results in a net positive charge on the cell-wall,
440 which is crucial for many functions of Gram-positive bacteria (139). As mentioned before, one important effect of this biochemical modification is its influence on the resistance to antimicrobial peptides and antibiotics. For example, D-alanylation of cell-wall components is somehow involved in the resistance of *Clostridium butyricum* to some cationic antimicrobial peptides and lysozyme (140). Similarly, it has been shown
445 that the D-alanylation of WTAs reduces vancomycin binding to the cell-wall in *S. aureus* (141). In this bacterium, as well as in *S. pneumoniae*, loss of one or more WTAs

genes resulted in not only a reduced cell-wall Pi content or aberrant cell morphology but also in an increased susceptibility of the cells to several antibiotics (142-145). Therefore, WTAs constitute very important compounds in Gram-positive bacteria as: i) structural components of the cell-wall, ii) inducers of essential nutritional starvation responses (such as the Pho response), and iii) protective agents against cell-wall damaging compounds.

10. Links between glycopeptide resistance and: a) cell envelope stress, b) autolytic activity, c) antibiotic synthesis

a) Cell envelope stress

As described before, resistance to vancomycin can be acquired by several different mechanisms. The most common involves conversion of cell-wall precursors ending in D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser (146). Another mechanism by which resistance can be acquired is through mutation of sigma factors and endogenous two-component signal transduction systems that control normal cell-wall metabolism (such as WalRK), or the cellular response to cell-wall damage, like *VraRS* and *GraRS* (147-150). For an in-depth review of the link between stress and antimicrobial resistance refer to Poole (151).

VraSR is a cell envelope stress responsive two-component system that is induced by cell-wall-active antibiotics (including glycopeptides). Inactivation of this system increases β -lactam, bacitracin, fosfomycin and glycopeptide (vancomycin and teicoplanin) susceptibility in MRSA strains (152). Mutations in *vraRS* leading to up-regulation of the *VraSR*-dependent cell-wall stress stimulon are also commonly observed in VISA strains (153). The *VraSR* homologues in *B. subtilis* and *Streptococcus mutans* (*LiaRS* and *LiaFRS*, respectively) are also induced by cell-wall-

active antibiotics and their inactivation also generate susceptible phenotypes to these agents (154, 155).

GraRS is another cell envelope stress responsive two-component system in *S. aureus*, which has been linked to vancomycin resistance (156, 157). Overexpression of *graRS* is reported in clinical VISA strains and, conversely, *graRS* knockout mutants show increasing susceptibility to vancomycin (156, 158). Although it is not yet completely known, glycopeptide resistance supported by GraRS appears to arise from an increased net positive surface charge as a consequence of the induction of genes associated with D-alanylation of WTAs (among other GraRS-dependent cell-wall modifications). This positive net charge would reduce the binding capabilities of cationic agents such as vancomycin (158). Nevertheless, in most cases, the stress induced effectors that promote resistance and/or recruit resistance determinants are yet unknown.

Links between cell envelope stress and glycopeptide resistance have also been described in different species of actinobacteria such as in *Corynebacterium*, *Mycobacterium* and *Streptomyces*. In *M. tuberculosis*, the SigE protein has been shown to be involved in the protection from cell envelope stress and to confer basal levels of resistance to vancomycin. *sigE* was induced by subinhibitory concentrations of vancomycin in this bacterium (159). The authors showed that a *M. tuberculosis sigE* mutant was more sensitive to vancomycin when compared to the wild-type parental strain. In *Corynebacterium glutamicum*, deletion of *sigE* increased cellular sensitivity to magnesium deficiency, lysozyme, SDS, EDTA, heat and antibiotics, including nalidixic acid, penicillin and vancomycin (160). In *S. coelicolor*, cell-wall hydrolytic enzymes and cell-wall acting antibiotics (such as penicillins and glycopeptides) also induce the *sigE* signal transduction system (161). In a similar manner to *M. tuberculosis* and *C. glutamicum*, vancomycin resistance is significantly decreased in a *S. coelicolor sigE*

null mutant, pointing to a conserved high influence of this sigma factor in glycopeptide resistance (162).

Other cell envelope stress responsive proteins that might be implicated in vancomycin resistance are SigW (a sigma factor that responds to a variety of cell envelope stresses),
500 SigB (a sigma factor involved in general stress response) and WhiB (a transcriptional regulator involved in diverse aspects of the biology of actinomycetes, including pathogenesis, antibiotic resistance and the control of development). SigW has been linked to fosfomycin, vancomycin and ampicillin resistance in *B. subtilis*, with *sigW* deletion mutants showing increased susceptibility to these agents (148). Disruption of
505 *sigB* highly increased vancomycin susceptibility in *S. coelicolor* (162) and *S. aureus* (163). Finally, *whiB* was one of the genes more transcriptionally induced by vancomycin (apart from the *van* genes) in a highly vancomycin-resistant *S. coelicolor* mutant (134). Although WhiB has not been described as a specific cell envelope stress regulator in *Streptomyces* (164), it appears to be linked to cell-wall stress in other
510 species. For example, WhiB homologs in *Corynebacterium diphtheriae* and *M. tuberculosis* have been implicated in key stages of pathogenesis, protection to cell-wall damaging agents and antibiotic resistance (165, 166). A summary of the link between stress response regulators and glycopeptide resistance is shown in table 1.

b) Autolytic activity

515 The complexities of the regulation of autolytic activity versus glycopeptide resistance are manifested by many examples in the scientific literature. For example, it has been noted that the autolytic activity of some glycopeptide-intermediate *S. aureus* strains (GISA) is reduced compared to that of the glycopeptide-susceptible strains (167). In this sense, vancomycin-dependent inhibition of cell-wall turnover and autolysis was

described in a highly vancomycin-resistant *S. aureus* mutant, resulting in the accumulation of cell-wall material and the inhibition of daughter cell separation (168). Vancomycin was also described to block the *in vitro* hydrolysis of cell-walls by autolytic enzyme extracts and some lytic enzymes such as lysostaphin and mutanolysin. Furthermore, it has been reported that decreased autolytic activity is involved in the reduced tolerance of a series of GISA isolates to vancomycin (169). All together, these findings indicate an important interconnection between glycopeptide resistance and cell-wall lytic activity.

c) Antibiotic synthesis

Glycopeptide antibiotics also affect other important cellular processes, such as antibiotic synthesis. This is not unexpected since antimicrobials themselves are growth-inhibiting stressing agents that normally elicit protective responses in bacteria, including (as previously mentioned) stress-dependent recruitment of resistance determinants and (as shown in this section) eventual activation of antibiotic biosynthesis. Using transcriptomic analyses in *S. coelicolor*, Hesketh and co-workers (162) observed the upregulation of genes involved in the biosynthesis of the antibiotics actinorhodin (Act), undecylprodigiosin (Red) and the calcium-dependent antibiotic (CDA) 30 min after the exposure of the cells to vancomycin. In another study, Santos-Beneit and co-workers (134) also reported a large induction of Act and Red synthesis in response to vancomycin, as well as a significant increase in the antibiotic production yields in a highly vancomycin-resistant mutant strain (see Figure 4).

11. Future perspectives

The phenomenon of antimicrobial resistance is a critical health issue today as the World Health Organization currently recognizes. Indeed, most bacteria have developed some

level of resistance to antibiotics. Worse still is the fact that many clinically important
545 pathogens have developed resistance to many, or even all, available antibiotics. The
problem of antimicrobial resistance is further complicated by the continued, decades
long, lack of significant numbers of novel antimicrobials in drug development pipelines
or clinical practice, making the development of alternative strategies to prevent
pathogenesis even more crucial. One possibility is the screening of novel antimicrobial
550 compounds generated by either chemical synthesis or natural production. Another
outlook is the development of new derivatives of the most important antibiotics used in
clinics, such as glycopeptides. For example, distinct glycopeptide derivatives with
increased antibiotic properties have been reported (170-172).

Alternatively, the very strong Pi regulation of vancomycin resistance reported in *S.*
555 *coelicolor* and the transcriptional induction of putative sRNAs within its *van* cluster
constitutes an unprecedented phenomenon of interconnection between antibiotic
resistance, nutritional stress and sRNA regulation. Characterization of new players
(including sRNAs) involved in antibiotic resistance could contribute to the elucidation
of new mechanisms of antibiotic action and to the designing of novel antibiotics and/or
560 antimicrobial strategies, such as “antisense RNA therapy” (173-175). Since antibiotic
discovery is economically challenging for pharmaceutical companies (due to the
development of resistance, among other obstacles), blocking and/or reducing the
problems of antimicrobial resistance will depend on developing new effective strategies.

565

Legend to the Figures

Figure 1: Scheme showing the targets of the most important cell-wall acting antibiotics.

Figure 2: Structure of the main compounds cited in this review.

570 **Figure 3: A model for the function of the vancomycin resistance VanSR two-component signal transduction system in VanA and VanB-type strains.** In the absence of antibiotic, VanS acts as a phosphatase to suppress the levels of VanR~P. In the presence of antibiotic, VanS switches the phosphatase activity into a kinase activity, leading to the accumulation of VanR~P (the activated form of VanR) and to the
575 activation of the *van* genes. Activation of the *van* genes is responsible for the substitution of the normal cell-wall precursors ending in D-Ala-D-Ala to D-Ala-D-Lac which reduces vancomycin binding affinity ~ 1000-fold.

Figure 4: Vancomycin disc diffusion assay of *S. coelicolor* SCO2594::Tn5062. For the assay, using MMCGT+P medium (134), *Streptomyces* spores were homogenized
580 into 5 mL of warm medium (containing 7 gr L⁻¹ of agar) and poured as a thin layer over a solid layer (20 mL) of medium containing 14 gr L⁻¹ of agar. After solidification of the added layer, a commercial vancomycin disc (containing 30 µg of the antibiotic) was placed on the plate. The result was evaluated after 3 days of incubation at 30 °C. Note the Red induction (i.e. undecylprodigiosin production) of the embedded bacteria around
585 the diffusion zone of the vancomycin disc (Santos-Beneit F; unpublished photograph).

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References

1. E.D. Brown, G.D. Wright, Antibacterial drug discovery in the resistance era, Nature 529 (2016) 336-343.
- 595 2. A. Fleming, On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenza*, Br. J. Exp. Pathol. 10 (1929) 226–236.
3. D. Hansman, M.M. Bullen, A resistant pneumococcus, Lancet 2 (1967) 264-265.
4. I. Nikolaidis, S. Favini-Stabile, A. Dessen, Resistance to antibiotics targeted to the bacterial cell-wall, Protein Sci. 23 (2014) 243-259.
- 600 5. Y.-Y. Liu, Y. Wang, T.R. Walsh, L.-X. Yi, R. Zhang, J. Spencer, et al., Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study, Lancet Infect. Dis. 16 (2016) 161-168.
- 605 6. A. Cannatelli, T. Giani, A. Antonelli, L. Principe, F. Luzzaro, G.M. Rossolini, First detection of the mcr-1 colistin resistance gene in *Escherichia coli* in Italy, Antimicrob. Agents Chemother. 60 (2016) 3257-3258.
7. C.J.H. von Wintersdorff, J. Penders, J.M. van Niekerk, N.D. Mills, S. Majumder, L.B. van Alphen, et al., Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer, Front. Microbiol. 7 (2016) 173.
- 610 8. L.L. Cavalli-Sforza, J. Lederberg, Isolation of Pre-Adaptive Mutants in Bacteria by Sib Selection, Genetics 41 (1956) 367-381.
9. V.M. D'Costa, C.E. King, L. Kalan, M. Morar, W.W.L. Sung, C. Schwarz, et al., Antibiotic resistance is ancient, Nature 477 (2011) 457-461.
- 615 10. K. Bhullar, N. Waglechner, A. Pawlowski, K. Koteva, E.D. Banks, M.D.

Johnston, et al., Antibiotic resistance is prevalent in an isolated cave microbiome, PLoS One 7 (2012) e34953.

11. J. O'Neill, Review on antimicrobial resistance: Tackling a crisis for the health and wealth of nations, (2014) (Available at <http://amr-review.org/>).

12. T.J. Louie, M.A. Miller, K.M. Mullane, K. Weiss, A. Lentnek, Y. Golan, et al., Fidaxomicin versus vancomycin for *Clostridium difficile* infection, N. Engl. J. Med. 364 (2011) 422-431.

13. O.A. Cornely, D.W. Crook, R. Esposito, A. Poirier, M.S. Somero, K. Weiss, et al., Fidaxomicin versus vancomycin for infection with *Clostridium difficile* in Europe, Canada, and the USA: a double-blind, non-inferiority, randomised controlled trial, Lancet Infect Dis 12 (2012) 281-289.

14. F. Harrison, A.E.L. Roberts, R. Gabriliska, K.P. Rumbaugh, C. Lee, S.P. Diggle, A 1,000-Year-Old Antimicrobial Remedy with Antistaphylococcal Activity, MBio 6 (2015) e01129.

15. L.B. Rice, Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE, J. Infect. Dis. 197 (2008) 1079-1081.

16. S. Behroozian, S.L. Svensson, J. Davies, Kisameet Clay Exhibits Potent Antibacterial Activity against the ESKAPE Pathogens, MBio 7 (2016).

17. W. Vollmer, D. Blanot, M.A. de Pedro, Peptidoglycan structure and architecture, FEMS Microbiol. Rev. 32 (2008) 149-167.

18. W. Vollmer, Structural variation in the glycan strands of bacterial peptidoglycan, FEMS Microbiol. Rev. 32 (2008) 287-306.

19. H. Barreteau, A. Kovac, A. Boniface, M. Sova, S. Gobec, D. Blanot, Cytoplasmic steps of peptidoglycan biosynthesis, FEMS Microbiol. Rev. 32 (2008) 168-207.

20. A. Bouhss, A.E. Trunkfield, T.D.H. Bugg, D. Mengin-Lecreulx, The biosynthesis of peptidoglycan lipid-linked intermediates, *FEMS Microbiol. Rev.* 32 (2008) 208-233.
- 645 21. N. Ruiz, Lipid Flippases for Bacterial Peptidoglycan Biosynthesis, *Lipid Insights* 8 (2015) 21-31.
22. E. Sauvage, F. Kerff, M. Terrak, J.A. Ayala, P. Charlier, The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis, *FEMS Microbiol. Rev.* 32 (2008) 234-258.
- 650 23. L.L. Silver, Viable screening targets related to the bacterial cell-wall, *Ann. NY Acad. Sci.* 1277 (2013) 29-53.
24. F.M. Kahan, J.S. Kahan, P.J. Cassidy, H. Kropp, The mechanism of action of fosfomycin (phosphonomycin), *Ann. NY Acad. Sci.* 235 (1974) 364-386.
25. C.J. Andres, J.J. Bronson, S.V. D'Andrea, M.S. Deshpande, P.J. Falk, K.A. Grant-Young, et al., 4-Thiazolidinones: novel inhibitors of the bacterial enzyme MurB, *Bioorg. Med. Chem. Lett.* 10 (2000) 715-717.
- 655 26. J.J. Bronson, K.L. DenBleyker, P.J. Falk, R.A. Mate, H.-T. Ho, M.J. Pucci, L.B. Snyder, Discovery of the first antibacterial small molecule inhibitors of MurB, *Bioorg. Med. Chem. Lett.* 13 (2003) 873-875.
- 660 27. E.Z. Baum, S.M. Crespo-Carbone, B.D. Foleno, L.D. Simon, J. Guillemont, M. Macielag, K. Bush, MurF inhibitors with antibacterial activity: effect on muropeptide levels, *Antimicrob. Agents Chemother.* 53 (2009) 3240-3247.
28. S. Favini-Stabile, C. Contreras-Martel, N. Thielens, A. Dessen, MreB and MurG as scaffolds for the cytoplasmic steps of peptidoglycan biosynthesis, *Environ. Microbiol.* 15 (2013) 3218-3228.
- 665 29. J.A. Caminero, G. Sotgiu, A. Zumla, G.B. Migliori, Best drug treatment for

multidrug-resistant and extensively drug-resistant tuberculosis, Lancet. Infect. Dis. 10 (2010) 621-629.

30. P. Scotto, P. Monaco, V. Scardi, V. Bonavita, Neurochemical studies with l-cycloserine, a central depressant agent, J. Neurochem. 10 (1963) 831-839.

31. W.W. Yew, C.F. Wong, P.C. Wong, J. Lee, C.H. Chau, Adverse neurological reactions in patients with multidrug-resistant pulmonary tuberculosis after coadministration of cycloserine and ofloxacin, Clin. Infect. Dis. 17 (1993) 288-289.

32. A.D. Elbein, The role of lipid-linked saccharides in the biosynthesis of complex carbohydrates, Annual Review of Plant Physiology 30 (1979) 239-272.

33. M. Inukai, F. Isono, A. Takatsuki, Selective inhibition of the bacterial translocase reaction in peptidoglycan synthesis by mureidomycins, Antimicrob. Agents Chemother. 37 (1993) 980-983.

34. C. Dini, P. Collette, N. Drochon, J.C. Guillot, G. Lemoine, P. Mauvais, J. Aszodi, Synthesis of the nucleoside moiety of liposidomycins: elucidation of the pharmacophore of this family of MraY inhibitors, Bioorg. Med. Chem. Lett. 10 (2000) 1839-1843.

35. T. Koga, T. Fukuoka, N. Doi, T. Harasaki, H. Inoue, H. Hotoda, et al., Activity of capuramycin analogues against *Mycobacterium tuberculosis*, *Mycobacterium avium* and *Mycobacterium intracellulare* in vitro and in vivo, J. Antimicrob. Chemother. 54 (2004) 755-760.

36. A. Mihalyi, S. Jamshidi, J. Slikas, T.D.H. Bugg, Identification of novel inhibitors of phospho-MurNAc-pentapeptide translocase MraY from library screening: Isoquinoline alkaloid michellamine B and xanthene dye phloxine B, Bioorg. Med. Chem. 22 (2014) 4566-4571.

37. Y. van Heijenoort, M. Leduc, H. Singer, J. van Heijenoort, Effects of moenomycin on *Escherichia coli*, J. Gen. Microbiol. 133 (1987) 667-674.
38. Y. Hu, J.S. Helm, L. Chen, X.-Y. Ye, S. Walker, Ramoplanin inhibits bacterial
695 transglycosylases by binding as a dimer to lipid II, J. Am. Chem. Soc. 125
(2003) 8736-8737.
39. Y.-Y. Tseng, J.-M. Liou, T.-L. Hsu, W.-C. Cheng, M.-S. Wu, C.-H. Wong,
Development of bacterial transglycosylase inhibitors as new antibiotics:
moenomycin A treatment for drug-resistant *Helicobacter pylori*, Bioorg. Med.
700 Chem. Lett. 24 (2014) 2412-2414.
40. H. Mathur, M.C. Rea, P.D. Cotter, R.P. Ross, C. Hill, The potential for
emerging therapeutic options for *Clostridium difficile* infection, Gut Microbes 5
(2014) 696-710.
41. C.N. Kraus, M.W. Lyerly, R.J. Carman, Ambush of *Clostridium difficile* spores
705 by ramoplanin: activity in an in vitro model, Antimicrob. Agents Chemother. 59
(2015) 2525-2530.
42. E. Jr Wise, J.T. Park, Penicillin: its basic site of action as an inhibitor of a
peptide cross-linking reaction in cell-wall mucopeptide synthesis, Proc. Natl.
Acad. Sci. USA 54 (1965) 75-81.
- 710 43. D.J. Tipper, J.L. Strominger, Mechanism of action of penicillins: a proposal
based on their structural similarity to acyl-D-alanyl-D-alanine, Proc. Natl. Acad.
Sci. USA 54 (1965) 1133-1141.
44. D.J. Waxman, R.R. Yocum, J.L. Strominger, Penicillins and cephalosporins are
active site-directed acylating agents: evidence in support of the substrate
715 analogue hypothesis, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 289 (1980) 257-
271.

45. K.J. Stone, J.L. Strominger, Mechanism of action of bacitracin: complexation with metal ion and C 55 -isoprenyl pyrophosphate, Proc. Natl. Acad. Sci. USA 68 (1971) 3223-3227.
- 720 46. J.A. Silverman, N.G. Perlmutter, H.M. Shapiro, Correlation of daptomycin bactericidal activity and membrane depolarization in *Staphylococcus aureus*, Antimicrob. Agents Chemother. 47 (2003) 2538-2544.
47. L.L. Ling, T. Schneider, A.J. Peoples, A.L. Spoering, I. Engels, B. Conlon, et al., A new antibiotic kills pathogens without detectable resistance, Nature 517 725 (2015) 455-459.
48. C. Brown, Antibiotic discovery heralds new world of drugs, CMAJ 187 (2015) 241-242.
49. C.A. Arias, B.E. Murray, A new antibiotic and the evolution of resistance, N. Engl. J. Med. 372 (2015) 1168-1170.
- 730 50. K.C. Nicolaou, C.N. Boddy, S. Bräse, N. Winssinger, Chemistry, Biology, and Medicine of the Glycopeptide Antibiotics, Angew. Chem. Int. Ed. Engl. 38 (1999) 2096-2152.
51. M. Othman, H. Kaur, J. Emsley, Platelet-type von Willebrand disease: new insights into the molecular pathophysiology of a unique platelet defect, Semin. 735 Thromb. Hemost. 39 (2013) 663-673.
52. P.E. Reynolds, Structure, biochemistry and mechanism of action of glycopeptide antibiotics, Eur. J. Clin. Microbiol. Infect. Dis, 8 (1989) 943-950.
53. J.P. Mackay, U. Gerhard, D.A. Beauregard, R.A. Maplestone, D.H. Williams, Dissection of the contributions toward dimerisation of glycopeptide antibiotics, 740 Am. Chem. Sot. 116 (1994) 4573-4580.

54. J.P. Mackay, U. Gerhard, D.A. Beauregard, M.S. Westwell, M.S. Searle, D.H. Williams, Glycopeptide antibiotic activity and the possible role of dimerization: a model for biological signalling, *J. Am. Chem. Soc.* 116 (1994) 4581-4590.
- 745 55. D.A. Beauregard, D.H. Williams, M.N. Gwynn, D.J. Knowles, Dimerization and membrane anchors in extracellular targeting of vancomycin group antibiotics, *Antimicrob. Agents Chemother.* 39 (1995) 781-785.
56. H. Chmara, S. Ripa, F. Mignini, E. Borowski, Bacteriolytic effect of teicoplanin, *J. Gen. Microbiol.* 137 (1991) 913-919.
- 750 57. C.S. Lunde, S.R. Hartouni, J.W. Janc, M. Mammen, P.P. Humphrey, B.M. Benton, Telavancin disrupts the functional integrity of the bacterial membrane through targeted interaction with the cell-wall precursor lipid II, *Antimicrob. Agents Chemother.* 53 (2009) 3375-3383.
- 755 58. M.H. McCormick, J.M. McGuire, G.E. Pittenger, R.C. Pittenger, W.M. Stark, Vancomycin, a new antibiotic. I. Chemical and biologic properties, *Antibiot. Annu.* 3 (1955) 606-611.
59. H.A. Kirst, D.G. Thompson, T.I. Nicas, Historical yearly usage of vancomycin, *Antimicrob. Agents Chemother.* 42 (1998) 1303-1304.
- 760 60. R.C. Jr Moellering, Current treatment options for community-acquired methicillin-resistant *Staphylococcus aureus* infection, *Clin. Infect. Dis.* 46 (2008) 1032-1037.
61. A.A. Venugopal, S. Johnson, Current state of *Clostridium difficile* treatment options, *Clin. Infect. Dis.* 55 (2012) S71--S76.
- 765 62. F. Parenti, G. Beretta, M. Berti, V. Arioli, Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* Nov. Sp. I. Description of the producer strain, fermentation studies and biological properties, *J. Antibiot. (Tokyo)* 31 (1978)

276-283.

63. A. Borghi, C. Coronelli, L. Faniuolo, G. Allievi, R. Pallanza, G.G. Gallo, Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. IV. Separation and characterization of the components of teichomycin (teicoplanin),
770 J. Antibiot. (Tokyo) 37 (1984) 615-620.
64. A. Borghi, D. Edwards, L.F. Zerilli, G.C. Lancini, Factors affecting the normal and branched-chain acyl moieties of teicoplanin components produced by *Actinoplanes teichomyceticus*, J. Gen. Microbiol. 137 (1991) 587-592.
65. A.J. Brink, G.A. Richards, G. Colombo, F. Bortolotti, P. Colombo, F. Jehl,
775 Multicomponent antibiotic substances produced by fermentation: implications for regulatory authorities, critically ill patients and generics, Int. J. Antimicrob. Agents 43 (2014) 1-6.
66. J.A. Roberts, V. Stove, J.J. De Waele, B. Sipinkoski, B. McWhinney, J.P.J. Ungerer, et al., Variability in protein binding of teicoplanin and achievement of
780 therapeutic drug monitoring targets in critically ill patients: lessons from the DALI Study, Int. J. Antimicrob. Agents 43 (2014) 423-430.
67. G.J. Patti, S.J. Kim, T.-Y. Yu, E. Dietrich, K.S.E. Tanaka, T.R. Jr Parr, et al., Vancomycin and oritavancin have different modes of action in *Enterococcus faecium*, J. Mol. Biol. 392 (2009) 1178-1191.
- 785 68. A. Belley, G.A. McKay, F.F. Arhin, I. Sarmiento, S. Beaulieu, I. Fadhil, et al., Oritavancin disrupts membrane integrity of *Staphylococcus aureus* and vancomycin-resistant enterococci to effect rapid bacterial killing, Antimicrob. Agents Chemother. 54 (2010) 5369-5371.
69. D.L. Higgins, R. Chang, D.V. Debabov, J. Leung, T. Wu, K.M. Krause, et al.,
790 Telavancin, a multifunctional lipoglycopeptide, disrupts both cell-wall synthesis

- and cell membrane integrity in methicillin-resistant *Staphylococcus aureus*,
Antimicrob. Agents Chemother. 49 (2005) 1127-1134.
70. L.D. Saravolatz, G.E. Stein, L.B. Johnson, Telavancin: a novel lipoglycopeptide,
Clin. Infect. Dis. 49 (2009) 1908-1914.
- 795 71. A. Malabarba, B.P. Goldstein, Origin, structure, and activity in vitro and in vivo
of dalbavancin, J. Antimicrob. Chemother. 55 (2005) ii15-ii20.
72. R. Leclercq, E. Derlot, M. Weber, J. Duval, P. Courvalin, Transferable
vancomycin and teicoplanin resistance in *Enterococcus faecium*, Antimicrob.
Agents Chemother. 33 (1989) 10-15.
- 800 73. A.H. Uttley, R.C. George, J. Naidoo, N. Woodford, A.P. Johnson, C.H. Collins,
et al., High-level vancomycin-resistant enterococci causing hospital infections,
Epidemiol. Infect. 103 (1989) 173-181.
74. M. Arthur, F. Depardieu, P. Reynolds, P. Courvalin, Quantitative analysis of the
metabolism of soluble cytoplasmic peptidoglycan precursors of glycopeptide-
805 resistant enterococci, Mol. Microbiol. 21 (1996) 33-44.
75. P.E. Reynolds, Control of peptidoglycan synthesis in vancomycin-resistant
enterococci: D,D-peptidases and D,D-carboxypeptidases, Cell Mol. Life Sci. 54
(1998) 325-331.
76. T.D. Bugg, G.D. Wright, S. Dutka-Malen, M. Arthur, P. Courvalin, C.T. Walsh,
810 Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147:
biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin
resistance proteins VanH and VanA, Biochemistry 30 (1991) 10408-10415.
77. C.C. McComas, B.M. Crowley, D.L. Boger, Partitioning the loss in vancomycin
binding affinity for D-Ala-D-Lac into lost H-bond and repulsive lone pair
815 contributions, J. Am. Chem. Soc. 125 (2003) 9314-9315.

78. M. Arthur, P. Reynolds, P. Courvalin, Glycopeptide resistance in enterococci, Trends Microbiol. 4 (1996) 401-407.
79. P. Courvalin, Vancomycin resistance in gram-positive cocci, Clin. Infect. Dis. 42 (2006) S25-S34.
80. V. Cattoir, R. Leclercq, Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce? J. Antimicrob. Chemother. 68 (2013) 731-742.
81. A. Reipert, K. Ehlert, T. Kast, G. Bierbaum, Morphological and genetic differences in two isogenic *Staphylococcus aureus* strains with decreased susceptibilities to vancomycin, Antimicrob. Agents Chemother. 47 (2003) 568-576.
82. W.C. Noble, Z. Virani, R.G. Cree, Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*, FEMS Microbiol. Lett. 72 (1992) 195-198.
83. S. Chang, D.M. Sievert, J.C. Hageman, M.L. Boulton, F.C. Tenover, F.P. Downes, et al., Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene, N. Engl. J. Med. 348 (2003) 1342-1347.
84. A.A. Alzolibani, A.A. Al Robaee, H.A. Al Shobaili, J.A. Bilal, M. Issa Ahmad, G. Bin Saif, Documentation of vancomycin-resistant *Staphylococcus aureus* (VRSA) among children with atopic dermatitis in the Qassim region, Saudi Arabia, Acta Dermatovenerol. Alp. Pannonica. Adriat. 21 (2012) 51-53.
85. C.G. Gemmell, D.I. Edwards, A.P. Fraise, F.K. Gould, G.L. Ridgway, R.E. Warren, Guidelines for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the UK, J. Antimicrob. Chemother. 57 (2006) 589-608.

86. G.G. Zhanel, D. Calic, F. Schweizer, S. Zelenitsky, H. Adam, P.R.S. Lagacé-Wiens, et al., New lipoglycopeptides: a comparative review of dalbavancin, oritavancin and telavancin, *Drugs* 70 (2010) 859-886.
87. N.E. Allen, T.I. Nicas, Mechanism of action of oritavancin and related glycopeptide antibiotics, *FEMS Microbiol. Rev.* 26 (2003) 511-532.
88. S.J. Kim, K.S.E. Tanaka, E. Dietrich, A. Rafai Far, J. Schaefer, Locations of the hydrophobic side chains of lipoglycopeptides bound to the peptidoglycan of *Staphylococcus aureus*, *Biochemistry* 52 (2013) 3405-3414.
89. M. Arthur, F. Depardieu, P. Reynolds, P. Courvalin, Moderate-level resistance to glycopeptide LY333328 mediated by genes of the *vanA* and *vanB* clusters in enterococci, *Antimicrob. Agents Chemother.* 43 (1999) 1875-1880.
90. R. Muthyala, N. Rastogi, W.S. Shin, M.L. Peterson, Y.Y. Sham, Cell permeable *vanX* inhibitors as vancomycin re-sensitizing agents, *Bioorg Med Chem Lett* 24 (2014) 2535-2538.
91. S. Handwerger, L. Discotto, J. Thanassi, M.J. Pucci, Insertional inactivation of a gene which controls expression of vancomycin resistance on plasmid pHKK100, *FEMS Microbiol. Lett.* 71 (1992) 11-14.
92. M. Arthur, C. Molinas, T.D. Bugg, G.D. Wright, C.T. Walsh, P. Courvalin, Evidence for in vivo incorporation of D-lactate into peptidoglycan precursors of vancomycin-resistant enterococci. *Antimicrob. Agents Chemother.* 36 (1992) 867-869.
93. T.D. Bugg, S. Dutka-Malen, M. Arthur, P. Courvalin, C.T. Walsh, Identification of vancomycin resistance protein VanA as a D-alanine:D-alanine ligase of altered substrate specificity, *Biochemistry* 30 (1991) 2017-2021.
94. P.E. Reynolds, H.A. Snaith, A.J. Maguire, S. Dutka-Malen, P. Courvalin,

Analysis of peptidoglycan precursors in vancomycin-resistant *Enterococcus gallinarum* BM4174, *Biochemi. J.* 301 (1994) 5-8.

95. K. Koteva, H.J. Hong, X.D. Wang, I. Nazi, D. Hughes, M.J. Naldrett, et al., A vancomycin photoprobe identifies the histidine kinase VanSsc as a vancomycin
870 receptor. *Nat. Chem. Biol.* 6 (2010) 327-329.

96. B.L. Wanner, B.D. Chang, The *phoBR* operon in *Escherichia coli* K-12, *J. Bacteriol.* 169 (1987) 5569-5574.

97. J. Tommassen, P. de Geus, B. Lugtenberg, J. Hackett, P. Reeves, Regulation of the *pho* regulon of *Escherichia coli* K-12. Cloning of the regulatory genes *phoB*
875 and *phoR* and identification of their gene products, *J. Mol. Biol.* 157 (1982) 265-274.

98. F.M. Hulett, J. Lee, L. Shi, G. Sun, R. Chesnut, E. Sharkova, et al., Sequential action of two-component genetics witches regulates the Pho regulon in *Bacillus subtilis*, *J. Bacteriol.* 176 (1994) 1348-1358.

880 99. D.O. Carmany, K. Hollingsworth, W.R. McCleary, Genetic and biochemical studies of phosphatase activity of PhoR, *J. Bacteriol.* 185 (2003) 1112-1115.

100. S.L. Fisher, W. Jiang, B.L. Wanner, C.T. Walsh, Cross-talk between the histidine protein kinase VanS and the response regulator PhoB, Characterization and identification of a VanS domain that inhibits activation of PhoB, *J. Biol.*
885 *Chem.* 270 (1995) 23143-23149.

101. M. Schau, A. Eldakak, F.M. Hulett, Terminal oxidases are essential to by pass the requirement for ResD for full Pho induction in *Bacillus subtilis*, *J. Bacteriol.* 186 (2004) 8424-8432.

102. G. Sun, S.M. Birkey, F.M. Hulett, Three two-component signal-transduction systems interact for Pho regulation in *Bacillus subtilis*, *Mol.*
890

Microbiol. 19 (1996) 941-948.

103. F.M. Hulett, The signal-transduction network for Pho regulation in *Bacillus subtilis*, Mol. Microbiol. 19 (1996) 933-939.

104. B. Kaushal, S. Paul, F.M. Hulett, Direct regulation of *Bacillus subtilis* *phoPR* transcription by transition state regulator ScoC, J. Bacteriol. 192 (2010) 3103-3113.

105. E. Botella, S.K. Devine, S. Hubner, L.I. Salzberg, R.T. Gale, E.D. Brown, et al., PhoR autokinase activity is controlled by an intermediate in wall teichoic acid metabolism that is sensed by the intracellular PAS domain during the PhoPR-mediated phosphate limitation response of *Bacillus subtilis*, Mol. Microbiol. 94 (2014) 1242-1259.

106. K. Makino, H. Shinagawa, M. Amemura, S. Kimura, A. Nakata, A. Ishihama, Regulation of the phosphate regulon of *Escherichia coli*. Activation of *pstS* transcription by PhoB protein in vitro, J. Mol. Biol. 203 (1988) 85-95.

107. A.G. Blanco, M. Solà, F.X. Gomis-Rüth, M. Coll, Tandem DNA recognition by PhoB, a two-component signal transduction transcriptional activator, Structure 10 (2002) 701-713.

108. F. Santos-Beneit, The Pho regulon: a huge regulatory network in bacteria, Front. Microbiol. 30 (2015) 402.

109. J.F. Martin, F. Santos-Beneit, A. Sola-Landa, P. Liras, Cross-talk of global regulators in *Streptomyces*. In: Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria, pp.257-267. Edited by Frans J. de Bruijn. Published by John Wiley & Sons, Inc., Hoboken, New Jersey. (2016).

110. M.G. Lamarche, B.L. Wanner, S. Crépin, J. Harel, The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate

homeostasis and pathogenesis, FEMS Microbiol. Rev. 32 (2008) 461-473.

111. S. Crépin, S.M. Chekabab, G. Le Bihan, N. Bertrand, C.M. Dozois, J. Harel, The Pho regulon and the pathogenesis of *Escherichia coli*, Vet. Microbiol. 153 (2011) 82-88.

- 920 112. S.M. Chekabab, J. Harel, C.M. Dozois, Interplay between genetic regulation of phosphate homeostasis and bacterial virulence, Virulence 5 (2014) 786-793.

113. E. Suziedeliene, K. Suziedelis, V. Garbenciute, S. Normark, The acid-inducible *asr* gene in *Escherichia coli*: transcriptional control by the *phoBR* operon, J. Bacteriol. 181 (1999) 2084-2093.

- 925 114. J.T. Pratt, A.M. Ismail, A. Camilli, PhoB regulates both environmental and virulence gene expression in *Vibrio cholerae*, Mol. Microbiol. 77 (2010) 1595-1605.

115. R.D. Monds, M.W. Silby, H.K. Mahanty, Expression of the Pho regulon negatively regulates biofilm formation by *Pseudomonas aureofaciens* PA147-2, Mol. Microbiol. 42 (2001) 415-426.

116. T. Danhorn, M. Hentzer, M. Givskov, M.R. Parsek, C. Fuqua, Phosphorus limitation enhances biofilm formation of the plant pathogen *Agrobacterium tumefaciens* through the PhoR-PhoB regulatory system, J. Bacteriol. 186 (2004) 4492-4501.

- 935 117. R.D. Monds, P.D. Newell, R.H. Gross, G.A. O'Toole, Phosphate-dependent modulation of c-di-GMP levels regulates *Pseudomonas fluorescens* Pf0-1 biofilm formation by controlling secretion of the adhesin LapA, Mol. Microbiol. 63 (2007) 656-679.

- 940 118. G.A. O'May, S.M. Jacobsen, M. Longwell, P. Stoodley, H.L. Mobley,

- M.E. Shirtliff, The high-affinity phosphate transporter Pst in *Proteus mirabilis* HI4320 and its importance in biofilm formation, *Microbiology* 155 (2009) 1523-1535.
119. H.C. Flemming, J. Wingender, The biofilm matrix, *Nat. Rev. Microbiol.* 8 (2010) 623-633.
945
120. T.F. Mah, Biofilm-specific antibiotic resistance, *Future Microbiol.* 7 (2012) 1061-1072.
121. J.F. Martin, Phosphate control of the biosynthesis of antibiotics and other secondary metabolites is mediated by the PhoR–PhoP system: an unfinished
950 story, *J. Bacteriol.* 186 (2004) 5197-5201.
122. K.J. McDowall, A. Thamchaipenet, I.S. Hunter, Phosphate control of oxytetracycline production by *Streptomyces rimosus* is at the level of transcription from promoters overlapped by tandem repeats similar to those of the DNA-binding sites of the OmpR family, *J. Bacteriol.* 181 (1999) 3025-3032.
- 955 123. H. Soualhin, V. Brochu, F. Ménard, B. Papadopoulou, K. Weiss, M.G. Bergeron, et al., A proteomic analysis of penicillin resistance in *Streptococcus pneumoniae* reveals a novel role for PstS, a subunit of the phosphate ABC transporter, *Mol. Microbiol.* 58 (2005) 1430-1440.
124. L. Mechler, A. Herbig, K. Paprotka, M. Fraunholz, K. Nieselt, R. Bertram, A novel point mutation promotes growth phase-dependent daptomycin
960 tolerance in *Staphylococcus aureus*, *Antimicrob. Agents Chemother.* 59 (2015) 5366-5376.
125. W. Shi, Y. Zhang, PhoY2 but not PhoY1 is the PhoU homologue involved in persisters in *Mycobacterium tuberculosis*, *J. Antimicrob. Chemother.* 65 (2010) 1237-1242.
965

126. H.A. Sutterlin, S. Zhang, T.J. Silhavy, Accumulation of phosphatidic acid increases vancomycin resistance in *Escherichia coli*, *J. Bacteriol.* 196 (2014) 3214-3220.
127. H. Nishi, H. Komatsuzawa, T. Fujiwara, N. McCallum, M. Sugai,
970 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.* 48 (2004) 4800-4807.
128. D. Nguyen, A. Joshi-Datar, F. Lepine, E. Bauerle, O. Olakanmi, K. Beer,
975 et al., Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science* 334 (2011) 982-986.
129. U.K. Sharma, D. Chatterji, Transcriptional switching in *Escherichia coli* during stress and starvation by modulation of sigma activity, *FEMS Microbiol. Rev.* 34 (2010) 646-657.
- 980 130. J. Abranches, A.R. Martinez, J.K. Kajfasz, V. Chávez, D.A. Garsin, J.A. Lemos, The molecular alarmone (p)ppGpp mediates stress responses, vancomycin tolerance, and virulence in *Enterococcus faecalis*, *J. Bacteriol.* 191 (2009) 2248 -2256.
- 985 131. F. Santos-Beneit, J.F. Martín, C. Barreiro, Glycopeptides and bacterial cell-walls. *In: Antimicrobial Compounds: Current Strategies and New Alternatives.* Chapter 11 (p. 285-311). Ed. T.G. Villa and P. Veiga-Crespo in Springer. (2014).
132. F. Santos-Beneit, J.F. Martín, Vancomycin resistance in *Streptomyces coelicolor* is phosphate-dependent but is not mediated by the PhoP regulator, *J. Global Antimicrob. Resist.* 1 (2013) 109-113.
990

133. H.-J. Hong, M.I. Hutchings, J.M. Neu, G.D. Wright, M.S. Paget, M.J. Buttner, Characterization of an inducible vancomycin resistance system in *Streptomyces coelicolor* reveals a novel gene (*vanK*) required for drug resistance, *Mol. Microbiol.* 52 (2004) 1107-1121.
- 995 134. F. Santos-Beneit, L.T. Fernández-Martínez, A. Rodríguez-García, S. Martín-Martín, M. Ordóñez-Robles, P. Yagüe, et al., Transcriptional response to vancomycin in a highly vancomycin-resistant *Streptomyces coelicolor* mutant, *Future Microbiol.* 9 (2014) 603-622.
- 1000 135. A. Toledo-Arana, F. Repoila, P. Cossart, Small noncoding RNAs controlling pathogenesis, *Curr. Opin. Microbiol.* 10 (2007) 182-188.
136. C.H. Hoe, C.A. Raabe, T.S. Rozhdestvensky, T.H. Tang, Bacterial sRNAs: regulation in stress, *Int. J. Med. Microbiol.* 303 (2013) 217-229.
137. G.D. Shockman, J.F. Barrett, Structure, function, and assembly of cell-walls of gram-positive bacteria, *Annu. Rev. Microbiol.* 37 (1983) 501-527.
- 1005 138. W. Liu, S. Eder, F.M. Hulett, Analysis of *Bacillus subtilis tagAB* and *tagDEF* expression during phosphate starvation identifies a repressor role for PhoP-P, *J. Bacteriol.* 180 (1998) 753–758.
- 1010 139. M. Li, D.J. Cha, Y. Lai, A.E. Villaruz, D.E. Sturdevant, M. Otto, The antimicrobial peptide-sensing system *aps* of *Staphylococcus aureus*, *Mol. Microbiol.* 66 (2007) 1136-1147.
140. S. Wydau-Dematteis, M. Louis, N. Zahr, R. Lai-Kuen, B. Saubaméa, M.J. Butel, J.L. Pons, The functional *dlt* operon of *Clostridium butyricum* controls the D-alanylation of cell-wall components and influences cell septation and vancomycin-induced lysis, *Anaerobe* 35 (2015) 105-114.

- 1015 141. K. Sieradzki, A. Tomasz, Alterations of cell-wall structure and
metabolism accompany reduced susceptibility to vancomycin in an isogenic
series of clinical isolates of *Staphylococcus aureus*, J. Bacteriol. 185 (2003)
7103-7110.
142. M. Schlag, R. Biswas, B. Krismer, T. Kohler, S. Zoll, W. Yu, et al., Role
1020 of staphylococcal wall teichoic acid in targeting the major autolysin, Atl. Mol.
Microbiol. 75 (2010) 864-873.
143. B. Over, R. Heusser, N. McCallum, B. Schulthess, P. Kupferschmied,
J.M. Gaiani, et al., LytR-CpsA-Psr proteins in *Staphylococcus aureus* display
partial functional redundancy and the deletion of all three severely impairs
1025 septum placement and cell separation, FEMS Microbiol. Lett. 320 (2011) 142-
151.
144. V. Dengler, P.S. Meier, R. Heusser, P. Kupferschmied, J. Fazekas, S.
Friebe, et al., Deletion of hypothetical wall teichoic acid ligases in
Staphylococcus aureus activates the cell-wall stress response, FEMS Microbiol.
1030 Lett. 333 (2012) 109-120.
145. O. Johnsborg, L.S. Havarstein, Pneumococcal LytR, a protein from the
LytR-CpsA-Psr family, is essential for normal septum formation in
Streptococcus pneumoniae, J. Bacteriol. 191 (2009) 5859-5864.
146. P.E. Reynolds, P. Courvalin, Vancomycin resistance in enterococci due
1035 to synthesis of precursors terminating in D-alanyl-D-serine, Antimicrob. Agents
Chemother. 49 (2005) 21-25.
147. N. McCallum, G. Spehar, M. Bischoff, B. Berger-Bächi, Strain
dependence of the cell-wall damage induced stimulon in *Staphylococcus aureus*,
Biochim. Biophys. Acta 1760 (2006) 1475-1481.

- 1040 148. T. Mascher, A.B. Hachmann, J.D. Helmann, Regulatory overlap and
functional redundancy among *Bacillus subtilis* extracytoplasmic function sigma
factors, *J. Bacteriol.* 189 (2007) 6919-6927.
149. B.P. Howden, T.P. Stinear, D.L. Allen, P.D. Johnson, P.B. Ward, J.K.
Davies, Genomic analysis reveals a point mutation in the two-component sensor
1045 gene *graS* that leads to intermediate vancomycin resistance in clinical
Staphylococcus aureus, *Antimicrob. Agents Chemother.* 52 (2008) 3755-3762.
150. Y. Kato, T. Suzuki, T. Ida, K. Maebashi, Genetic changes associated with
glycopeptide resistance in *Staphylococcus aureus*: predominance of amino acid
substitutions in YvqF/VraSR, *J. Antimicrob. Chemother.* 65 (2010) 37-45.
- 1050 151. K. Poole, Bacterial stress responses as determinants of antimicrobial
resistance, *J. Antimicrob. Chemother.* 67 (2012) 2069-2089.
152. S. Boyle-Vavra, S. Yin, R.S. Daum, The VraS/VraR two-component
regulatory system required for oxacillin resistance in community-acquired
methicillin-resistant *Staphylococcus aureus*, *FEMS Microbiol. Lett.* 262 (2006)
1055 163-171.
153. M. Kuroda, H. Kuroda, T. Oshima, F. Takeuchi, H. Mori, K. Hiramatsu,
Two-component system VraSR positively modulates the regulation of cell-wall
biosynthesis pathway in *Staphylococcus aureus*, *Mol. Microbiol.* 49 (2003) 807-
821.
- 1060 154. T. Mascher, S.L. Zimmer, T.A. Smith, J.D. Helmann, Antibiotic-
inducible promoter regulated by the cell envelope stress-sensing two-component
system LiaRS of *Bacillus subtilis*, *Antimicrob. Agents Chemother.* 48 (2004)
2888-2896.
155. P. Suntharalingam, M.D. Senadheera, R.W. Mair, C.M. Lévesque, D.G.

- 1065 Cvitkovitch, The LiaFSR system regulates the cell envelope stress response in
Streptococcus mutans, J. Bacteriol. 191 (2009) 2973-2984.
156. L. Cui, H.M. Neoh, M. Shoji, K. Hiramatsu, Contribution of *vraSR* and
graSR point mutations to vancomycin resistance in vancomycin-intermediate
Staphylococcus aureus, Antimicrob. Agents Chemother. 53 (2009) 1231-1234.
- 1070 157. S.J. Yang, A.S. Bayer, N.N. Mishra, M. Meehl, N. Ledala, M.R.
Yeaman, et al., The *Staphylococcus aureus* two-component regulatory system,
GraRS, senses and confers resistance to selected cationic antimicrobial peptides,
Infect. Immun. 80 (2012) 74-81.
158. M. Meehl, S. Herbert, F. Gotz, A. Cheung, Interaction of the GraRS two-
1075 component system with the VraFG ABC transporter to support vancomycin-
intermediate resistance in *Staphylococcus aureus*, Antimicrob. Agents
Chemother. 51 (2007) 2679-2689.
159. R. Provvedi, F. Boldrin, F. Falciani, G. Palù, R. Manganelli, Global
transcriptional response to vancomycin in *Mycobacterium tuberculosis*,
1080 Microbiology 155 (2009) 1093–1102.
160. S.D. Park, J.W. Youn, Y.J. Kim, S.M. Lee, Y. Kim, H.S. Lee,
Corynebacterium glutamicum sigE is involved in responses to cell surface
stresses and its activity is controlled by the anti-sigma factor CseE,
Microbiology 154 (2008) 915-923.
- 1085 161. H.-J. Hong, M.S.B. Paget, M.J. Buttner, A signal transduction system in
Streptomyces coelicolor that activates the expression of a putative cell-wall
glycan operon in response to vancomycin and other cell-wall-specific
antibiotics, Mol. Microbiol. 44 (2002) 1199-1211.
162. A. Hesketh, C. Hill, J. Mokhtar, G. Novotna, N. Tran, M. Bibb, H.J.

- 1090 Hong, Genome-wide dynamics of a bacterial response to antibiotics that target
the cell envelope, *BMC Genomics* 12 (2011) 226.
163. C.T. Price, V.K. Singh, R.K. Jayaswal, B.J. Wilkinson, J.E. Gustafson,
Pine oil cleaner-resistant *Staphylococcus aureus*: reduced susceptibility to
vancomycin and oxacillin and involvement of SigB, *Appl. Environ. Microbiol.*
1095 68 (2002) 5417-5421.
164. M.J. Bush, G. Chandra, M.J. Bibb, K.C. Findlay, M.J. Buttner, Genome-
Wide Chromatin Immunoprecipitation Sequencing Analysis Shows that WhiB Is
a Transcription Factor That Cocontrols Its Regulon with WhiA To Initiate
Developmental Cell Division in *Streptomyces*, *MBio* 7 (2016).
- 1100 165. D.E. Geiman, T.R. Raghunand, N. Agarwal, W.R. Bishai, Differential
gene expression in response to exposure to antimycobacterial agents and other
stress conditions among seven *Mycobacterium tuberculosis whiB*-like genes,
Antimicrob. Agents Chemother. 50 (2006) 2836–2841.
166. S. Ramón-García, C. Ng, P.R. Jensen, M. Dosanjh, J. Burian, R.P.
1105 Morris, et al., WhiB7, an Fe-S-dependent transcription factor that activates
species-specific repertoires of drug resistance determinants in Actinobacteria, *J.*
Biol. Chem. 288 (2013) 34514-34528.
167. S. Utaida, R.F. Pfeltz, R.K. Jayaswal, B.J. Wilkinson, Autolytic
properties of glycopeptide intermediate *Staphylococcus aureus* Mu50,
1110 *Antimicrob. Agents Chemother.* 50 (2006) 1541-1545.
168. K. Sieradzki, A. Tomasz, Inhibition of cell-wall turnover and autolysis
by vancomycin in a highly vancomycin-resistant mutant of *Staphylococcus*
aureus, *J. Bacteriol.* 179 (1997) 2557-2566.

169. J.L. Koehl, A. Muthaiyan, R.K. Jayaswal, K. Ehlert, H. Labischinski,
1115 B.J. Wilkinson, Cell-wall composition and decreased autolytic activity and
lysostaphin susceptibility of glycopeptide-intermediate *Staphylococcus aureus*,
Antimicrob. Agents Chemother. 48 (2004) 3749-3757.
170. M.J. Kwun, H.J. Hong, The activity of glycopeptide antibiotics against
resistant bacteria correlates with their ability to induce the resistance system,
1120 Antimicrob. Agents Chemother. 58 (2014) 6306-6310.
171. V. Yarlagadda, P. Sarkar, S. Samaddar, J.A. Haldar, Vancomycin
Derivative with a Pyrophosphate-Binding Group: A Strategy to Combat
Vancomycin-Resistant Bacteria, Angew. Chem. Int. Ed. Engl. 55 (2016) 7836-
7840.
- 1125 172. M.S. Butler, K.A. Hansford, M.A. Blaskovich, R. Halai, M.A. Cooper,
Glycopeptide antibiotics: back to the future, J. Antibiot. (Tokyo) 67 (2014) 631-
644.
173. G.D. Wright, Making sense of antisense in antibiotic drug discovery, Cell
Host Microbe 6 (2009) 197-198.
- 1130 174. S.B. Singh, J.W. Phillips, J. Wang, Highly sensitive target-based whole-
cell antibacterial discovery strategy by antisense RNA silencing, Curr. Opin.
Drug Discov. Devel. 10 (2007) 160-166.
175. H. Bai, X. Xue, Z. Hou, Y. Zhou, J. Meng, X. Luo, Antisense antibiotics:
a brief review of novel target discovery and delivery, Curr. Drug Discov.
1135 Technol. 7 (2010) 76-85.