New Bacterial Targets and Computational Methods Against Bacterial Resistance

Authors:

Tarek M. Mahfouz

Michael J. Young

Raabe College of Pharmacy, Ohio Northern University, Ada, OH 45810

Author e-mails:

Tarek M. Mahfouz: t-mahfouz@onu.edu

Michael J. Young: m-young@onu.edu

Abstract

The increasing number of resistant strains of pathogenic bacteria results in a growing number of infections becoming harder to treat. The over and misuse of antibiotics have caused the emergence and spread of multidrug resistant "superbugs" by selecting against sensitive organisms. An example that highlights the problem of multidrug resistant bacteria is the recent report by ABC news of a Nevada woman who died following septic shock caused by the bacteria *K. pneumonaiae*[1]. This bacterium was among the carbapenem-resistant Enterobacteriaceae (CRE) and was resistant to all available antibiotics in the U.S. In 2013, the CDC characterized CRE infections as an urgent threat, meaning the bacteria are an "immediate public health threat that requires urgent and aggressive action." Exacerbating the problem of drug resistance is the scaling down of funding allocated to new antibacterial development by the pharmaceutical industry due to increased cost and low return on investment compared to other groups of medications that are used for life such as cholesterol lowering medications. However, despite the growth of multidrug resistant bacteria and scaling down of funding towards it, there is still hope. The cost of developing new antibiotics can be reduced by focusing on the well validated bacterial targets and by utilizing the available computational resources to efficiently maximize the number of successful leads that make it to the market as new antibiotics. The focus in this article is on simple and cost efficient strategies to develop novel antibiotics or to revive old ones. To assist in this effort, presented in this article is a review of computational techniques and strategies that can be employed to develop safe and effective novel antibacterial therapies. This is followed by a review of resistance mechanisms in bacteria and validated bacterial targets amenable for drug design.

Introduction

Antibiotic resistance is a global public health challenge[2, 3]. Two factors contributing to this problem are the increased antibiotic use/abuse[4] and the dissemination of antibiotic resistance genes among bacteria[5]. This, together with the decreased interest by the pharmaceutical industry in new antibiotic development, has prompted governments in the United States and worldwide to draft strategies to combat this problem. In the United States, the national strategy for combating antibacterial resistance as explained by the Center for Disease Control outlines five goals for action to combat the increasing antibacterial resistance. One of the goals outlined is to "accelerate basic and applied research and development for new antibiotic, other therapeutics, and vaccines"[6].

The battle against antibiotic resistance can be fought on two fronts; 1) advancing research efforts toward the discovery of novel antibiotic structures, or 2) enhancing the effectiveness of the currently available ones, which can be achieved either by chemical modifications or by coadministration with "antiresistance" drugs. In either case, the great advances in computer sciences and programming have made available a diverse variety of programs that can facilitate the identification of new antiresistance lead compounds and their further development into drugs which will greatly advance our efforts to develop new therapies against resistant bacteria. The computational resources available for lead identification are classified into two main approaches: structure-based and ligand-based approaches. As the names imply, structure-based approaches, which may involve homology modeling techniques, are used in cases where structural models are available for the drug target of interest. A drug target is a protein or any other molecule that is essential for bacterial growth and survival. On the other hand, ligand-based approaches are used in cases when structural models are not available. In addition to lead identification, there are auxiliary programs that allow researchers to examine important properties of the identified leads such as absorption and other pharmacokinetic properties to exclude problematic compounds from further development efforts. All those computational resources, when used properly, can reduce the time and cost of developing new antibiotics. In the next few paragraphs, the computational techniques are presented first followed by a short review of some of the promising bacterial targets.

Computational Methods

Structure-based Approaches

The basic idea behind structure-based approaches is to develop a novel therapeutic agent utilizing chemical and structural information obtained from within the binding site of the target protein. That requires the availability of a structural model of the target protein. A great number of computer programs are also available to assist with the different steps involved in the structure-based modeling process such as binding site visualization and analysis, inhibitor design, and binding affinity predictions. Examples of such programs will be provided in the next few paragraphs.

In general, structures of the target protein with a ligand (whether a substrate, an inhibitor, an activator, an agonist, or an antagonist) bound in the active site are ideal and the most helpful because they reveal clearly the important interactions stabilizing the ligand in place. Such interactions, once identified, can be extracted and incorporated into pharmacophore models for virtual screening of chemical databases to identify new lead compounds with improved potency or enhanced properties such as selectivity[7]. A number of computer programs that can be used to develop structure-based

pharmacophore models as well as pharmacophore-based virtual screening of databases are commercially available such as PHASE[8].

In absence of ligand-bound structures of the bacterial target, structures of the unbound free targets are also useful because they allow for the *de novo* design of novel structures tailored to fit in a particular active site. Fragment-based drug design is a structure-based technique that is used to develop custom-based drugs. In this technique, novel chemical structures are built to fit in a particular binding site by connecting small chemical fragments together and estimating the binding energies of the resulting structures[9]. Among the programs that can be used in fragment-based drug design are SPROUT[10] and MUSIC[11]. A drawback, however, of the fragment-based approach is the difficulty, sometimes, in synthesizing these novel structures.

If chemical synthesis of structures resulting from fragment-based approaches proved to be challenging, an alternative approach is to search databases of commercially or otherwise available chemical compounds by docking into the target protein's vacant binding site to identify compounds with good binding affinities that can be potential leads. Docking programs usually employ scoring functions to rank the group of compounds based on their estimated binding affinities. Several docking programs can be used to dock compound libraries; some are freely available online, as have been reviewed[12, 13], e.g. AutoDock4[14-16].

With binding sites structures available, ligands with varying degrees of selectivity can be developed. For example, antibacterial drugs that interfere with human biochemistry can be made more selective towards their bacterial targets if the structures of the bacterial target and the human protein are available. In such cases, the two binding sites can be superimposed to identify features unique to the bacterial binding site that can be utilized to bias the ligand toward the bacterial target. On the other hand, oftentimes resistance in bacteria result from a mutant form of the bacterial target that has no or weakened affinity to the antibiotic. In such cases, ligands with decreased selectivity that target both the wild type and the mutant form simultaneously can be developed by superimposing the wild type and mutant binding sites to target features conserved between the two. Using this approach, or through docking of chemical databases, compounds with similar affinities towards the two binding sites can be identified. For all those reasons, structure-based approaches represent powerful tools in the battle against resistant bacteria.

Two freely available internet resources that are indispensable to computational structure-based drug design efforts are the Protein Data Bank (PDB)[17, 18] and the ZINC database[19, 20]. The PDB is a database of protein structures which, as of October 2016, contained 123,456 protein structures. Of those, approximately 45,000 are bacterial proteins that are either bound to their ligands or in the unbound states. The ZINC database, on the other hand, is a database of commercially available compounds that contains over 13 million drug-like structures for docking or virtual screening using pharmacophore models to identify new potential therapeutic agents. Both databases are available for free download. Other chemical databases are also available from multiple resources online[21].

In cases where a structural model of the bacterial target is not available, it is possible to develop one using comparative (homology) modeling. Homology modeling is the process of developing a structural model for an unknown protein (called the target) that has its primary structure (amino acid sequence) available using as a template

a high resolution structure of a highly similar protein (called the template) which has at least 25% sequence identity to the target[22,23]. The process of homology modeling is a four step process that involves 1) template selection, 2) target-template alignment, 3) model building and 4) model evaluation/validation. If the target has been cloned, then its primary structure can be obtained from different web-based resources such as the UniProt database[24] or the SWISS-MODEL workspace[25,26]. Once amino acid sequence information of the target is obtained, the next step is to identify an appropriate template. In this step, databases of proteins with known structures, such as the PDB, can be searched to identify the protein with the highest sequence identity to the target. It has been indicated that homology models built with over 50% target-template sequence identities are accurate enough for drug design applications[22]. Once a suitable template is identified, the target and the template sequences are then aligned and atomic coordinates of the template are copied to the target to construct a raw structural model which is then optimized and validated in the last step by, for example, testing its ability to reproduce experimental binding results. Once the model proves to be accurate, any of the structure-based approaches explained above can be employed to identify novel antibacterial compounds. Several computer programs are available, some for free and some are licensed, that can help with the homology modeling process. The SWISS-MODEL workspace is a powerful web site for comparative modeling that is freely available. It integrates together programs and databases required for comparative modeling and allows the user to perform all the four steps mentioned above using a web-based modeling workbench. A number of other homology modeling programs are either freely[27,28] or commercially available[29].

Ligand-based Approaches

In the absence of any structural information of a particular bacterial target, new and optimized lead compounds may still be developed using ligand-based approaches. In ligand-based approaches, binding information to a particular target can be inferred by superpositioning of a group of experimentally identified ligands that all bind to the same site on the target of interest but with varying affinities[30]. Such information can then be incorporated into pharmacophore models or quantitative structure-based activity relationship (QSAR) models which are then used as queries to virtually screen chemical databases such as the ZINC database. An important aspect of ligand-based models is that they can be used to assign an experimental activity to the identified compounds so they could be prioritized for further evaluation and selection. The computer program PHASE can also be used for ligand-based pharmacophore building and the subsequent database screening[8]. In addition, some ligand-based pharmacophore extraction programs are also freely available for download from the internet such as PHARMER[31].

Absorption, Distribution, Metabolism, Excretion (ADME) and Toxicity of Lead Compounds

One of the pitfalls to drug development is poor ADME properties. Poor ADME properties, side effects and toxicity of potential drug candidates are frequent causes of failures in clinical trials[32]. Identifying and eliminating those problematic compounds at early stages of the drug development process, even before animal testing, should significantly reduce the cost and save time. Several computer programs have been developed to screen candidate molecules for ADME and toxicity properties, some of them are licensed

software, e.g. QikProp[33], and some are freely available on the internet[21, 34]. In addition, the FAF-Drugs3 is an online service that allows users to process their own compound collections via simple ADME/toxicity filtering[35,36].

In the remaining sections of this article, a group of bacterial proteins that are valid targets for new antibacterial design and that have not been targeted before are introduced with the appropriate drug design approach in each case. The list is not meant to be inclusive and is only suggestive.

Bacterial Elements of Resistance

One of the earliest approaches to combat antibiotic resistance is to target bacterial elements of resistance which in that case was the beta lactamase enzyme which deactivates penicillin and the other beta lactam antibiotics. This was accomplished through the coadministration of a beta lactamase inhibitor together with the beta lactam antibiotic[37]. However, bacterial resistance is not just limited to the production of beta lactamases and an increasing number of bacterial proteins implicated in resistance are being uncovered regularly. What is facilitating this are the technological advances in molecular and structural biology and the availability of genomes from pathogenic bacteria. Antibiotic resistance mechanisms in bacteria can be grouped into four groups: 1) biofilm formation, 2) alterations of cell wall or cell wall metabolites, 3) modification/mutations in the antibiotic target, and 4) antibiotic deactivation by bacterial enzymes. Among those, more attention is given to biofilms because of the multiple antibiotic targets it presents that have not been utilized yet.

1- Biofilm Formation

Bacterial growth is characterized by two phenotypes; single cells (planktonic form) or sessile aggregates (biofilm form). Biofilms account for a great proportion of all microbial infections in the human body[38]. Bacterial biofilms are highly resistant to the host's antibodies[39]. In addition, antibiotics that kill planktonic cells often times fail to kill bacterial cells within a biofilm[39]. Among the mechanisms proposed to explain this increased resistance of biofilms to antibiotics are delayed antibiotic diffusion in biofilms, persister cells that exist within a biofilm[40], stress responses[38], quorum sensing[41], and multidrug efflux pumps[42]. Biofilm components behind each of these resistance mechanisms, therefore, represent attractive targets for novel antibiotic design.

- Delayed antibiotics diffusion in biofilms

Transport of some antibiotics across bacterial biofilms was found to be significantly impeded by the biofilm[43]. Not all antibiotics, however, are equally affected by the biofilm matrix polymers[44,45]. Mathematical models have been formulated to predict the diffusion of different types of antibiotics into biofilms under different conditions[46]. Despite the fact that these models did not include parameters pertinent to structural and physicochemical properties of the individual antibiotic, they can be updated to incorporate such parameters so that they can be included as part of the screening step to exclude problematic compounds allowing only hits with good biofilm penetration properties to move forward. In addition, since hydrolyzing the complex polymeric structures forming the biofilm matrix, especially the polysaccharides, has been shown to enhance antibiotic activity[47], an alternative approach is to block their production. Glucan synthase is the enzyme that allows bacteria to produce the polysaccharides in biofilms[48,49]. Consequently, glucan synthase inhibitors could be coadministered with the antibiotic to facilitate antibiotic diffusion into the biofilm. Unfortunately, as of this point, this

enzyme has no structural models available but homology modeling techniques could be used to develop one using any of the relevant glycosyltransferases structures deposited in the PDB[17].

- Persister cells

"Persister cells" denotes a subpopulation of bacterial cells in a biofilm that are tolerant to antibiotics. Tolerant means they neither grow nor die in the presence of bactericidal antibiotics[50]. Persister cells are also not mutants and are not resistant to antibiotics[51, 52]. Antibiotic treatment usually eliminates the majority of both planktonic and biofilm cells except the persisters. Upon discontinuation of treatment, apparently due to resolution of symptoms, persisters repopulate the biofilm, releasing new planktonic cells and leading to relapsing biofilm infections. Persister cells are not killed by antibiotics because they do not express the respective targets due to shut down of protein and DNA synthesis[40]. The shift of bacterial cells to the persister state is controlled by several regulatory proteins and targeting of those regulators, therefore, constitutes an attractive strategy to fight bacteria in biofilms. In this context, an antipersisters molecule can be coadministered with the antibiotic and the combination therapy would prevent relapsing infections due to its activity against bacterial biofilms. Obtaining an antipersisters molecule, however, could prove to be a difficult task because of redundancies in persistence genes[53, 54].

An important regulator of the persister state are the toxin-antitoxin (TA) modules. TA modules consist of a stable toxin, a protein whose activation promotes the switching to the persister state or even bacterial death, and a degradation-prone antitoxin, which is either a protein or a small RNA molecule that either binds to and directly inhibit the toxin or down regulates its production[55]. A total of five types of TA modules have been identified in bacteria[56] with type II TA modules being the most relevant to persistence[55]). The HipA-HipB protein pair is a type II TA module that was the first to be implicated in bacterial persistence[57]. HipA, the toxin, is a serine protein kinase that phosphorylates glutamyl-tRNA synthase thus inhibiting protein biosynthesis and driving cells to dormancy and persistence[58]. Normally, this does not happen because of HipB, the antitoxin, which binds tightly to HipA blocking its function and preventing cells from shifting to the persister state[59,60]. Disrupting the function of HipA, therefore, should stop, or at least reduce the frequency of, shifting to the persister state. In fact, a group of HipA inhibitors have been shown to remarkably reduce *E. coli* persistence[61], further validating HipA as a target for antipersister drug design. There are several structures for HipA and HipB in the PDB from different bacterial species both in the free (e.g. HipA, PDB code 3DNU[62], and HipB, PDB code 3DNV[62]), and the bound (e.g. PDB codes 4YG7[63], 4PU3[64]) states as well.

Another novel approach to eliminating persister cells could be through utilizing the ToxN-ToxI system which is a type III TA module. In this system, ToxN is the protein toxin which promotes bacterial cell death while ToxI is an RNA antitoxin that neutralizes the effects of ToxN[65]. The ToxN-ToxI complex has a heterohexameric triangular arrangement where the ToxN monomers are at the corners of the triangle and are connected by the ToxI monomers that bind on either side of the toxin. The ToxI monomers fold into an H-type pseudoknot structure followed by two single stranded tails. It is these tails that bind to ToxN binding sites and act to form and stabilize the triangular arrangement, all the while neutralizing ToxN. Crystal structures are available for the ToxN-ToxI complex (e.g. PDB codes for $ToxIN_{Pa}$ is 2XDD[66] and

for $ToxIN_{Bt}$ is 4ATO[67]). Through the analysis of these crystal structures, it is possible to design a drug molecule to bind onto the tails of ToxI to block its binding to ToxN which will allow ToxN to perform its function of promoting bacterial cell death.

The induction of persister state in bacteria through TA modules involve the production of the signaling nucleotide ppGpp which, in turn, exerts its action by involving the polyphosphate kinase and the Lon protease which degrade the antitoxins leaving the toxins free in the active state to shut down translation and promote the induction of the persistence state[60,68]. Another antipersister approach, therefore, could involve small molecule inhibitors of either the polyphosphate kinase or the Lon protease. Structures for the Lon protease in different states are available (e.g. PDB codes 1RR9[69] and 5E7S[70]) as well as structures for the polyphosphate kinase (e.g. PDB code 4YEG[71]). Other TA modules in bacteria have been identified[55,72] and provide a wealth of other targets for antipersister drugs.

An example of a bacterial regulator of persistence that is not part of a TA module is the PhoU protein which is a negative regulator for phosphate metabolism in bacteria[73] that shuts down genes involved in energy production to facilitate persister formation. PhoU protein is highly expressed in bacteria when nutrients are limited and upon exposure to ampicillin. Moreover, PhoU mutant strains of E. coli are deficient in persistence[74] which validates it as a drug target to block the persister formation. The structure of PhoU from different bacterial species, including pathogenic ones, are available (e.g. PDB code 4Q25[75]).

- Stress response

In addition to the inhibition of their respective bacterial targets, bactericidal antibiotics have been shown to kill bacteria by stimulating hydroxyl radical formation and inducing oxidative stress[76]. The oxidative stress caused by bactericidal antibiotics generates reactive oxygen species (ROS) that cause oxidative damage to nucleic acids (DNA) and proteins within bacterial cells. This has been supported by the observation that mutants deficient in the function of RecA, a protein required for DNA damage repair mechanisms[77], showed increased sensitivity to antibiotics[78] and by further *in vivo* experiments[79] as well. In addition, the biofilm associated tolerance to ofloxacin has been shown to require a functional SOS response[80], which adds proteins of the SOS response to the list of anti-biofilm drug targets. In fact, several genes encoding proteins involved in the generation of ROS are downregulated in persister cells while genes encoding proteins involved in ROS detoxification are upregulated[81]. These results demonstrated the significance of the ROS detoxifying and the damage repair proteins to survival of persister cells in biofilms which makes them viable targets for antipersister drug design.

The SOS response is regulated mainly by two proteins; the transcription repressor Lex and the inducer RecA recombinase. Small molecule inhibitors of RecA should make bacterial biofilms more susceptible to antibiotics and, to assist with this effort, structures for the RecA are available for structure-based design of inhibitors, e.g. PDB code 4TWZ[82]. In addition, small molecule inhibitors of RecA have been identified[79,83] if ligand-based approaches are desired or more feasible.

Catalase and superoxide dismutase, which are two enzymes that allow bacteria to neutralize ROS, have been shown to significantly decrease the persister fraction in bacterial biofilms[81]. Multiple crystal structures for the catalase and superoxide dismutase enzymes from different bacterial species are available to support structure-

based inhibitor design efforts, e.g. PDB codes 3N3N[84], 4ENP[85], 1QWL[86], and for superoxide dismutase PDB codes 3OT7[87] and 4YIP[88]. Care should be taken, however, because some antibacterial drugs require activation by certain catalases, such as KatG first, before they show antibacterial effect[89] as will be explained in a later section.

Similar to DNA damage repair mechanisms, chaperones, which are proteins involved in the regulation of misfolded proteins, have been shown to be upregulated due to oxidative damage[78]. Targeting of such bacterial chaperones, in particular DnaK, also have been shown to enhance the killing effect of bactericidal antibiotics[90]. Structures for DnaK are also available, for example PDB code 4R5G[91] and all relevant structures.

Other bacterial enzymes that were found to be upregulated under stress are enzymes of the glyoxalate shunt, in particular isocitrate lyase[81]. The crystal structure of isocitrate lyase from multiple bacterial species is available (e.g. PDB code 1IGW[92] and all relevant structures).

- Quorum sensing

Bacterial cells in the planktonic form produce a group of signaling molecules at small amounts that are not enough to stimulate gene expression. However, when the population size reaches a certain level, the concentration of those molecules becomes high enough to induce the expression of genes that promote biofilm formation and antibiotic resistance[93]. This phenomenon is termed quorum sensing and the common classes of quorum signaling molecules in bacteria include oligopeptides in Gram-positive bacteria, N-acyl homoserine lactones (AHL) in Gram-negative bacteria, and the two major systems of autoinducers, autoinducer-1 (AI-1) and autoinducer-2 (AI-2), which exists in both Gram-negative and Gram-positive bacteria[94]. Disrupting quorum sensing pathways would, therefore, disrupt biofilm formation and make bacterial populations more vulnerable. In fact it has been shown that mBTL, which is an antagonist of the quorum sensing receptors LasR and RhiR, blocks virulence and biofilm formation in *P. aeruginosa*[95]. In addition, molecules that inhibit the production of AHLs have been shown to inhibit the swarming motility of *P. aeruginosa*[96].

Inhibition of quorum sensing in bacteria can be achieved through several mechanisms that may include entrapment of the signaling molecules, inhibition of proteins/enzymes required for the biosynthesis of the involved signaling molecules, or through the use of antagonists that competitively bind to quorum sensing receptors and block their activation[94]. Several inhibitors have been developed that inhibit quorum sensing in multiple bacterial species[97-99] which provides important information for ligand-based efforts to identify new more efficient inhibitors suitable for clinical use in humans. In addition, and to facilitate structure-based design of quorum sensing inhibitors, structural models of proteins involved in quorum sensing have been made available. For example, the LuxS protein, which is required for the biosynthesis of AI-2 and is wide spread in bacteria, has three crystal structures from three different bacterial species (PDB codes 1J6X, 1J6W, 1INN, and 1J6V[100]). In addition, the crystal structures of the quorum sensing receptors LuxP and its variants from multiple species are available both in the unbound free form and bound to AI-2 are also available (PDB codes 1JX6[101], 1TM2[102], 3EJW[103], and [104]). Although some of those structures are from nonpathogenic species, homology modeling techniques can be employed to generate structural models for the receptor from any desired pathogenic species.

- Efflux pumps

Efflux pumps are membrane proteins that pump out a wide range of waste products/harmful compounds to maintain their cytoplasmic concentrations below a certain safety threshold. Different families of antibiotics are substrates for bacterial efflux pumps making efflux pumps an important antibiotic resistance mechanism by bacteria in both the planktonic and biofilm forms. Inhibition of efflux pumps have been shown to interfere with bacterial biofilm formation validating bacterial efflux pumps as targets for novel antibiotic discovery[105-108].

Bacterial efflux pumps that confer multidrug resistance are classified into five different families based on their structure and the energy source utilized to drive the transport[109]. These are: the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, the resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family (which is a member of the much larger drug/metabolite transporter (DMT) superfamily), and the multidrug and toxic compound extrusion (MATE) family. Of those five families, the RND family is found in Gram-negative organisms whereas the other four families are found in both Gram-negative and Gram-positive organisms[110]. One bacterial cell may contain several efflux pumps from several different families where a single antibiotic may be pumped out by more than one pump type. For example, on the planktonic level, several efflux pumps have been identified in bacteria that allow them to resist the effects of the tetracyclines and these have been grouped into seven different groups[111]. They share high degree of sequence identity among themselves with the most dissimilar pair sharing 29% sequence identity; high enough to allow for the development of structural models using homology modeling for use in structure-based approaches with the possibility of developing a universal inhibitor.

Multiple approaches have been utilized to identify bacterial efflux pump inhibitors that resulted in the identification of two main structural classes of inhibitors: the peptidomimetics and the pyrido-pyrimidines[112]. However, no efflux pump inhibitor has been approved for clinical use possibly due to their toxicities in humans and/or unsuitability for clinical use as some of those inhibitors are actually drugs that are in use to treat other conditions, e.g. reserpine and phenothiazines[113]. Nonetheless, the availability of structural and inhibitory data for those inhibitors provides enough means for ligand-based drug design approaches to develop new more potent inhibitors with decreased toxicities which are also more suitable for use in humans.

Crystal structures for efflux pumps from the different families (in the free and in some cases with inhibitors bound in the active site) have been made available for structure-based approaches to develop safer, more potent, and more clinically relevant inhibitors. One of those that has been recently released is the AcrAB-TolC (PDB codes 4ZLJ, 5EN5, 4U96 and related structures)[114-116]. Other structures include, from the RND family, the MtrD pump (PDB code 4MT0[117]) and from the MFS family is pump adapter EmrA (PDB code 4TKO[118]).

2- Alterations of Cell Wall or Cell Wall Metabolites

Bacterial cells differ from mammalian cells in having an additional protective layer which is the peptidoglycan cell wall layer. It plays an essential role in protecting bacterial cells from osmotic lysis. Because of this, it has been the target for several antibiotic groups that interfere with its biosynthesis such as the penicillins, cephalosporins, and the glycopeptides such as vancomycin. However, structural changes in bacterial cell

walls can contribute to bacterial resistance. For example, the vancomycin resistant entercocci produce altered peptidoglycan precursors terminating with the precursor depsipeptide D-Ala-D-lactate which has significantly lower affinity to vancomycin than the normal dipeptide precursor D-Ala-D-Ala[119,120]. Production of the D-Ala-D-lactate precursor with the reduced affinity to vancomycin is attributed to the cumulative functions of three different proteins, VanA (a variant of the normal D-Ala-D-Ala ligase (ddl) that specifically binds to D-lactate resulting in the production of the depsipeptide D-Ala-D-lactate)[121], VanH (a D-hydroxy acid dehydrogenase that makes available the D-lactate required for the VanA ligase reaction)[122], and VanX (a D,D-dipeptidase that specifically cleaves the D-Ala-D-Ala dipeptides but lacks activity against the depsipeptide)[123]. These proteins work together to produce the depsipeptide that is then incorporated into the peptidoglycan layer even in the presence of vancomycin. An inhibitor to any of those enzymes would be expected to abolish the vancomycin resistant phenotype of this mutant strain. Moreover, the expression of those three proteins is regulated by the activity of the VanR protein which regulates the transcription of the *vanHAX* operon[124], representing an additional target to abolish the vancomycin resistant phenotype. Other variants of the VanA ligase have been identified in other vancomycin resistant enterococci but they all share between 44% to 76% sequence identity to VanA[125,126]. The percentage sequence identity drops to around 30% between VanA and the similar D-Ala-D-lactate/D-serine ligases from other bacteria[127]. However, 30% sequence identity is still high enough to develop homology models for this family of ligases for use in structure-based inhibitor design which offers the possibility of developing a universal inhibitor against vancomycin resistance in the enterococci and other species as well[128]. What may facilitate this is the availability of structures for VanA from different bacterial species (PDB codes 1E4E[129] and 1EHI[130]) in addition to structures of other similar ligases such as that of the ddl enzyme (PDB code 2DLN[131]), and the similar VanG which has D-Ala-D-serine ligase activity (PDB code 4FU0[132]). Structures of Van X and VanY peptidases are also available (PDB codes 1R44[133], 5HNM, 4OAK[134], and all related structures). That, in addition to the availability of inhibitors for ddl, VanA[135], and VanX[136,137] for ligand-based approaches. This allows for the use of multiple approaches to develop inhibitors to those resistance enzymes which maximizes the chances of identifying good leads.

3- Modifications/Mutations in Antibiotic Targets

One of the fastest and most common mechanisms that allow bacteria to resist antibiotics are spontaneous mutations that arise in antibiotic targets. Within the cytoplasm, antibiotic molecules kill bacteria by binding to and inhibiting specific protein targets that are essential for bacterial survival. In response, mutational changes in the targets that reduce their affinity for the antibiotic can occur whilst retaining the targets cellular functions. Alternatively, evolution of bacterial targets can produce different variants with different susceptibilities to inhibition with the same antibiotic. A good approach in these situations would be to develop structural models for the mutant form of the enzyme (assuming it does not have one), utilizing any of the homology modeling programs outlined above, and using the wild type enzyme as the template and to compare the mutant and wild type structures to develop a non-selective inhibitor capable of inhibiting the two forms of the enzyme.

- Penicillin binding proteins (PBPs)

The beta-lactam antibiotics act by blocking bacterial peptidoglycan biosynthesis by inhibiting the group of membrane bound enzymes known as penicillin binding proteins (PBPs) which are responsible for peptidoglycan polymerization and insertion into the preexisting cell wall. The PBPs constitute a large family of enzymes and, typically, bacterial cells contain varied numbers of PBPs each with different affinity toward the different members of the beta-lactam antibiotics[138]. Bacteria that produce mainly PBPs with reduced or no affinity to the beta-lactams are resistant to this class of antibiotics such as the Methicillin Resistant *S. aureus* (MRSA)[139]. The crystal structures of several PBPs are available (for example PDB codes 1MWR[140], 3EQU[141], and 3OC2[142]). Of special interest are the resistant ones PBP1a, (PDB code 2C5W[143]), PBPb2 (PDB code 2WAD[144]), and PBP2x (PDB code1RP5[145]). This should facilitate structure-based drug design studies to develop a universal or a broad spectrum PBP inhibitor. If needed, homology models could also be developed for those PBPs of interest that have no structure available using any of the above structures as a template.

- DNA gyrase and topoisomerase IV

Fluoroquinolones act by inhibiting DNA gyrase and topoisomerase IV which are enzymes that relieve supercoiling in bacterial DNA[146]. Mutations in both the DNA gyrase and topoisomerase IV can confer resistance to the organism through decreased drug affinity[147,148]. Structures for bacterial DNA gyrase and topoisomerases are available (PDB codes 4CKK[149], 5IWI[150] and all similar structures) together with inhibitory activities for newly developed inhibitors[150].

- RNA polymerase

Resistance to the antibiotic rifampicin results from mutations in its target site which is the beta-subunit of RNA polymerase[151]. To facilitate structural studies, the structure of *E. coli* RNA polymerase is available in complex with rifampin (PDB code 4KMU[152]).

- Ribosomes

Bacterial ribosomes are the target for antibiotic groups that interfere with bacterial protein biosynthesis such as the tetracyclines, chloramphenicols, macrolides, and aminoglycosides. Resistance mechanisms involving ribosomal targets include ribosomal masking (which will be discussed in more detail in the sections below), ribosomal mutations, and ribosomal modifications. Resistance to the macrolide antibiotics results from a post transcriptional methylation of adenine bases in the peptidyl transferase functional domain of the 23S rRNA component of the 50S ribosomal subunit[153]. One practical approach to combat resistance resulting from ribosomal modifications could be to develop inhibitors of the modifying enzymes, i.e. the bacterial methylases, to be coadministered with the antibiotic.

Several structures of the bacterial ribosomal 50S subunit in complex with different families of antibiotics are available, for example the macrolides (PDB code 1K8A[154]), chloramphenicol and others (PDB codes 1NJI, 1KC8, and 1M90[155]). Structures of mutant ribosomes are also available (e.g. PDB code 1JZY[156]) which could allow for the development of non-selective inhibitors targeting both wild type and mutant ribosomes using structure-based approaches. The aminoglycosides, on the other hand, bind to the 16S rRNA in the smaller 30S subunit of the bacterial ribosome and structures of representative aminoglycosides bound to the bacterial 30S subunit are

available (e.g. PDB codes 2QAL, 2QAM, 2QAN and all other structures[157]).

Mutations also can arise in enzymes that are required for the activation of prodrug antibiotics such as the antitibercular drugs isoniazid[158] and pyrazinamide[159]. Such mutations weaken the affinity of the enzyme to the prodrug blocking its conversion to the active antibiotic, thus, conferring resistance to the organism. Structures of the wild type and mutant KatG (PDB codes 1SJ2[160] and 4C50[161]; respectively), required to activate isoniazid, and pyrazinamidase[162], required to activate pyrazinamide are available for structure-based antibiotic design.

- Ribosomal/Target Protection

Another resistance mechanism in bacteria involving antibacterial targets is masking of the bacterial target using protection proteins such as the ribosomal protection protein Tet(O)[163]. Tetracycline antibiotics block protein biosynthesis in bacteria by binding to the 30S subunit of bacterial ribosomes[164]. Ribosomal protection proteins like Tet(O) act by binding to the ribosome and decreasing its affinity for tetracycline[165]. Molecules that can interfere with or inhibit binding of Tet(O) and similar proteins to the ribosome could presumably abolish resistance to tetracyclines. The structure of the 30S ribosomal subunit in complex with tetracycline is available (PDB code 1HNW)[166] together with the structure of Tet(O) bound to the 70S ribosome(PDB code 4V6V[167]) which makes this system suitable for structure-based antiresistance drug design.

4- Antibiotic Deactivation by Bacterial Enzymes

Bacterial cells typically produce several families of enzymes that can chemically modify widely diverse classes of chemical molecules including antibiotics. By chemically modifying the antibiotic molecule, the relevant bacterial target loses its affinity toward it which confers resistance to the organism. Bacterial enzymes involved in resistance through antibiotic deactivation by chemical modification have been classified into five main groups; hydrolases, group transferases such as kinases and acetyl transferases, lyases, and oxidoreductases[168]. Two approaches can be utilized to combat resistance by modifying enzyme. The first is to coadminister the antibiotic with an inhibitor of the modifying enzyme, and the other is to change the modification site on the antibiotic so that it is no longer recognizable by the modifying enzyme. Both approaches have been used successfully to fight the deactivating effect of the beta-lactamases against penicillins and cephalosprins. Preparations containing a beta lactam antibiotic in combination with a beta lactamase inhibitor are on the market[37] in addition to newer classes of beta lactams that are resistant to the hydrolyzing effect of the beta lactamases[169]. The case could be applied to other antibiotic classes as well.

Several members of the antibiotic deactivating enzyme families have structures available in the protein databank. For example, the group of hydrolases known as beta lactamases, which allow resistant bacteria to deactivate the beta lactam antibiotics by hydrolyzing the beta lactam ring, have multiple structures available (e.g. PDB code 5EOE[170]). On the other hand, among many known resistance mechanisms against the chloramphenicols and the aminoglycosides is acetylation catalyzed by different groups of acetyl transferases [171,172]. Relevant structures available for antiresistance drug design include the crystal structure of chloramphenicol acetyl transferase in complex with chloramphenicol (PDB code 3U9F[173]) and, more importantly, the structure of the bifunctional aminoglycoside acetyl transferase, known as AAC(6')-Ib variant, AAC(6')-Ib-cr which catalyzes the acetylation and deactivation of

both the fluoroquinolones as well as the aminoglycosides (PDB code 1V0C and related structures[174]) offering the chance for structure-based inhibitor design against bacterial species resistant to these two classes of antibiotics. Here, again, care should be taken because, in general, aminoglycoside acetyl transferases belong to the GCN5 super family, which also includes histone acetyl transferases among others, which can limit their clinical use. Nonetheless, having all relevant structures available, whether experimentally determined structures or homology models, should facilitate the development of selective inhibitors by focusing on active site differences, a strength of structure-based approaches. Interested readers can search the PDB (www.rcsb.org) for their enzyme of interest to find out if it has structural models available for structure-based antiresistance drug design. Alternatively, if a structural model is not available, the amino acid sequence could be obtained from UniProt or GenBank (https://www.ncbi.nlm.nih.gov /genbank/) to develop a homology model of the target enzyme for structure-based approaches.

Conclusions

In conclusion, there are multiple validated bacterial targets and powerful computational tools with diverse capabilities for the design of new antibiotics to fight resistant organisms. This, combined with more funding being provided for new antibiotic research and discovery, should greatly advance efforts toward that goal. The one thing left to do is to employ accurate and more inclusive methods of estimating lead compounds' resistance potentials so that those compounds with estimated high resistance potential should be excluded from further investigation at earlier stages. Currently employed methods of assessing resistance potential are not inclusive because they, for one thing, ignore the risk of horizontal gene transfer and do not include a fitness cost and, hence, do not give an accurate estimate of a compound's resistance potential[175]. Such factors, if determined experimentally for different infection models, can be incorporated into mathematical models to give better estimate of a lead compound's resistance potential.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Mohney, G. What You Need to Know About the Deadly Superbug Infection Resistant to All FDA-Approved Antibiotics. *https://gma.yahoo.com/know-deadly-superbug-infection-resistant-fda-approved-antibiotics-202932850.html* (accessed Jan 31, 2017).

2. Bush, K.; Courvalin, P.; Dantas, G.; Davies, J.; Eisenstein, B.; Huovinen, P.; Jacoby, G. A.; Kishony, R.; Kreiswirth, B. N.; Kutter, E.; Lerner, S. A.; Levy, S.; Lewis, K.; Lomovskaya, O.; Miller, J. H.; Mobashery, S.; Piddock, L. J. V.; Projan, S.; Thomas, C. M.; Tomasz, A.; Tulkens, P. M.; Walsh, T. R.; Watson, J. D.; Witkowski, J.; Witte, W.; Wright, G.; Yeh, P.; Zgurskaya, H. I. Tackling antibiotic resistance. *Nat. Rev. Microbiol.* **2011**, *9*, 894-896.

3. Roca, I.; Akova, M.; Baquero, F.; Carlet, J.; Cavaleri, M.; Coenen, S.; Cohen, J.; Findlay, D.; Gyssens, I.; Heure, O. E.; Kahlmeter, G.; Kruse, H.; Laxminarayan, R.; Liébana, E.; López-Cerero, L.; MacGowan, A.; Martins, M.; Rodríguez-Baño, J.; Rolain, J. -.; Segovia, C.; Sigauque, B.; Tacconelli, E.; Wellington, E.; Vila, J. The global threat of antimicrobial resistance: science for intervention. *New Microbe. and New Infect.* **2015**, *6*, 22-29.

4. Holmes, A. H.; Moore, L. S. P.; Sundsfjord, A.; Steinbakk, M.; Regmi, S.; Karkey, A.; Guerin, P. J.; Piddock, L. J. V. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* **2016**, *387*, 176-187.

5. Savage, V. J.; Chopra, I.; O'Neill, A. J. Staphylococcus aureus Biofilms Promote Horizontal Transfer of Antibiotic Resistance. *Antimicrob. Agents Chemother.* **2013**, *57*, 1968-1970.

6. Centers for Disease Control and Prevention. National strategy for combating antibiotic-resistant bacteria. 2014. http://www.cdc.gov/drugresistance/pdf/carb_national_strategy.pdf. Accessed 20 September 2016. *Centers for Disease Control and Prevention* **2014**, 1-37.

7. Anderson, A. C. The process of structure-based drug design. *Chem. Biol.* **2003**, *10*, 787-797.

8. Dixon, S. L.; Smondyrev, A. M.; Rao, S. N. PHASE: A Novel Approach to Pharmacophore Modeling and 3D Database Searching. *Chem. Biol. Drug Des.* **2006**, *67*, 370-372.

9. Zoete, V.; Grosdidier, A.; Michielin, O. Docking, virtual high throughput screening and in silico fragment-based drug design. *J. Cell. Mol. Med.* **2009**, *13*, 238-248.

10. Gillet, V.; Johnson, A. P.; Mata, P.; Sike, S.; Williams, P. SPROUT: A program for structure generation. *J. Comput. Aided Mol. Des.* **1993**, *7*, 127-153.

11. Carlson, H. A.; Masukawa, K. M.; Rubins, K.; Bushman, F. D.; Jorgensen, W. L.; Lins, R. D.; Briggs, J. M.; McCammon, J. A. Developing a Dynamic Pharmacophore Model for HIV-1 Integrase. *J. Med. Chem.* **2000**, *43*, 2100-2114.

12. Sperandio, O.; Miteva, M. A.; Delfaud, F.; Villoutreix, B. O. Receptor-based computational screening of compound databases: the main docking-scoring engines. *Curr. Protein Pept. Sci.* **2006**, *7*, 369-393.

13. Leach, A. R.; Shoichet, B. K.; Peishoff, C. E. Prediction of protein−ligand interactions. docking and scoring: successes and gaps. *J. Med. Chem.* **2006**, *49*, 5851-5855.

14. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639-1662.

15. The Scripps Research Institute. Autodock [online]. Available from URL: http://autodock.scripps.edu/ [Accessed 2010 Mar 24].

16. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J Comput Chem* **2009**, *30*, 2785-2791.

17. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235-242.

18. The Research Collaboratory for Structural Bioinformatics. Protein Data Bank [online]. Available from URL: http://www.rcsb.org/pdb [Accessed 2010 Mar 24].

19. Irwin, J. J.; Shoichet, B. K. ZINC - A free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* **2005**, *45*, 177-182.

20. The Bioinformatics and Chemical Informatics Research Center (BCIRC). ZINC database [online]. Available from URL: http://zinc.docking.org/ [Accessed 2010 Mar 24].

21. Villoutreix, B. O.; Renault, N.; Lagorce, D.; Sperandio, O.; Montes, M.; Miteva, M. A. Free resources to assist structure-based virtual ligand screening experiments. *Curr. Protein Pept. Sci.* **2007**, *8*, 381-411.

22. Cavasotto, C. N.; Phatak, S. S. Homology modeling in drug discovery: current trends and applications. *Drug Discov. Today* **2009**, *14*, 676-683.

23. Hillisch, A.; Pineda, L. F.; Hilgenfeld, R. Utility of homology models in the drug discovery process. *Drug Discovery Today* **2004**, *9*, 659-669.

24. Consortium, T. U. UniProt: a hub for protein information. *Nucl. Acids Res.* **2015**, *43*, D212.

25. Arnold, K.; Bordoli, L.; Kopp, J.; Schwede, T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* **2006**, *22*, 195-201.

26. Swiss Institute of Bioinformatics (SIB). Available from URL: http://swissmodel.expasy.org/ [Accessed 2010 Mar 24].

27. Sali, A.; Blundell, T. L. Comparative Protein Modelling by Satisfaction of Spatial Restraints. *J. Mol. Biol.* **1993**, *234*, 779-815.

28. MODELLER [online]. Available from URL: http://salilab.org/modeller/ [Accessed 2016 Oct 14].

29. Schrodinger LLC. PRIME, version 1.5. **2005**.

30. Acharya, C.; Coop, A.; Polli, J. E.; MacKerell, A. D. Recent advances in ligand-based drug design: relevance and utility of the conformationally sampled pharmacophore approach. *Curr. Comput. Aided Drug Des.* **2011**, *7*, 10-22.

31. Koes, D. R.; Camacho, C. J. Pharmer: Efficient and Exact Pharmacophore Search. *J. Chem. Inf. Model.* **2011**, *51*, 1307-1314.

32. Selick, H. E.; Beresford, A. P.; Tarbit, M. H. The emerging importance of predictive ADME simulation in drug discovery. *Drug Discov. Today* **2002**, *7*, 109-116.

33. Schrodinger LLC. QIKPROP, version 3.1 and user manual. **2008**.

34. Valerio, L. G. J. In silico toxicology for the pharmaceutical sciences. *Toxicol. Appl. Pharmacol.* **2009**, *241*, 356-370.

35. Lagorce, D.; Sperandio, O.; Baell, J. B.; Miteva, M. A.; Villoutreix, B. O. FAF-Drugs3: a web server for compound property calculation and chemical library design. *Nucl. Acids Res.* **2015**, *43*, W207.

36. FAF-Drugs3 [online]. Available from URL: http://fafdrugs3.mti.univ-paris-diderot.fr. [Accessed 2016 Oct 14].

37. Lee, N.; Yuen, K.; Kumana, C. R. Clinical role of β-lactam/β-lactamase inhibitor combinations. *Drugs* **2012**, *63*, 1511-1524.

38. Sun, F.; Qu, F.; Ling, Y.; Mao, P.; Xia, P.; Chen, H.; Zhou, D. Biofilm-associated infections: antibiotic resistance and novel

therapeutic strategies. *Future Microbiol* **2013**, *8*, 877-886.

39. Donlan, R. M.; Costerton, J. W. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* **2002**, *15*, 167-193.

40. Lewis, K. Multidrug Tolerance of Biofilms and Persister Cells. In *Bacterial Biofilms;* Romeo, T., Ed.; Springer Berlin Heidelberg: 2008; pp 107-131.

41. Miller, M. B.; Bassler, B. L. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* **2001**, *55*, 165-199.

42. Marquez, B. Bacterial efflux systems and efflux pumps inhibitors. *Biochimie* **2005**, *87*, 1137-1147.

43. Suci, P. A.; Mittelman, M. W.; Yu, F. P.; Geesey, G. G. Investigation of ciprofloxacin penetration into *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother.* **1994**, *38*, 2125-2133.

44. Souli, M.; Giamarellou, H. Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. *Antimicrob. Agents Chemother.* **1998**, *42*, 939-941.

45. Gordon, C. A.; Hodges, N. A.; Marriott, C. Antibiotic interaction and diffusion through alginate and exopolysaccharide of cystic fibrosis-derived Pseudomonas aeruginosa. *J. Antimicrob. Chemother.* **1988**, *22*, 667-674.

46. Stewart, P. S. Theoretical aspects of antibiotic diffusion into microbial biofilms. *Antimicrob. Agents Chemother.* **1996**, *40*, 2517-2522.

47. Hatch, R. A.; Schiller, N. L. Alginate lyase promotes diffusion of aminoglycosides through the extracellular polysaccharide of mucoid *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **1998**, *42*, 974-977.

48. McIntosh, M.; Stone, B. A.; Stanisich, V. A. Curdlan and other bacterial (1→3)-β-D-glucans. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 163-173.

49. Walling, E.; Gindreau, E.; Lonvaud-Funel, A. A putative glucan synthase gene dps detected in exopolysaccharide-producing *Pediococcus damnosus* and *Oenococcus oeni* strains isolated from wine and cider. *Int. J. Food Microbiol.* **2005**, *98*, 53-62.

50. Qu, Y.; Daley, A. J.; Istivan, T. S.; Rouch, D. A.; Deighton, M. A. Densely adherent growth mode, rather than extracellular polymer substance matrix build-up ability, contributes to high resistance of *Staphylococcus epidermidis* biofilms to antibiotics. *J. Antimicrob. Chemother.* **2010**, *65*, 1405-1411.

51. Lewis, K. Programmed death in bacteria. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 503-514.

52. Spoering, A. L.; Lewis, K. Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J. Bacteriol.* **2001**, *183*, 6746-6751.

53. Van Melderen, L. Toxin–antitoxin systems: why so many, what for? *Curr. Opin. Microbiol.* **2010**, *13*, 781-785.

54. Fasani, R. A.; Savageau, M. A. Molecular mechanisms of multiple toxin–antitoxin systems are coordinated to govern the persister phenotype. *PNAS* **2013**, *110*, E2537.

55. Wen, Y.; Behiels, E.; Devreese, B. Toxin–antitoxin systems: their role in persistence, biofilm formation, and pathogenicity. *Pathog. Dis.* **2014**, *70*, 240-249.

56. Ramage, H. R.; Connolly, L. E.; Cox, J. S. Comprehensive functional analysis of *Mycobacterium tuberculosis* toxin-antitoxin systems: implications for pathogenesis, stress responses, and evolution. *PLoS Genet.* **2009**, *5*, e1000767.

57. Moyed, H. S.; Bertrand, K. P. hipA, a newly recognized gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis. *J. Bacteriol.* **1983**, *155*, 768-775.

58. Correia, F. F.; D'Onofrio, A.; Rejtar, T.; Li, L.; Karger, B. L.; Makarova, K.; Koonin, E.

V.; Lewis, K. Kinase activity of overexpressed HipA is required for growth arrest and multidrug tolerance in *Escherichia coli*. *J. Bacteriol.* **2006**, *188*, 8360-8367.

59. Kaspy, I.; Rotem, E.; Weiss, N.; Ronin, I.; Balaban, N. Q.; Glaser, G. HipA-mediated antibiotic persistence via phosphorylation of the glutamyl-tRNA-synthetase. *Nat. Commun.* **2013**, *4*, 3001.

60. Germain, E.; Castro-Roa, D.; Zenkin, N.; Gerdes, K. Molecular mechanism of bacterial persistence by HipA. *Mol. Cell* **2013**, *52*, 248-254.

61. Li, T.; Yin, N.; Liu, H.; Pei, J.; Lai, L. Novel inhibitors of toxin HipA reduce multidrug tolerant persisters. *ACS Med. Chem. Lett.* **2016**, *7*, 449-453.

62. Schumacher, M. A.; Piro, K. M.; Xu, W.; Hansen, S.; Lewis, K.; Brennan, R. G. Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. *Science* **2009**, *323*, 396-401.

63. Schumacher, M. A.; Balani, P.; Min, J.; Chinnam, N. B.; Hansen, S.; Vulić, M.; Lewis, K.; Brennan, R. G. HipBA-promoter structures reveal the basis of heritable multidrug tolerance. *Nature* **2015**, *524*, 59-64.

64. Wen, Y.; Behiels, E.; Felix, J.; Elegheert, J.; Vergauwen, B.; Devreese, B.; Savvides, S. N. The bacterial antitoxin HipB establishes a ternary complex with operator DNA and phosphorylated toxin HipA to regulate bacterial persistence. *Nucleic Acids Res.* **2014**, *42*, 10134-10147.

65. Goeders, N.; Chai, R.; Chen, B.; Day, A.; Salmond, G. P. C. Structure, evolution, and functions of bacterial type III toxin-antitoxin systems. *Toxins (Basel)* **2016**, *8*, .

66. Fineran, P. C.; Blower, T. R.; Foulds, I. J.; Humphreys, D. P.; Lilley, K. S.; Salmond, G. P. C. The phage abortive infection system, ToxIN, functions as a protein-RNA toxin-antitoxin pair. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 894-899.

67. Short, F. L.; Pei, X. Y.; Blower, T. R.; Ong, S.; Fineran, P. C.; Luisi, B. F.; Salmond, G. P. C. Selectivity and self-assembly in the control of a bacterial toxin by an antitoxic noncoding RNA pseudoknot. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 241.

68. Maisonneuve, E.; Castro-Camargo, M.; Gerdes, K. (p)ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity. *Cell* **2013**, *154*, 1140-1150.

69. Botos, I.; Melnikov, E. E.; Cherry, S.; Tropea, J. E.; Khalatova, A. G.; Rasulova, F.; Dauter, Z.; Maurizi, M. R.; Rotanova, T. V.; Wlodawer, A.; Gustchina, A. The catalytic domain of *Escherichia coli* Lon protease has a unique fold and a Ser-Lys dyad in the active site. *J. Biol. Chem.* **2004**, *279*, 8140-8148.

70. Su, S.; Lin, C.; Tai, H.; Chang, M.; Ho, M.; Babu, C. S.; Liao, J.; Wu, S.; Chang, Y.; Lim, C.; Chang, C. Structural basis for the magnesium-dependent activation and hexamerization of the Lon AAA+ protease. *Structure* **2016**, *24*, 676-686.

71. Batten, L. E.; Parnell, A. E.; Wells, N. J.; Murch, A. L.; Oyston, P. C. F.; Roach, P. L. Biochemical and structural characterization of polyphosphate kinase 2 from the intracellular pathogen Francisella tularensis. *Biosci. Rep.* **2015**, *36*, e00294.

72. Mutschler, H.; Meinhart, A. ε/ζ systems: their role in resistance, virulence, and their potential for antibiotic development. *J. Mol. Med.* **2011**, *89*, 1183-1194.

73. Wanner, B. L. Phosphorus assimilation and its control of gene expression in *Escherichia coli*. In *The Molecular Basis of Bacterial Metabolism;* Hauska, P. D. G., Thauer, Professor Dr Rudolf K, Eds.; Springer Berlin Heidelberg: 1990; 152-163.

74. Li, Y.; Zhang, Y. PhoU Is a persistence switch involved in persister formation and tolerance to multiple antibiotics and stresses in *Escherichia coli*. *Antimicrob. Agents Chemother.* **2007**, *51*, 2092-2099.

75. Lee, S. J.; Park, Y. S.; Kim, S.; Lee, B.; Suh, S. W. Crystal structure of PhoU from *Pseudomonas aeruginosa*, a negative regulator of the Pho regulon. *J. Struct. Biol.* **2014**, *188*, 22-29.

76. Dwyer, D. J.; Belenky, P. A.; Yang, J. H.; MacDonald, I. C.; Martell, J. D.; Takahashi, N.; Chan, C. T. Y.; Lobritz, M. A.; Braff, D.; Schwarz, E. G.; Ye, J. D.; Pati, M.; Vercruysse, M.; Ralifo, P. S.; Allison, K. R.; Khalil, A. S.; Ting, A. Y.; Walker, G. C.; Collins, J. J. Antibiotics induce redox-related physiological alterations as part of their lethality. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 2100.

77. Courcelle, J.; Hanawalt, P. C. RecA-dependent recovery of arrested DNA replication forks. *Annu. Rev. Genet.* **2003**, *37*, 611-646.

78. Kohanski, M. A.; Dwyer, D. J.; Hayete, B.; Lawrence, C. A.; Collins, J. J. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **2007**, *130*, 797-810.

79. Peterson, E. J. R.; Janzen, W. P.; Kireev, D.; Singleton, S. F. High-throughput screening for RecA inhibitors using a transcreener adenosine 5'-O-diphosphate assay. *Assay Drug Dev. Technol.* **2012**, *10*, 260-268.

80. Bernier, S. P.; Lebeaux, D.; DeFrancesco, A. S.; Valomon, A.; Soubigou, G.; Coppée, J.; Ghigo, J.; Beloin, C. Starvation, together with the SOS response, mediates high biofilm-specific tolerance to the fluoroquinolone ofloxacin. *PLoS Genet.* **2013**, *9*, 1-14.

81. Van Acker, H.; Sass, A.; Bazzini, S.; De Roy, K.; Udine, C.; Messiaen, T.; Riccardi, G.; Boon, N.; Nelis, H. J.; Mahenthiralingam, E.; Coenye, T. Biofilm-grown *Burkholderia cepacia* complex cells survive antibiotic treatment by avoiding production of reactive oxygen species. *PLoS ONE* **2013**, *8*, e58943.

82. Shinohara, T.; Ikawa, S.; Iwasaki, W.; Hiraki, T.; Hikima, T.; Mikawa, T.; Arai, N.; Kamiya, N.; Shibata, T. Loop L1 governs the DNA-binding specificity and order for RecA-catalyzed reactions in homologous recombination and DNA repair. *Nucleic Acids Res.* **2015**, *43*, 973-986.

83. Wigle, T. J.; Singleton, S. F. Directed molecular screening for RecA ATPase inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3249-3253.

84. Wiseman, B.; Carpena, X.; Feliz, M.; Donald, L. J.; Pons, M.; Fita, I.; Loewen, P. C. Isonicotinic acid hydrazide conversion to isonicotinyl-NAD by catalase-peroxidases. *J. Biol. Chem.* **2010**, *285*, 26662-26673.

85. Jha, V.; Chelikani, P.; Carpena, X.; Fita, I.; Loewen, P. C. Influence of main channel structure on H(2)O(2) access to the heme cavity of catalase KatE of *Escherichia coli*. *Arch. Biochem. Biophys.* **2012**, *526*, 54-59.

86. Loewen, P. C.; Carpena, X.; Rovira, C.; Ivancich, A.; Perez-Luque, R.; Haas, R.; Odenbreit, S.; Nicholls, P.; Fita, I. Structure of *Helicobacter pylori* catalase, with and without formic acid bound, at 1.6 A resolution. *Biochemistry* **2004**, *43*, 3089-3103.

87. Whittaker, M. M.; Lerch, T. F.; Kirillova, O.; Chapman, M. S.; Whittaker, J. W. Subunit dissociation and metal binding by *Escherichia coli* apo-manganese superoxide dismutase. *Arch. Biochem. Biophys.* **2011**, *505*, 213-225.

88. Krauss, I. R.; Merlino, A.; Pica, A.; Rullo, R.; Bertoni, A.; Capasso, A.; Amato, M.; Riccitiello, F.; Vendittis, E. D.; Sica, F. Fine tuning of metal-specific activity in the Mn-like group of cambialistic superoxide dismutases. *RSC Adv.* **2015**, *5*, 87876-87887.

89. Zhang, Y.; Heym, B.; Allen, B.; Young, D.; Cole, S. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* **1992**, *358*, 591-593.

90. Credito, K.; Lin, G.; Koeth, L.; Sturgess, M. A.; Appelbaum, P. C. Activity of levofloxacin alone and in combination with a DnaK inhibitor against Gram-negative rods, including levofloxacin-resistant

strains. *Antimicrob. Agents Chemother*. **2009**, *53*, 814-817.

91. Leu, J. I.; Zhang, P.; Murphy, M. E.; Marmorstein, R.; George, D. L. Structural basis for the inhibition of HSP70 and DnaK chaperones by small-molecule targeting of a C-terminal allosteric pocket. *ACS Chem. Biol.* **2014**, *9*, 2508-2516.

92. Britton, K. L.; Abeysinghe, I. S.; Baker, P. J.; Barynin, V.; Diehl, P.; Langridge, S. J.; McFadden, B. A.; Sedelnikova, S. E.; Stillman, T. J.; Weeradechapon, K.; Rice, D. W. The structure and domain organization of *Escherichia coli* isocitrate lyase. *Acta Crystallogr. D Biol. Crystallogr.* **2001**, *57*, 1209-1218.

93. Ahmer, B. M. M. Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica*. *Mol. Microbiol.* **2004**, *52*, 933-945.

94. Kalia, V. C. Quorum sensing inhibitors: an overview. *Biotechnol. Adv.* **2013**, *31*, 224-245.

95. O'Loughlin, C. T.; Miller, L. C.; Siryaporn, A.; Drescher, K.; Semmelhack, M. F.; Bassler, B. L. A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. *PNAS* **2013**, *110*, 17981-17986.

96. Norizan, S. N. M.; Yin, W.; Chan, K. Caffeine as a potential quorum sensing inhibitor. *Sensors* **2013**, *13*, 5117-5129.

97. Castang, S.; Chantegrel, B.; Deshayes, C.; Dolmazon, R.; Gouet, P.; Haser, R.; Reverchon, S.; Nasser, W.; Hugouvieux-Cotte-Pattat, N.; Doutheau, A. N-sulfonyl homoserine lactones as antagonists of bacterial quorum sensing. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5145-5149.

98. Frezza, M.; Castang, S.; Estephane, J.; Soulère, L.; Deshayes, C.; Chantegrel, B.; Nasser, W.; Queneau, Y.; Reverchon, S.; Doutheau, A. Synthesis and biological evaluation of homoserine lactone derived ureas as antagonists of bacterial quorum sensing. *Bioorg. Med. Chem.* **2006**, *14*, 4781-4791.

99. Morohoshi, T.; Shiono, T.; Takidouchi, K.; Kato, M.; Kato, N.; Kato, J.; Ikeda, T. Inhibition of quorum sensing in Serratia marcescens AS-1 by synthetic analogs of N-acylhomoserine lactone. *Appl. Environ. Microbiol.* **2007**, *73*, 6339-6344.

100. Lewis, H. A.; Furlong, E. B.; Laubert, B.; Eroshkina, G. A.; Batiyenko, Y.; Adams, J. M.; Bergseid, M. G.; Marsh, C. D.; Peat, T. S.; Sanderson, W. E.; Sauder, J. M.; Buchanan, S. G. A structural genomics approach to the study of quorum sensing: crystal structures of three LuxS orthologs. *Structure* **2001**, *9*, 527-537.

101. Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B. L.; Hughson, F. M. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* **2002**, *415*, 545-549.

102. Miller, S. T.; Xavier, K. B.; Campagna, S. R.; Taga, M. E.; Semmelhack, M. F.; Bassler, B. L.; Hughson, F. M. *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Mol. Cell* **2004**, *15*, 677-687.

103. Pereira, C. S.; McAuley, J. R.; Taga, M. E.; Xavier, K. B.; Miller, S. T. *Sinorhizobium meliloti*, a bacterium lacking the autoinducer-2 synthase, responds to AI-2 supplied by other bacteria. *Mol. Microbiol.* **2008**, *70*, 1223-1235.

104. Koch, B.; Liljefors, T.; Persson, T.; Nielsen, J.; Kjelleberg, S.; Givskov, M. The LuxR receptor: the sites of interaction with quorum-sensing signals and inhibitors. *Microbiology* **2005**, *151*, 3589-3602.

105. Kvist, M.; Hancock, V.; Klemm, P. Inactivation of efflux pumps abolishes bacterial biofilm formation. *Appl. Environ. Microbiol.* **2008**, *74*, 7376-7382.

106. Baugh, S.; Ekanayaka, A. S.; Piddock, L. J. V.; Webber, M. A. Loss of or inhibition of all multidrug resistance efflux pumps of *Salmonella enterica* serovar Typhimurium results in impaired ability to form a biofilm.

J. Antimicrob. Chemother. **2012**, *67*, 2409-2417.

107. Baugh, S.; Phillips, C. R.; Ekanayaka, A. S.; Piddock, L. J. V.; Webber, M. A. Inhibition of multidrug efflux as a strategy to prevent biofilm formation. *J. Antimicrob. Chemother.* **2014**, *69*, 673-681.

108. Imuta, N.; Nishi, J.; Tokuda, K.; Fujiyama, R.; Manago, K.; Iwashita, M.; Sarantuya, J.; Kawano, Y. The *Escherichia coli* efflux pump TolC promotes aggregation of enteroaggregative *E. coli* 042. *Infect. Immun.* **2008**, *76*, 1247-1256.

109. Putman, M.; Veen, H. W. v.; Konings, W. N. Molecular properties of bacterial multidrug transporters. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 672-693.

110. Handzlik, J.; Matys, A.; Kieć-Kononowicz, K. Recent advances in multi-drug resistance (MDR) efflux pump inhibitors of Gram-positive bacteria *S. aureus*. *Antibiotics* **2013**, *2*, 28-45.

111. Thaker, M.; Spanogiannopoulos, P.; Wright, G. D. The tetracycline resistome. *Cell. Mol. Life Sci.* **2009**, *67*, 419-431.

112. Sun, J.; Deng, Z.; Yan, A. Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. *Biochem. Biophys. Res. Commun.* **2014**, *453*, 254-267.

113. Van Bambeke, F.; Pagès, J.; Lee, V. J. Inhibitors of bacterial efflux pumps as adjuvants in antibiotic treatments and diagnostic tools for detection of resistance by efflux. *Recent Pat Antiinfect Drug Discov* **2006**, *1*, 157-175.

114. Eicher, T.; Seeger, M. A.; Anselmi, C.; Zhou, W.; Brandstätter, L.; Verrey, F.; Diederichs, K.; Faraldo-Gómez, J. D.; Pos, K. M. Coupling of remote alternating-access transport mechanisms for protons and substrates in the multidrug efflux pump AcrB. *Elife* **2014**, *3*, .

115. Sjuts, H.; Vargiu, A. V.; Kwasny, S. M.; Nguyen, S. T.; Kim, H.; Ding, X.; Ornik, A. R.; Ruggerone, P.; Bowlin, T. L.; Nikaido, H.; Pos, K. M.; Opperman, T. J. Molecular basis for inhibition of AcrB multidrug efflux pump by novel and powerful pyranopyridine derivatives. *PNAS* **2016**, *113*, 3509-3514.

116. Ababou, A.; Koronakis, V. Structures of gate loop variants of the AcrB drug efflux pump bound by erythromycin substrate. *PLoS One* **2016**, *11*, .

117. Lei, H.; Chou, T.; Su, C.; Bolla, J. R.; Kumar, N.; Radhakrishnan, A.; Long, F.; Delmar, J. A.; Do, S. V.; Rajashankar, K. R.; Shafer, W. M.; Yu, E. W. Crystal structure of the open state of the *Neisseria gonorrhoeae* MtrE outer membrane channel. *PLoS One* **2014**, *9*, .

118. Hinchliffe, P.; Greene, N. P.; Paterson, N. G.; Crow, A.; Hughes, C.; Koronakis, V. Structure of the periplasmic adaptor protein from a major facilitator superfamily (MFS) multidrug efflux pump. *FEBS Lett.* **2014**, *588*, 3147-3153.

119. Bugg, T. D.; Wright, G. D.; Dutka-Malen, S.; Arthur, M.; Courvalin, P.; Walsh, C. T. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry* **1991**, *30*, 10408-10415.

120. Handwerger, S.; Pucci, M. J.; Volk, K. J.; Liu, J.; Lee, M. S. The cytoplasmic peptidoglycan precursor of vancomycin-resistant *Enterococcus faecalis* terminates in lactate. *J. Bacteriol.* **1992**, *174*, 5982-5984.

121. Bugg, T. D.; Dutka-Malen, S.; Arthur, M.; Courvalin, P.; Walsh, C. T. Identification of vancomycin resistance protein VanA as a D-alanine:D-alanine ligase of altered substrate specificity. *Biochemistry* **1991**, *30*, 2017-2021.

122. Arthur, M.; Molinas, C.; Dutka-Malen, S.; Courvalin, P. Structural relationship between the vancomycin resistance protein VanH and 2-hydroxycarboxylic acid dehydrogenases. *Gene* **1991**, *103*, 133-134.

123. Reynolds, P. E.; Depardieu, F.; Dutka-Malen, S.; Arthur, M.; Courvalin, P. Glycopeptide resistance mediated by enterococcal transposon Tn1546 requires production of VanX for hydrolysis of D-alanyl-D-alanine. *Mol. Microbiol.* **1994**, *13*, 1065-1070.

124. Arthur, M.; Depardieu, F.; Gerbaud, G.; Galimand, M.; Leclercq, R.; Courvalin, P. The VanS sensor negatively controls VanR-mediated transcriptional activation of glycopeptide resistance genes of Tn1546 and related elements in the absence of induction. *J. Bacteriol.* **1997**, *179*, 97-106.

125. Evers, S.; Sahm, D. F.; Courvalin, P. The vanB gene of vancomycin-resistant *Enterococcus faecalis* V583 is structurally related to genes encoding D-Ala:D-Ala ligases and glycopeptide-resistance proteins VanA and VanC. *Gene* **1993**, *124*, 143-144.

126. Fines, M.; Perichon, B.; Reynolds, P.; Sahm, D. F.; Courvalin, P. VanE, a new type of acquired glycopeptide resistance in *Enterococcus faecalis* BM4405. *Antimicrob. Agents Chemother.* **1999**, *43*, 2161-2164.

127. Evers, S.; Reynolds, P. E.; Courvalin, P. Sequence of the vanB and ddl genes encoding d-alanine:d-lactate and d-alanine:d-alanine ligases in vancomycin-resistant *Enterococcus faecalis* V583. *Gene* **1994**, *140*, 97-102.

128. Meziane-Cherif, D.; Saul, F. A.; Moubareck, C.; Weber, P.; Haouz, A.; Courvalin, P.; Périchon, B. Molecular basis of vancomycin dependence in VanA-type *Staphylococcus aureus* VRSA-9. *J. Bacteriol.* **2010**, *192*, 5465-5471.

129. Roper, D. I.; Huyton, T.; Vagin, A.; Dodson, G. The molecular basis of vancomycin resistance in clinically relevant enterococci: crystal structure of d-alanyl-d-lactate ligase (VanA). *PNAS* **2000**, *97*, 8921-8925.

130. Kuzin, A. P.; Sun, T.; Jorczak-Baillass, J.; Healy, V. L.; Walsh, C. T.; Knox, J. R. Enzymes of vancomycin resistance: the structure of D-alanine-D-lactate ligase of naturally resistant *Leuconostoc mesenteroides*. *Structure* **2000**, *8*, 463-470.

131. Fan, C.; Moews, P. C.; Walsh, C. T.; Knox, J. R. Vancomycin resistance: structure of D-alanine:D-alanine ligase at 2.3 A resolution. *Science* **1994**, *266*, 439-443.

132. Meziane-Cherif, D.; Saul, F. A.; Haouz, A.; Courvalin, P. Structural and functional characterization of VanG D-Ala:D-Ser ligase associated with vancomycin resistance in *Enterococcus faecalis*. *J. Biol. Chem.* **2012**, *287*, 37583-37592.

133. Bussiere, D. E.; Pratt, S. D.; Katz, L.; Severin, J. M.; Holzman, T.; Park, C. H. The structure of VanX reveals a novel amino-dipeptidase involved in mediating transposon-based vancomycin resistance. *Mol. Cell* **1998**, *2*, 75-84.

134. Meziane-Cherif, D.; Stogios, P. J.; Evdokimova, E.; Savchenko, A.; Courvalin, P. Structural basis for the evolution of vancomycin resistance D,D-peptidases. *PNAS* **2014**, *111*, 5872-5877.

135. Ellsworth, B. A.; Tom, N. J.; Bartlett, P. A. Synthesis and evaluation of inhibitors of bacterial D-alanine: D-alanine ligases. *Chem. Biol.* **1996**, *3*, 37-44.

136. Yang, K.; Brandt, J. J.; Chatwood, L. L.; Crowder, M. W. Phosphonamidate and phosphothioate dipeptides as potential inhibitors of VanX. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1085-1087.

137. Jia, C.; Yang, K.; Liu, C.; Feng, L.; Xiao, J.; Zhou, L.; Zhang, Y. Synthesis, characterization and activity of new phosphonate dipeptides as potential inhibitors of VanX. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 482-484.

138. Sauvage, E.; Kerff, F.; Terrak, M.; Ayala, J. A.; Charlier, P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol. Rev.* **2008**, *32*, 234-258.

139. Fuda, C.; Suvorov, M.; Vakulenko, S. B.; Mobashery, S. The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant

Staphylococcus aureus. *J. Biol. Chem.* **2004**, *279*, 40802-40806.

140. Lim, D.; Strynadka, N. C. J. Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat. Struct. Biol.* **2002**, *9*, 870-876.

141. Powell, A. J.; Tomberg, J.; Deacon, A. M.; Nicholas, R. A.; Davies, C. Crystal structures of penicillin-binding protein 2 from penicillin-susceptible and -resistant strains of *Neisseria gonorrhoeae* reveal an unexpectedly subtle mechanism for antibiotic resistance. *J. Biol. Chem.* **2009**, *284*, 1202-1212.

142. Sainsbury, S.; Bird, L.; Rao, V.; Shepherd, S. M.; Stuart, D. I.; Hunter, W. N.; Owens, R. J.; Ren, J. Crystal structures of penicillin-binding protein 3 from *Pseudomonas aeruginosa*: comparison of native and antibiotic-bound forms. *J. Mol. Biol.* **2011**, *405*, 173-184.

143. Contreras-Martel, C.; Job, V.; Di Guilmi, A. M.; Vernet, T.; Dideberg, O.; Dessen, A. Crystal structure of penicillin-binding protein 1a (PBP1a) reveals a mutational hotspot implicated in β-lactam resistance in *Streptococcus pneumoniae*. *J. Mol. Biol.* **2006**, *355*, 684-696.

144. Contreras-Martel, C.; Dahout-Gonzalez, C.; Martins, A. D. S.; Kotnik, M.; Dessen, A. PBP active site flexibility as the key mechanism for β-lactam resistance in pneumococci. *J. Mol. Biol.* **2009**, *387*, 899-909.

145. Pernot, L.; Chesnel, L.; Gouellec, A. L.; Croizé, J.; Vernet, T.; Dideberg, O.; Dessen, A. A PBP2x from a clinical isolate of *Streptococcus pneumoniae* exhibits an alternative mechanism for reduction of susceptibility to β-lactam antibiotics. *J. Biol. Chem.* **2004**, *279*, 16463-16470.

146. Hooper, D. C. Mechanisms of action of antimicrobials: focus on fluoroquinolones. *Clin. Infect. Dis.* **2001**, *32 Suppl 1*, S15.

147. Willmott, C. J.; Maxwell, A. A single point mutation in the DNA gyrase A protein greatly reduces binding of fluoroquinolones to the gyrase-DNA complex. *Antimicrob. Agents Chemother.* **1993**, *37*, 126-127.

148. Blondeau, J. M. Fluoroquinolones: mechanism of action, classification, and development of resistance. *Surv. Ophthalmol.* **2004**, *49 Suppl 2*, S78.

149. Hearnshaw, S. J.; Edwards, M. J.; Stevenson, C. E.; Lawson, D. M.; Maxwell, A. A new crystal structure of the bifunctional antibiotic simocyclinone D8 bound to DNA gyrase gives fresh insight into the mechanism of inhibition. *J. Mol. Biol.* **2014**, *426*, 2023-2033.

150. Miles, T. J.; Hennessy, A. J.; Bax, B.; Brooks, G.; Brown, B. S.; Brown, P.; Cailleau, N.; Chen, D.; Dabbs, S.; Davies, D. T.; Esken, J. M.; Giordano, I.; Hoover, J. L.; Jones, G. E.; Kusalakumari Sukmar, S. K.; Markwell, R. E.; Minthorn, E. A.; Rittenhouse, S.; Gwynn, M. N.; Pearson, N. D. Novel tricyclics (e.g., GSK945237) as potent inhibitors of bacterial type IIA topoisomerases. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2464-2469.

151. Mariam, D. H.; Mengistu, Y.; Hoffner, S. E.; Andersson, D. I. Effect of rpoB mutations conferring rifampin resistance on fitness of *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **2004**, *48*, 1289-1294.

152. Molodtsov, V.; Nawarathne, I. N.; Scharf, N. T.; Kirchhoff, P. D.; Showalter, H. D. H.; Garcia, G. A.; Murakami, K. S. X-ray crystal structures of the *Escherichia coli* RNA polymerase in complex with benzoxazinorifamycins. *J. Med. Chem.* **2013**, *56*, 4758-4763.

153. Lambert, P. A. Bacterial resistance to antibiotics: modified target sites. *Adv. Drug Deliv. Rev.* **2005**, *57*, 1471-1485.

154. Hansen, J. L.; Ippolito, J. A.; Ban, N.; Nissen, P.; Moore, P. B.; Steitz, T. A. The structures of four macrolide antibiotics bound to the large ribosomal subunit. *Mol. Cell* **2002**, *10*, 117-128.

155. Hansen, J. L.; Moore, P. B.; Steitz, T. A. Structures of five antibiotics bound at the

peptidyl transferase center of the large ribosomal subunit. *J. Mol. Biol.* **2003**, *330*, 1061-1075.

156. Tu, D.; Blaha, G.; Moore, P. B.; Steitz, T. A. Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. *Cell* **2005**, *121*, 257-270.

157. Borovinskaya, M. A.; Pai, R. D.; Zhang, W.; Schuwirth, B. S.; Holton, J. M.; Hirokawa, G.; Kaji, H.; Kaji, A.; Cate, J. H. D. Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nat. Struct. Mol. Biol.* **2007**, *14*, 727-732.

158. Basso, L. A.; Zheng, R.; Musser, J. M.; Jacobs, W. R.; Blanchard, J. S. Mechanisms of isoniazid resistance in *Mycobacterium tuberculosis*: enzymatic characterization of enoyl reductase mutants identified in isoniazid-resistant clinical isolates. *J. Infect. Dis.* **1998**, *178*, 769-775.

159. Mestdagh, M.; Fonteyne, P. A.; Realini, L.; Rossau, R.; Jannes, G.; Mijs, W.; De Smet, K. A.; Portaels, F.; Van den Eeckhout, E. Relationship between pyrazinamide resistance, loss of pyrazinamidase activity, and mutations in the pncA locus in multidrug-resistant clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **1999**, *43*, 2317-2319.

160. Bertrand, T.; Eady, N. A. J.; Jones, J. N.; Jesmin, n.; Nagy, J. M.; Jamart-Grégoire, B.; Raven, E. L.; Brown, K. A. Crystal structure of *Mycobacterium tuberculosis* catalase-peroxidase. *J. Biol. Chem.* **2004**, *279*, 38991-38999.

161. Zhao, X.; Hersleth, H.; Zhu, J.; Andersson, K. K.; Magliozzo, R. S. Access channel residues Ser315 and Asp137 in *Mycobacterium tuberculosis* catalase-peroxidase (KatG) control peroxidatic activation of the pro-drug isoniazid. *Chem. Commun. (Camb.)* **2013**, *49*, 11650-11652.

162. Petrella, S.; Gelus-Ziental, N.; Maudry, A.; Laurans, C.; Boudjelloul, R.; Sougakoff, W. Crystal structure of the pyrazinamidase of *Mycobacterium tuberculosis*: insights into natural and acquired resistance to pyrazinamide. *PLoS ONE* **2011**, *6*, e15785.

163. Connell, S. R.; Tracz, D. M.; Nierhaus, K. H.; Taylor, D. E. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob. Agents Chemother.* **2003**, *47*, 3675-3681.

164. Anokhina, M. M.; Barta, A.; Nierhaus, K. H.; Spiridonova, V. A.; Kopylov, A. M. Mapping of the second tetracycline binding site on the ribosomal small subunit of *E.coli*. *Nucleic Acids Res.* **2004**, *32*, 2594-2597.

165. Trieber, C. A.; Burkhardt, N.; Nierhaus, K. H.; Taylor, D. E. Ribosomal protection from tetracycline mediated by Tet(O): Tet(O) interaction with ribosomes is GTP-dependent. *Biol. Chem.* **1998**, *379*, 847-855.

166. Brodersen, D. E.; Clemons Jr., W. M.; Carter, A. P.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. The structural basis for the action of the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. *Cell* **2000**, *103*, 1143-1154.

167. Li, W.; Atkinson, G. C.; Thakor, N. S.; Allas, Ü; Lu, C.; Chan, K.; Tenson, T.; Schulten, K.; Wilson, K. S.; Hauryliuk, V.; Frank, J. Mechanism of tetracycline resistance by ribosomal protection protein Tet(O). *Nat. Commun.* **2013**, *4*, 1477-1485.

168. Wright, G. D. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Adv. Drug Deliv. Rev.* **2005**, *57*, 1451-1470.

169. Birnbaum, J.; Kahan, F. M.; Kropp, H.; MacDonald, J. S. Carbapenems, a new class of beta-lactam antibiotics. Discovery and development of imipenem/cilastatin. *Am. J. Med.* **1985**, *78*, 3-21.

170. Pozzi, C.; De Luca, F.; Benvenuti, M.; Poirel, L.; Nordmann, P.; Rossolini, G. M.; Mangani, S.; Docquier, J. Crystal structure of the *Pseudomonas aeruginosa* BEL-1 extended-spectrum β-lactamase and its

complex with moxalactam and imipenem. *Antimicrob. Agents Chemother.* **2016**, .

171. Schwarz, S.; Kehrenberg, C.; Doublet, B.; Cloeckaert, A. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews* **2004**, *28*, 519-542.

172. Ramirez, M. S.; Tolmasky, M. E. Aminoglycoside modifying enzymes. *Drug Resist. Updat.* **2010**, *13*, 151-171.

173. Biswas, T.; Houghton, J. L.; Garneau-Tsodikova, S.; Tsodikov, O. V. The structural basis for substrate versatility of chloramphenicol acetyltransferase CATI. *Protein Sci.* **2012**, *21*, 520-530.

174. Vetting, M. W.; Park, C. H.; Hegde, S. S.; Jacoby, G. A.; Hooper, D. C.; Blanchard, J. S. Mechanistic and structural analysis of aminoglycoside N-acetyltransferase AAC(6')-Ib and its bifunctional, fluoroquinolone-active AAC(6')-Ib-cr variant. *Biochemistry* **2008**, *47*, 9825-9835.

175. Andersson, D. I. Improving predictions of the risk of resistance development against new and old antibiotics. *Clin. Microbiol. Infect.* **2015**, *21*, 894-898.

