Molecular mechanisms of antibiotic resistance

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Abstract | Antibiotic-resistant bacteria that are difficult or impossible to treat are becoming increasingly common and are causing a global health crisis. Antibiotic resistance is encoded by several genes, many of which can transfer between bacteria. New resistance mechanisms are constantly being described, and new genes and vectors of transmission are identified on a regular basis. This article reviews recent advances in our understanding of the mechanisms by which bacteria are either intrinsically resistant or acquire resistance to antibiotics, including the prevention of access to drug targets, changes in the structure and protection of antibiotic targets and the direct modification or inactivation of antibiotics.

Enoyl-ACP reductase
An enzyme key in the production of fatty acids which is the target for triclosan.

Lipopeptide
A natural or semi-synthetic fatty acid-linked peptide chain that targets the cell membrane (for example, daptomycin).

Glycopeptide
A natural or semi-synthetic amino sugar-linked peptide chain that targets terminal D-Ala-D-Ala dipeptides (for example, vancomycin).

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Antibiotics underpin modern medicine; their use has reduced childhood mortality and increased life expectancy, and they are crucial for invasive surgery and treatments such as chemotherapy. However, the number of infections caused by multidrug-resistant bacteria is increasing globally, and the spectre of untreatable infections is becoming a reality. The most recent World Economic Forum Global Risks reports have listed antibiotic resistance as one of the greatest threats to human health[1–3]. It is estimated that in Europe 25,000 people die each year as a result of multidrug-resistant bacterial infections and that this costs the European Union economy €1.5 billion annually[1]. In the United States more than 2 million people are infected with antibiotic-resistant bacteria annually, with 23,000 deaths as a direct result[4,5]. In addition to increased resistance to existing agents, there is a lack of new antibiotics in development. The word antibiotic has become synonymous with 'antibacterial drug': therefore, in this article the term antibiotic has been used throughout.

Bacteria can be intrinsically resistant to certain antibiotics but can also acquire resistance to antibiotics via mutations in chromosomal genes and by horizontal gene transfer. The intrinsic resistance of a bacterial species to a particular antibiotic is the ability to resist the action of that antibiotic as a result of inherent structural or functional characteristics (FIG. 1). The simplest example of intrinsic resistance in an individual species results from the absence of a susceptible target of a specific antibiotic; for example, the biocide triclosan has broad efficacy against Gram-positive bacteria and many Gram-negative bacteria, but it is unable to inhibit growth of members of the Gram-negative genus *Pseudomonas*. Although this was initially thought to be due to active efflux[6], it has more recently been shown that it is instead due to the carriage of an insensitive allele of *fabI* that encodes an additional enoyl-ACP reductase enzyme — the target for triclosan in sensitive species[7]. A second example relates to the lipopeptide daptomycin (first approved for clinical use in 2003), which is active against Gram-positive bacteria but is not effective against Gram-negative bacteria. This is due to an intrinsic difference in the composition of the cytoplasmic membrane; Gram-negative bacteria have a lower proportion of anionic phospholipids in the cytoplasmic membrane than do Gram-positive bacteria, which reduces the efficiency of the Ca^{2+}-mediated insertion of daptomycin into the cytoplasmic membrane that is required for its antibacterial activity[8]. The intrinsic resistance of some Gram-negative bacteria to many compounds is due to an inability of these agents to cross the outer membrane: for example, the glycopeptide antibiotic vancomycin inhibits peptidoglycan crosslinking by binding to target D-Ala-D-Ala peptides but is only normally effective in Gram-positive bacteria as, in Gram-negative organisms, it cannot cross the outer membrane and access these peptides in the periplasm[9].

Recent studies have led to the identification of many genes that are responsible for intrinsic resistance to antibiotics of different classes, including β-lactams, fluoroquinolones and aminoglycosides. This was achieved using high-throughput screens of high-density genome mutant libraries that were created by targeted insertion or random transposon mutagenesis in bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas*

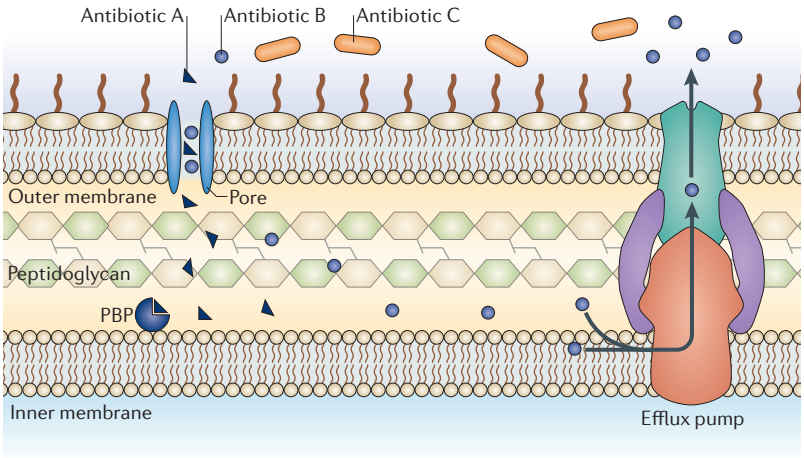

Figure 1 | **Intrinsic mechanisms of resistance.** The figure shows an overview of intrinsic resistance mechanisms. The example shown is of β-lactam antibiotics targeting a penicillin-binding protein (PBP). Antibiotic A can enter the cell via a membrane-spanning porin protein, reach its target and inhibit peptidoglycan synthesis. Antibiotic B can also enter the cell via a porin, but unlike Antibiotic A, it is efficiently removed by efflux. Antibiotic C cannot cross the outer membrane and so is unable to access the target PBP.

aeruginosa[10,11]. Screening these libraries for antibiotic susceptibility has identified possible novel drug combinations in which one agent can inhibit an intrinsic resistance mechanism and so increase the spectrum of activity of other antibiotics beyond normal target species. For example, analysis of the susceptibility phenotypes resulting from inactivation of all non-essential *E. coli* genes identified putative targets, including thioredoxin (TrxA), thioredoxin reductase (TrxB), FabI, RecQ, SapC and the D-Ala-D-Ala carboxypeptidase DacA, which, if inhibited, can greatly promote the activity of existing drugs, including rifampicin, triclosan, nitrofurantoin, aminoglycosides and some β-lactams[11]. Understanding the genetic basis of intrinsic bacterial resistance, and hence the spectrum of activity of an antibiotic, can therefore guide the development of new combinations of agents with improved or expanded activity against target species. Various studies have identified *in vitro* synergies between unconventional combinations of antibiotics that can be used to target particularly problematic pathogens such as *Acinetobacter baumannii* and *Neisseria gonorrhoeae*[12–14]. Combining information from studies seeking synergy with those that use genetic screens to identify interactions between biochemical pathways could extend the arsenal of antibiotics and enable the use of existing drugs against species that are thought to be intrinsically resistant.

In addition to intrinsic resistance, bacteria can acquire or develop resistance to antibiotics. This can be mediated by several mechanisms, which fall into three main groups: first, those that minimize the intracellular concentrations of the antibiotic as a result of poor penetration into the bacterium or of antibiotic efflux; second, those that modify the antibiotic target by genetic mutation or post-translational modification of the target; and third, those that inactivate the antibiotic by hydrolysis or modification.

β-lactams
An important class of antibiotics, members of which contain a β-lactam ring and inhibit peptidoglycan synthesis by covalent binding to the active-site Ser of penicillin-binding proteins. β-lactam subclasses include carbapenems, cephalosporins, penicillins, monobactams and clavams.

Fluoroquinolones
Synthetic compounds that target topoisomerases. Examples include nalidixic acid and ciprofloxacin.

Aminoglycosides
Natural or semi-synthetic amino sugars that target translation by binding to the 30S subunit of the ribosome. Examples include gentamicin, tobramycin, streptomycin and kanamycin.

Each of these mechanisms has been reviewed over the past decade[15–17]. Therefore, this Review provides an update of the latest research for each type of antibiotic resistance mechanism and puts it into global context in terms of prevalence, the biological impact on the bacterium and the potential impact on clinical treatment.

Prevention of access to target

Reduced permeability. Compared with Gram-positive species, Gram-negative bacteria are intrinsically less permeable to many antibiotics as their outer membrane forms a permeability barrier[18,19]. Hydrophilic antibiotics cross the outer membrane by diffusing through outer-membrane porin proteins. In most Enterobacteriaceae, the major porins, such as the outer-membrane proteins OmpF and OmpC of *E. coli*, are thought to function as non-specific channels; previous evidence that suggested drug-binding sites were present within these channels now seems to be incorrect[18,20]. Therefore, reducing the permeability of the outer membrane and limiting antibiotic entry into the bacterial cell is achieved by the down-regulation of porins or by the replacement of porins with more-selective channels. This well-established mechanism of intrinsic antibiotic resistance in Gram-negative bacteria has been reviewed previously (see, for example, REF. 21). However, recent data have shown that in Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp., reductions in porin expression significantly contribute to resistance to newer drugs such as carbapenems and cephalosporins, to which resistance is usually mediated by enzymatic degradation[21–25]. For example, clinically relevant resistance to carbapenems in Enterobacteriaceae can occur in the absence of carbapenemase production if mutations reduce porin production or if mutant porin alleles are present[22,26]. The selective pressure exerted by carbapenems to favour the emergence of mutations in porin genes, as well as in genes that regulate porin expression, has been shown by the rapid accumulation of mutations in these genes in *E. coli* and *Enterobacter* spp. after carbapenem exposure[23,27,28]. In addition, isolates of *Klebsiella pneumoniae* that express porin variants have been associated with clonal lineages that have caused global outbreaks of infection[24,29].

Increased efflux. Bacterial efflux pumps actively transport many antibiotics out of the cell and are major contributors to the intrinsic resistance of Gram-negative bacteria to many of the drugs that can be used to treat Gram-positive bacterial infections. When overexpressed, efflux pumps can also confer high levels of resistance to previously clinically useful antibiotics. Some efflux pumps have narrow substrate specificity (for example, the Tet pumps), but many transport a wide range of structurally dissimilar substrates and are known as multidrug resistance (MDR) efflux pumps.

There are well-studied examples of MDR efflux pumps that are present in all bacteria, and new pumps that export antibiotics continue to be described. In the past 2 years, these have included MdeA in *Streptococcus mutans*, FuaABC in *Stenotrophomonas maltophilia*, KexD in *K. pneumoniae* and LmrS in *S. aureus*[30–33].

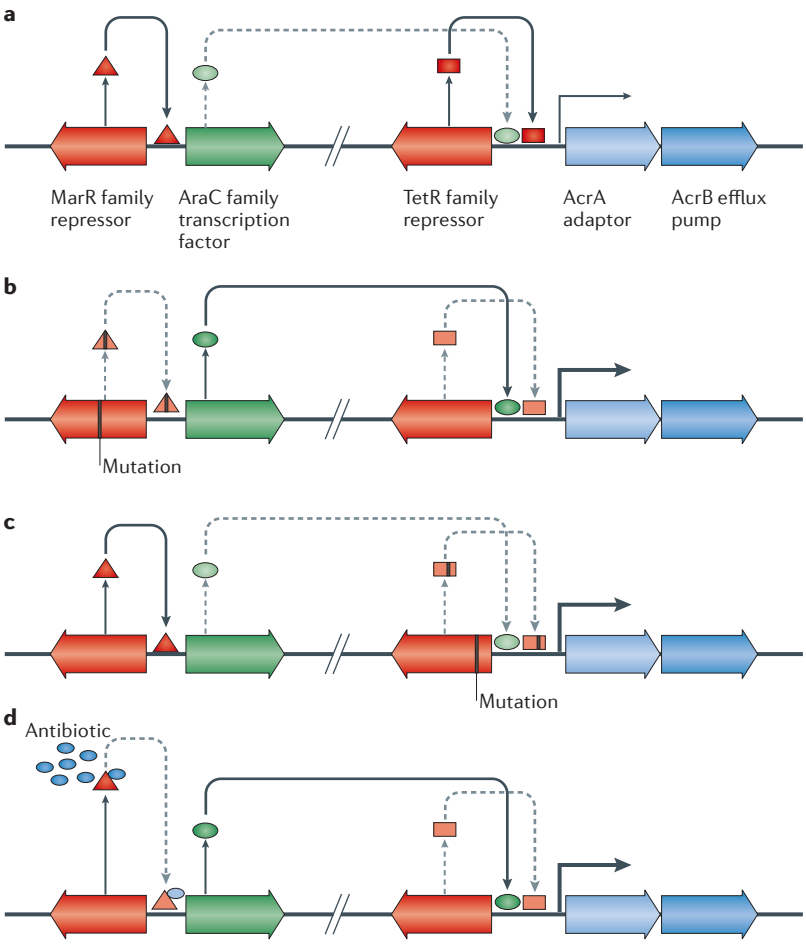

Figure 2 | **Pathways regulating multidrug efflux. a** | Baseline expression of resistance nodulation division (RND) efflux pumps is controlled by a locally encoded TetR family repressor, and levels of the AraC family transcription factor, which can relieve TetR-mediated repression are kept low by repression from the multiple antibiotic resistance protein (MarR) family repressor. **b** | Mutations in the *marR* family repressor gene cause the repressor to lose the ability to inhibit AraC family activators. Increased expression of the AraC activator confers increased expression of the RND efflux pump AcrB and the periplasmic adaptor AcrA. **c** | Mutation in the TetR family repressor gene reduces the repressor binding upstream of *acrA* and *acrB*, leading to increased transcription of *acrAB*. **d** | Antibiotics bind to MarR and cause conformational changes that prevent repression of the AraC activator. Increased expression of the AraC activator leads to increased transcription of *acrA* and *acrB*. Activated pathways are indicated by solid arrows and inhibited pathways are indicated by dashed arrows.

Although all bacteria carry multiple genes that encode MDR efflux pumps on their chromosomes, some have been mobilized: that is, they have been mobilized onto plasmids that can transfer between bacteria. Genes coding for a novel tripartite resistance nodulation division (RND) pump were recently found to be carried on an IncH1 plasmid that was isolated from a *Citrobacter freundii* strain that also carried the gene for the antibiotic-targeting enzyme New Delhi metallo-β-lactamase 1 (NDM1)[34]. This is a worrying development as it shows that this resistance mechanism is transmissible and could be rapidly disseminated to other clinically relevant pathogens.

The RND family of MDR efflux pumps is found in Gram-negative bacteria and is the best characterized of the clinically relevant MDR efflux transporters. When overexpressed, RND pumps confer clinically relevant levels of MDR and export an extremely wide range of substrates[35]. Well-studied examples include the multidrug efflux pump AcrB in *E. coli* and MexB in *P. aeruginosa*. RND pumps, such as AcrB, are homotrimers that reside in the inner membrane and form a tripartite complex with a periplasmic adaptor protein, such as AcrA or MexA, and an outer-membrane channel, such as TolC or OprM. Substantial progress has been made in understanding the structure and function of these efflux pumps (reviewed in REFS 36,37). Briefly, co-crystallization of *E. coli* AcrB in complex with substrates, along with computational studies, has shown that AcrB has two distinct binding pockets (distal and proximal). These pockets can accommodate substrates of different sizes and properties, which explains how the pumps can transport and provide resistance to such a broad range of antibiotics[19,38–42]. Further understanding of substrate binding has been gained from the detection of mutations occurring within the binding pockets; for example, a substitution at residue 288, within the distal binding pocket of AcrB, was found in clinical isolates of *Salmonella enterica* subsp. *enterica* serovar Typhimurium selected during antibiotic therapy. The substitution altered substrate binding and conferred antibiotic resistance, including to ciprofloxacin (J.M.A.B, and L.J.V.P, unpublished observations).

A structure of the whole tripartite system of *E. coli* AcrAB–TolC based on data produced with cryo-electron microscopy of an artificial assembly of mutant proteins has more recently been published, thereby addressing several outstanding questions about the pump structure and function[43]. Du and colleagues showed that the stoichiometry of the pump is 3/6/3 (AcrB/AcrA/TolC), which differs from previous models[44] but is in agreement with structures for RND pumps, including CusBA in other Gram-negative species, and with data regarding the stoichiometry of periplasmic adapter proteins[45–48].

Bacteria that overexpress efflux pumps, including Enterobacteriaceae, *P. aeruginosa* and *S. aureus*, have been isolated from patients since the 1990s[49–51]. Understanding the mechanism of overexpression is important as this is a common mechanism of resistance in clinical isolates, and thorough knowledge of the mechanism could enable the design of novel therapeutics to prevent the production of efflux-pump proteins. The transcription of genes that encode efflux pumps is controlled by local regulators, which are encoded alongside the efflux-pump genes, and by global regulators, which have broader biological functions (FIG. 2a). Global regulators include transcription factors of the AraC–XylS family, such as MtrA, which enhances transcription of *mtrCDE* in *N. gonorrhoeae*[52], and RamA, which activates transcription of *acrAB–tolC* in *Salmonella* spp.[53]. Many of the AraC–XylS family of regulators are encoded alongside a repressor of the multiple antibiotic resistance protein (MarR) family, including *marR*, which is encoded with *marA* in *E. coli*, and *ramR*, which is encoded with *ramA* in *S.* Typhimurium[54,55]. Overexpression of the AraC–XylS transcription factors follows loss of repression by the

corresponding MarR family protein[56–58]. The increased expression of these transcription factors, including MarA, SoxS, RamA or Rob in Enterobacteriaceae, confers MDR as a result of the increased expression of efflux pumps and repression of porin proteins[53,59].

The high-level expression of efflux genes seen in multidrug-resistant bacteria is often due to mutation in the regulatory network controlling efflux-pump expression (FIG. 2b,c). These mutations can be within a local repressor, a global transcription factor or intergenic sites that alter the expression of pump genes or their regulators[60–66]. Mutations can alter promoter activity; a recent example is the detection of a single-base-pair mutation in the consensus −10 sequence upstream of *mtrC* in *N. gonorrhoeae* that forms a new promoter with stronger activity, causing constitutive overexpression of the efflux pump and multidrug resistance[54].

Increased expression of efflux pumps can also occur as a result of induction in response to environmental signals and in conditions in which their function is required. For example, the *acrAB* genes in *E. coli* and *Salmonella* spp. are induced by small molecules that would be encountered during infection, such as indole and bile[57,67–69], whereas expression of MtrCDE of *N. gonorrhoeae* and NorA of *S. aureus* is responsive to iron limitation, which is a common hallmark of the host environment[70,71]. The most common mechanism of induction of efflux pump gene expression is the direct binding of a molecule to a transcriptional repressor protein, which decreases binding of the repressor to its target DNA (FIG. 2d). The structures of several regulatory repressor proteins have recently been solved, improving our understanding of this mechanism of resistance. For example, the structure of the *Mycobacterium tuberculosis* TetR family transcriptional repressor Rv1219c has been recently solved; this protein controls expression of the ABC family transporter RV1217c–Rv1218c, which is responsible for the export of isoniazid and rifampicin[72], and the structure revealed a large multidrug-binding pocket[73]. Similarly, the structure of RamR, the transcriptional repressor of the *ramA* gene, has been solved in complex with several ligands[74]. Inducing compounds reduced the binding affinity of RamR to DNA, increasing *ramA* expression. Understanding the molecular basis of induction of efflux could allow the development of chemical modulators to prevent efflux de-repression, and these modulators could be used in conjunction with antibiotics in novel therapeutic strategies.

Changes in antibiotic targets by mutation

Most antibiotics specifically bind to their targets with high affinity, thus preventing the normal activity of the target. Changes to the target structure that prevent efficient antibiotic binding, but that still enable the target to carry out its normal function, can confer resistance (FIG. 3). During the course of infection there are often large and diverse populations of pathogens, and if a single point mutation in the gene encoding an antibiotic target can confer resistance to the antibiotic, strains with this mutation can then proliferate. The genes that encode the targets of some antibiotics exist in multiple copies; for example, linezolid (the first oxazolidinone antibiotic to be developed, which entered clinical use just over 10 years ago) targets the 23S rRNA ribosomal subunit of Gram-positive bacteria, which is encoded by multiple, identical copies of its gene. Clinical use of linezolid has selected for resistance in *S. pneumoniae* and *S. aureus* by mutation in one of these copies, followed by recombination at high frequency between homologous alleles, which rapidly produces a population weighted in favour of carriage of the mutant allele[75–77].

Transformation — that is, uptake of DNA from the environment — can confer antibiotic resistance by target protein modification through the formation of 'mosaic' genes. The archetypal example of this is penicillin resistance in *S. pneumoniae*, which is conferred by mosaic penicillin-binding protein (*pbp*) genes encoding penicillin-insensitive enzymes. These mosaic alleles have arisen by recombination with DNA from the closely related species *Streptococcus mitis*. Mosaicism in the *penA* gene (which encodes a PBP) in *N. gonorrhoeae* has also been linked with high-level resistance to extended-spectrum cephalosporins. Infections with *N. gonorrhoeae* are currently one of the biggest challenges in clinical microbiology owing to the emergence of pan-resistant isolates and the resulting loss of the utility of ceftriaxone as a therapeutic option for these isolates[78].

Another example of a target change is acquisition of a gene homologous to the original target, such as in methicillin-resistant *S. aureus* (MRSA), in which methicillin resistance is conferred by acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*) element. This carries the *mecA* gene, which encodes the β-lactam-insensitive protein PBP2a; this protein enables cell wall biosynthesis to occur despite the native PBP being inhibited in the presence of antibiotic[79]. Many SCC*mec* elements have been identified in different *Staphylococcus* species, and there is evidence that the *mecA* allele has been mobilized several times[80]. The presence of a *mecA*-containing SCC*mec* element was thought to be the defining characteristic of MRSA. However, a divergent *mecA* allele, named *mecC*, has recently been identified in isolates from livestock and humans in the United Kingdom, Denmark and Ireland[80–82]. The *mecC* gene shares 70% nucleotide identity to *mecA* and is carried on a type XI SCC*mec* element. The presence of *mecC* MRSA presents a potential problem for diagnosis as it is not detected by standard PCR assays for *mecA* or by agglutination assays for PBP2a[83]. The antibiotic-resistance phenotype of *mecC* MRSA also differs slightly from *mecA* MRSA: isolates carrying *mecC* are more susceptible than *mecA* isolates to oxacillin but retain resistance to cefoxitin[32,84,85].

Modification (and protection) of targets

Protection by modification of the target can also be an effective means of antibiotic resistance that does not require a mutational change in the genes encoding the target molecules (FIG. 3). In recent years, protection of targets has been found to be a clinically relevant mechanism of resistance for several important antibiotics; for example, the erythromycin ribosome methylase (*erm*) family of genes methylate 16S rRNA and alter the drug-binding

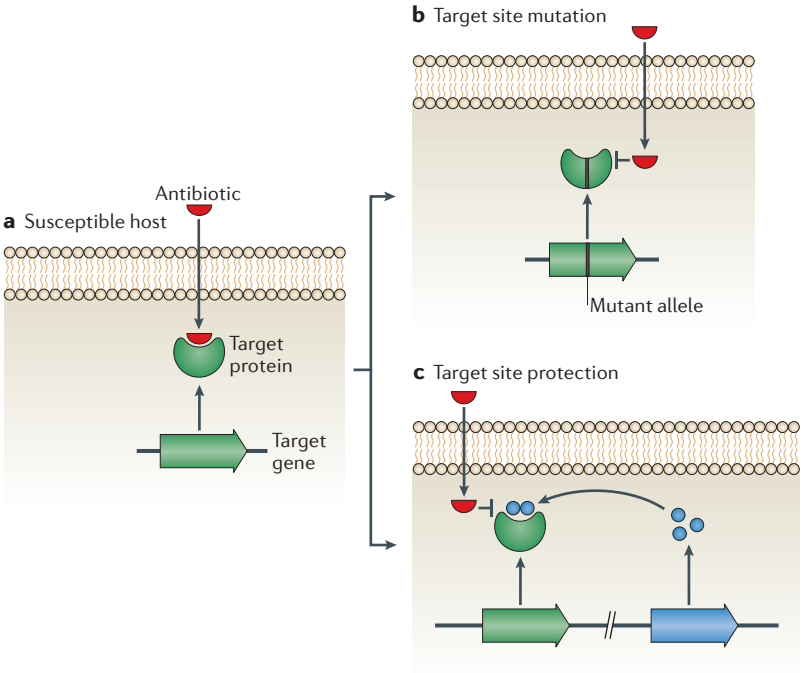

Figure 3 | **Target site changes.** **a** | A susceptible host in which an antibiotic is able to bind tightly to its specific target and exert an inhibitory effect. **b** | Mutation of the target site (for example, as found in mutations in topoisomerase genes in many species that confer fluoroquinolone resistance) or recombination to provide a mosaic allele (as found in the mosaic penicillin-binding proteins in pneumococci and gonococci that confer β-lactam resistance) results in a functional target with reduced affinity for the antibiotic, which does not bind efficiently and therefore has a reduced or negligible effect. **c** | Modification of the target by addition of a chemical group can also prevent antibiotic binding without altering the primary protein sequence of the target, which retains its activity.

site, thus preventing the binding of macrolides, lincosamines and streptogramins[73]. Another recently identified example is the chloramphenicol–florfenicol resistance (*cfr*) methyltransferase, which specifically methylates A2503 in the 23S rRNA; this confers resistance to a wide range of drugs that have targets near this site, including phenicols, pleuromutilins, streptogramins, lincosamides and oxazolidonones (including linezolid)[86]. Initially isolated in 1997 from a bovine staphylococcal isolate, the *cfr* gene was subsequently isolated from animal and human isolates of Gram-positive and Gram-negative bacteria, including *S. aureus* and *E. coli*[87]. The *erm* and *cfr* genes are both often carried on plasmids, which function as vectors to drive their wide dissemination[77,88]. The aminoglycoside antibiotics are protein synthesis inhibitors that function by binding to the ribosome. One mechanism of resistance to aminoglycosides is modification of the target ribosome by methylation. This was not previously thought to be a clinically relevant mechanism of resistance, but the enzymes that are responsible have recently been detected in several bacterial pathogens; for example, the *armA* gene, which encodes a methyltransferase, has been found in clinical isolates of Enterobacteriacae throughout North America, Europe and India, and *rmt* genes, which encode another methyltransferase, have been found in North America, Central and South America, and India[89,90].

The *qnr* families of quinolone resistance genes have also been found on plasmids in various pathogens. The *qnr* genes encode pentapeptide repeat proteins (PRPs), which bind to and protect topoisomerase IV and DNA gyrase from the lethal action of quinolones. A recent model of the mechanism of action of PRPs is based on structural data of QnrB1, which suggests that PRPs interact with topoisomerase–quinolone complexes after drug binding and promote release of the quinolone[91]. This rescues the topoisomerase and enables it to complete its normal activity and re-ligate DNA, thus preventing the release of double-stranded DNA breaks, which normally result from quinolone–topoisomerase interaction[91].

The polymyxin antibiotics, polymyxin B and polymyxin E (also known as colistin), are cyclic antimicrobial peptides with long, hydrophobic tails that target Gram-negative bacteria[92,93]. This specificity is based on their binding to lipopolysaccharide (LPS); antibacterial activity is conferred by the hydrophobic chain, which disrupts both cell membranes[72,73]. Owing to a lack of alternative therapies in recent years, colistin has become widely used in the treatment of infections by multidrug-resistant *P. aeruginosa*, *Acinetobacter* spp. and Enterobacteriaceae and, as a consequence, polymyxin resistance has developed. This resistance is often associated with changes in the expression of regulators affecting LPS production, which result in alterations in the target and reduce binding of the drug. For example, mutations that result in overexpression of *pmrC*, the product of which modifies lipid A, have been detected in the genes encoding the two-component regulatory system PmrAB[92,94–96]. These mutations cause the addition of phosphoethanolamine to lipid A and, as a result, colistin binding is reduced through lowering of the negative charge of the LPS[95]. Mutations in the genes encoding the PhoPQ two-component system, or its regulators, can also confer colistin resistance via increased expression of the PmrAB system[97,98]. This is a common resistance mechanism in *K. pneumoniae*[99].

Daptomycin specifically targets anionic phospholipids in the cytoplasmic membrane of Gram-positive bacteria and, in the presence of calcium ions, it inserts into the membrane, causing depolarization and the loss of intracellular contents[8,10]. In *S. aureus*, daptomycin resistance can occur as a result of point mutations in *mprF* (which encodes the multiple peptide resistance factor, a protein that decorates anionic phospholipid phosphatidylglycerol with L-Lys), resulting in remodelling of the phospholipid content of the membrane; this in turn alters the membrane charge and phospholipid composition, thus reducing binding of daptomycin[100]. One important use of daptomycin has been to treat serious infections caused by enterococci, particularly *Enterococcus faecium*. However, resistance can emerge during therapy; although the mechanisms by which enterococci can resist daptomycin are not fully understood, recent evidence has identified several genes and pathways that correlate strongly with daptomycin susceptibility[101,102]. Among these, mutations within the genes encoding the LiaFSR regulatory system, which coordinates membrane stress response, and the YycFGHIJ

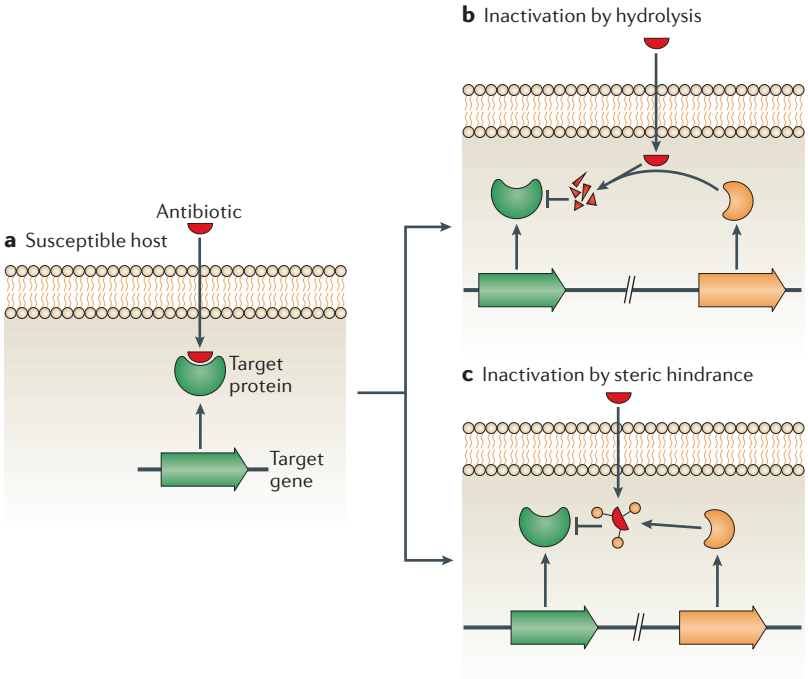

Figure 4 | **Direct interactions with antibiotics.** **a** | A susceptible host with a target that is efficiently inhibited by an antibiotic. **b** | Acquisition and production of an enzyme that destroys the antibiotic (for example, β-lactamases) prevents binding to the target and confers resistance. **c** | Acquisition and production of an enzyme that modifies the structure of the antibiotic (for example, aminoglycoside-modifying enzymes) can also prevent binding to the target and confer resistance.

system, which controls cell wall homeostasis, have been found in multiple isolates that show daptomycin non-susceptibility. Some isolates contain mutations affecting both systems, which shows that they are not mutually exclusive as resistance mechanisms[103]. Disruption of the LiaFSR system alters phospholipid content; this results in the redistribution of cardiolipin-rich domains in the membrane, which are the primary targets for daptomycin. The removal of primary binding sites for daptomycin from the septum, which is the primary cellular target in enterococci, is thought to confer daptomycin resistance; this is a novel example of how spatial reorganization of the target can confer resistance[104].

Direct modification of antibiotics

As well as preventing antibiotics from entering the cell or altering their targets, bacteria can destroy or modify antibiotics, thus resisting their action.

Inactivation of antibiotics by hydrolysis. The enzyme-catalysed modification of antibiotics is a major mechanism of antibiotic resistance that has been relevant since the first use of antibiotics, with the discovery of penicillinase (a β-lactamase), in 1940 (REF. 105) (FIG. 4). Thousands of enzymes have since been identified that can degrade and modify antibiotics of different classes, including β-lactams, aminoglycosides, phenicols and macrolides. There are also subclasses of enzymes that can degrade different antibiotics within the same class; for example the β-lactam antibiotics, such as penicillins, cephalosporins, clavams, carbapenems and monobactams, are hydrolysed by a diverse range of β-lactamases[106–109]. The expansion of antibiotic classes to include derivatives that have improved properties has been reflected by the emergence of hydrolytic enzymes that have altered spectra of activity. The early β-lactamases, which were active against first-generation β-lactams, were followed by extended-spectrum β-lactamases (ESBLs) that have activity against oxyimino-cephalosporins[110]. The carriage of diverse ESBLs and carbapenemases, including the IMP (imipenemase), VIM (Verona integron encoded metallo β-lactamase), *K. pneumoniae* carbapenemase (KPC), OXA (oxacillinase) and NDM enzymes in Gram-negative bacteria such as *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *A. baumannii*, has underpinned the emergence of isolates that are resistant to all β-lactam antibiotics; this has serious implications for the treatment of severe infections, particularly in hospital patients[108,110,111]. In some cases, the expansion of resistant bacterial clones has been responsible for increased rates of resistance, whereas in others, plasmids that carry resistance genes have spread widely. Irrespective of the mechanism, the common feature has been the rapid spread and expansion of resistance in a relatively short period of time.

There are hundreds of variants of the CTX-M genes (which encode ESBLs that are notable for their greater activity against cefotaxime than other oxyimino-β-lactams). These genes are classified into different subgroups, which have resulted from the escape of genes from the chromosome of *Kluyvera* species found in soil[112]. This escape has been facilitated by insertion sequences, notably IS*Ecp1*, followed by transfer on conjugative plasmids into many bacterial species[112]. The CTX-M14 and CTX-M15 enzymes have become the most widely isolated ESBLs worldwide[113,114], especially in cephalosporin-resistant *E. coli* and *K. pneumoniae* isolates. CTX-M15-producing *K. pneumoniae* isolates are mainly a nosocomial problem, whereas CTX-M15-producing *E. coli* strains are disseminated in patients in the community[115]. Plasmids that belong to the IncFII incompatibility group, which is found widely in Gram-negative pathogens, have been particularly associated with the spread of $bla_{CTX\text{-}M15}$, in some cases with particular clonal lineages (for example, the ST131-H30 clone of *E. coli*)[115].The $bla_{CTX\text{-}M14}$ gene is often associated with the IncK plasmid pCT in human, animal and environmental isolates of Enterobacteriaceae[116–118].

As a result of the increased numbers of bacteria carrying ESBL genes, the clinical use of carbapenem antibiotics has increased over the past decade. In turn, this has been associated with increasing numbers of clinical isolates carrying β-lactamases with carbapenem-hydrolysing activity (known as carbapenemases)[119–121]. Carbapenemases mainly include members of the class A, B and D β-lactamases[119–121]. The hallmark of these enzymes is the ability to inactivate a broad range of β-lactams, including carbapenems and extended-spectrum cephalosporins. Although first identified on the chromosomes of single species, many carbapenemases are now plasmid-mediated and have been reported in Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*[121].

Extended-spectrum β-lactamases
(ESBLs). β-lactamase enzymes that are able to hydrolyse extended-spectrum oxyimino cephalosporins.

The spread of carbapenemases has occurred in different ways, as exemplified by the *kpc* and *ndm* genes. The serine carbapenemase KPC was first identified in *K. pneumoniae* in 1996 (REF. 122) and has since been described in several Enterobacteriaceae[123]. The *kpc* gene is plasmid-borne and is associated with a dominant clone of KPC-producing *K. pneumoniae*, ST258, which is found worldwide[124]. This gene is often carried on the pKP-Qil plasmid or on closely related variants[125], and there are several variants of the *kpc* gene that encode proteins that can be differentiated by single-amino-acid substitutions (although most retain similar activity); KPC2- and KPC3-producing strains have been responsible for outbreaks in the United States, Greece, Israel and the United Kingdom[126,127]. Since its first description in India in 2009 (REF. 128), the NDM carbapenemase has become one of the most widespread carbapenemases and is found in Gram-negative pathogens including *A. baumannii*, *K. pneumoniae* and *E. coli* throughout the world. NDM confers resistance to all β-lactams except aztreonam. The *ndm* genes are often found on broad-host-range conjugative plasmids belonging to several incompatibility or replicon types, including IncA, IncC, IncF, IncHI1 and IncL–IncM[129–131], and in conjunction with other antibiotic-resistance genes[132]. Interestingly, the spread of NDM has been underpinned by the extreme mobility of the bla_{NDM} gene itself, which is mediated by an IS*Aba125* element upstream of the *ndm* gene rather than by expansion of an epidemic clone of bacteria or plasmid, as seen with the *kpc* genes[107]. NDM-producing isolates have been found in many species: the genes are located both on plasmids and on the host chromosome and able to move between the two at high frequency[107,133]. Initially, infections with NDM-carrying bacteria were epidemiologically linked to the Indian subcontinent, Balkan states and Middle East[107]. However, recent reports have identified outbreaks that have occurred and been sustained in other parts of the world[106,134].

Inactivation of antibiotic by transfer of a chemical group. The addition of chemical groups to vulnerable sites on the antibiotic molecule by bacterial enzymes causes antibiotic resistance by preventing the antibiotic from binding to its target protein as a result of steric hindrance (FIG. 4). Various different chemical groups can be transferred, including acyl, phosphate, nucleotidyl and ribitoyl groups, and the enzymes that are responsible form a large and diverse family of antibiotic-resistance enzymes[135].

Aminoglycoside antibiotics are particularly susceptible to modification as they tend to be large molecules with many exposed hydroxyl and amide groups. Aminoglycoside-modifying enzymes confer high levels of resistance to the antibiotic (or antibiotics) that they modify. There are three main classes of aminoglycoside-modifying enzymes: acetyltransferases, phosphotransferases and nucleotidyltransferases. These classes are evolutionarily diverse and vary in the aminoglycosides that they can modify and in the part of the molecule that is modified[136]. However, recent molecular dynamic studies have suggested that all three are able to bind to aminoglycosides as their active sites mimic the target environment of the ribosomal binding cleft[137]. The substrate specificity is due to protein flexibility and the size of the active site, in combination with the modulation of the thermodynamic properties of the enzyme when in complex with bound substrate[136]. A worrying recent development is the discovery of a novel genomic island in *Campylobacter coli* isolated from broiler chickens in China: this genomic island encodes six aminoglycoside-modifying enzymes, including members of all three classes, and confers resistance to several aminoglycoside antibiotics that are used in the treatment of *Campylobacter* infections, including gentamicin[138].

Another recent discovery is the widespread presence of a family of rifamycin-resistance genes in Actinomycetes and pathogenic bacteria. These genes were discovered by identifying a common sequence element (rif-associated-element (RAE)) that is present in various genera of Actinomycetes upstream of rifamycin-resistance genes. A bioinformatic analysis of sequences deposited in GenBank identified the presence of the RAE in a wide range of species and also identified a novel rifamycin phosphotransferase that is associated with the RAE. This use of phylogenetics and functional assays has identified a previously unknown reservoir of rifamycin-resistance genes[139].

Summary and conclusions

As a result of the widespread use of antibiotics in human medicine — as well as in animal treatment, horticulture, beekeeping, anti-fouling paints (used in the marine and oil industries) and laboratories carrying out genetic manipulation — the evolutionary pressure for the emergence of antibiotic resistance is great. Antibiotic resistance is a natural phenomenon, and bacteria have been evolving to resist the action of natural antibacterial products for billions of years (BOX 1). Although the ability of bacteria to become resistant to antibiotics has long been appreciated, our knowledge of the remarkable diversity of mechanisms involved has increased greatly in recent years. Advances in genomics, systems biology and structural biology have dissected many of the precise events

Box 1 | **The ancient resistome**

Although much attention has been focused on antibiotic resistance in pathogens found in hospital patients and on the bacteria that are directly responsible for adverse effects on human health, the development of resistance to antibiotics is a natural ecological phenomenon and is the product of billions of years of evolution. Studies of microorganisms from pristine sites, including isolated caves and permafrost, have shown that resistance occurs in the absence of human activity[141–143]. Most antibiotics that are used in human medicine are derived from antibiotic-producing microorganisms that expose other species in their local environment to antimicrobial molecules. This favours selection of resistance in environmental species as well as generating resistance or 'immunity' genes in the producer organisms. Although the presence of resistance in natural environments might be a natural phenomenon, it is not innocuous because this reservoir of resistance genes can be mobilized and can transfer into human pathogens[144–146]. Human use of antibiotics has selected for the escape of genes from the soil 'resistome' into human pathogens, as demonstrated by the presence of the same genes in soil bacteria and human bacteria. Furthermore, environmental microorganisms carry genes that encode resistance to newly licensed antibiotics, such as daptomycin, even before the first clinical use of such antibiotics[147–149].

underpinning resistance and will continue to provide greater understanding. This information, if used properly, should aid the discovery and development of new agents that can circumvent or neutralize existing resistance mechanisms. Indeed, increased understanding of resistance has also provided new targets for discovery; for example, inhibitors of MarA, which regulates the expression of genes that encode for MDR efflux pumps, have been identified and have been shown to limit the severity of *E. coli* infection in a mouse urinary tract infection model[140].

Studies of resistance development and mechanisms of resistance must be a mandatory requirement at an early stage of drug development; such studies will enable academic institutions and industry to work together. We now have the ability to rapidly evaluate the potential for the emergence of resistance to novel drugs, identify where and when this might occur and determine the mechanisms responsible. The early identification of naturally occurring resistance mechanisms and targets that can accommodate numerous structural changes should lead to the discontinuation of the development of agents that are likely to fail in the clinic as a result of resistance. Resources could instead be focused on agents that are less likely to drop out of the pipeline for microbiological reasons. Knowledge about how and when resistance occurs and potential synergies with combinations of agents will also facilitate the development of dosing regimens that can help to minimize the emergence of resistance to current and new antibiotics, enabling these drugs to be used to best effect. This is crucial in the short term as new agents are not likely to enter widespread clinical practice in the immediate future. The challenge for the field is now to make best use of the available technologies, information and expertise to ensure the impact of resistance is fully accounted for in the urgent development of next-generation antibacterial drugs.

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The authors declare no competing interests.