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Bacterial defences: mechanisms, evolution and antimicrobial resistance

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Abstract

Throughout their evolutionary history, bacteria have faced diverse threats from other microorganisms, including competing bacteria, bacteriophages and predators. In response to these threats, they have evolved sophisticated defence mechanisms that today also protect bacteria against antibiotics and other therapies. In this Review, we explore the protective strategies of bacteria, including the mechanisms, evolution and clinical implications of these ancient defences. We also review the countermeasures that attackers have evolved to overcome bacterial defences. We argue that understanding how bacteria defend themselves in nature is important for the development of new therapies, and for minimising resistance evolution.

[H1] Introduction

Bacteria are amongst the most ancient organisms on Earth[1], but across virtually every ecosystem, they are threatened by **competitor [G]** bacteria[2–5], **bacteriophages**[6] **[G]** and **predators**[7] **[G]** , which are all equipped with a broad range of means to attack them. Whereas the widespread human use of antibiotics dates back a mere century, these three biotic threats have been shaping the evolution and physiology of bacteria for billions of years.

Bacteria have evolved a panoply of **defence mechanisms [G]** to avoid or mitigate harm from biotic threats. Understanding these defences is important for several reasons. They offer insights into bacterial biology, illustrating ecological challenges that bacteria faced in the past and the mechanisms that evolved to overcome them. These mechanisms are phylogenetically widespread and influence the physiology of diverse bacterial species; some components of animal innate immune systems even trace their origins to bacterial defence mechanisms[8]. Ancient defences are also central to how modern bacteria respond to antimicrobial therapies. Many defences offer broad protection against various threats, which means that bacteria often have **preadaptations [G]** that potentiate resistance to antimicrobials in the clinic. Moreover, as we search for new **biotherapeutic [G]** alternatives to antibiotics, including probiotic bacteria and phage therapy, we face many of the same challenges from these preadaptations that render bacteria hard to kill[9].

In this Review, we explore bacterial defence mechanisms through an evolutionary lens and discuss their relevance for treating bacterial infections. We discuss the threats that bacteria face from microbial predators, competitors and viruses, and then identify common principles of defence that protect against these threats. The set of known bacterial defences is large and ever-growing, such that exhaustively cataloguing every mechanism is beyond the scope of this article. Instead, we select examples that illustrate different categories of defence, and discuss their regulation and their evolution. We close by examining how attackers have evolved to overcome bacterial defences, and discuss how the study of defences can inform on the treatment of bacterial disease (Box 1).

[H1] Bacteria face myriad threats

In a given environment, abiotic factors (for example, light, salinity or heat) produce **stressors [G]** , and for host-associated bacteria, immune cells and responses may contribute others (for example, antimicrobial peptides). In this Review, however, our focus centres on the biotic challenges presented by bacterial competitors, phages, and predation by eukaryotes and specialised bacteria (Figure 1).

[H2] Bacterial competitors.

Most bacteria live in dense, multi-species communities, where competition for space and nutrient resources is severe[2–4]. Commensurately, bacteria have evolved diverse strategies for inhibiting and killing their competitors, many of which involve the use of specialised **weaponry [G]** (recently reviewed in Ref. [10]). Antibacterial weapons are extraordinarily diverse, encompassing molecular toxins[11], antimicrobial peptides[12] and proteins[13,14], toxin-injecting[15] and membrane-puncturing[16] nanomachines, and even weaponized phages[17]. These myriad weapons harm a target bacterium by attacking its key cellular structures and processes, which results in growth inhibition or cell death. For example, diffusible peptide-based toxins (bacteriocins) often damage DNA and RNA[18], or compromise cell envelopes via pore-forming[19] or wall-degrading activity[20]. Protein toxins injected via the type VI secretion system (T6SS) frequently attack the bacterial cell wall or membrane(s)[21], lysing intoxicated cells quickly and thereby clearing a path to new targets[22]. Antibiotics, a diverse group of secondary metabolite toxins, have broad but overlapping activities, and common targets include gene transcription and protein translation, DNA synthesis and replication, and the cell envelope[23,24].

[H2] Bacteriophages.

Phages are the most numerous biological entities in the biosphere[25], and are a leading cause of bacterial mortality in many environments[26] (for a recent review, see Ref. [6]). Phages differ widely in their evolutionary relationships with hosts, spanning a continuum from **parasitism [G]** to **mutualism [G]** [27]. To replicate all phages must inject their genetic material into bacterial hosts. For lytic phages, the injected genetic material is immediately copied and transcribed to assemble progeny phage particles, which kill and burst the host cell to disperse. Temperate phages (for example, λ-coliphages) also reproduce via host lysis under certain conditions, but have the additional ability to lysogenize host bacteria[28], whereby the phage inserts its genome into the bacterial chromosome, which enables it to replicate vertically alongside its host as it grows and divides. A third class of phages (for example, filamentous phages) exhibit a chronic replicative cycle, whereby new phages are continuously extruded from the host[29]. Lysis of cells infected with lytic phages is triggered by envelope-degrading endolysins and holins[30]; temperate phages kill via similar mechanisms but may lie dormant for long periods before those mechanisms are induced. Cells with chronic phage infections are generally not killed[29], but still suffer from reduced fitness owing to the diversion of cellular resources towards phage assembly[31].

[H2] Eukaryotic and bacterial predators.

As well as viral infection, bacteria have long faced the threat of predation, particularly from free-living protozoa that feed via phagocytosis in soil and aquatic environments[7]. Some

bacteria are also facultative or obligate bacterial predators: the soil bacterium *Myxococcus xanthus* moves rapidly in large groups, digesting encountered prey with secreted hydrolytic enzymes[32]. *Bdellovibrio* and like organisms (BALOs) are small bacteria that burrow inside Gram-negative bacteria: once inside the periplasm, a BALO cell grows by digesting the cytosolic contents of the host with hydrolytic enzymes, fueling rapid growth[33]. Once the resources of the host are exhausted, the BALO cell divides to form multiple progeny cells, which are released via host-cell lysis[34]. Meanwhile, the Candidate Phyla Radiation, a diverse group of small-celled bacteria representing approximately 15% of all bacterial diversity[35], may incorporate other new types of predatory or parasitic bacteria. Although the biology of this group remains poorly understood, members often have reduced genomes, and seem to rely on other bacteria to survive and reproduce[36].

[H1] Classes of bacterial defence

Bacteria have a wide range of defensive mechanisms against competitors, phages and predators. These mechanisms operate at a range of spatial scales, from molecular and cellular defences, to those that require bacteria to work as a group (Figure 2).

[H2] Molecular-scale defences.

[H3] Target modification and protection. To kill a bacterium, attackers deploy harmful **agents [G]** that interact with specific molecular targets to disrupt vital cellular processes of the target cell. Modification[37] or protection[38] of a target structure can attenuate these interactions and prevent or lessen harm. □-lactam antibiotics, such as penicillin, kill bacteria by inhibiting cell-wall cross-linking enzymes. In methicillin-resistant *Staphylococcus aureus* (MRSA), the genes *mecA* and *mecC* encode modified cross-linking enzymes that are insensitive to almost all □-lactam drugs[39]. Modification can be post-translational as well as genetic; for instance, the enzymatic methylation of bacterial ribosomes can prevent multiple classes of antibiotics from binding with this target[37].

[H3] Target repair and compensation. Cells can compensate for the presence of a harmful agent via generalized physiological responses that repair damaged targets. Exposure of bacteria to antibiotics, other competitor toxins, or phages, often results in oxidative DNA damage[40,41]. Subsequently, repair of oxidised DNA occurs via the base excision repair (BER) and nucleotide excision repair (NER) systems, which are both highly conserved and ancient pathways[42,43]. Apart from chromosomal repair, some species possess RNA ligases that can mend 16S rRNA damage caused by ribotoxic bacteriocins[44]. Similarly, the extrusion of filamentous phages can compromise the inner membrane of *Escherichia coli*, but the expression of membrane-binding phage-shock proteins suppresses proton leakage and

maintains the proton-motive force[45]. Sometimes it suffices to simply replace lost targets: when intoxicated with cell-wall-degrading T6SS toxins, *Vibrio cholerae* responds by increasing peptidoglycan synthesis to compensate[46].

[H3] Agent modification, binding and degradation. Harmful agents can be neutralised before they inflict damage. Multiple classes of antibiotics are neutralized through modification, via the enzymatic addition of acetyl, phosphoryl or adenyl groups[47]. Toxic agents can also be inactivated via binding to other molecules: the expression of cognate immunity proteins confers resistance to many bacteriocins[19], T6SS[48] and Cdi[49] effectors, and enables cells to safely use these toxic proteins as weapons[10]. In the same way, expression of orphan immunity proteins (that is, those for which a bacterium does not produce a cognate toxin) enables bacteria to survive attacks from non-kin cells[50,51].

Bacteria also have diverse systems to degrade harmful agents. □-lactamases are ancient proteins that hydrolyse the ring structure of beta-lactam antibiotics, such as penicillin[52]. Restriction-modification (RM) systems encode restriction endonucleases, which bind to and cleave phage and other foreign DNA at specific recognition sites. Target modification also has a role here, but is directed at host DNA: recognition sequences on host DNA are modified (e.g. via methylation) to protect them from degradation, while unmodified phage DNA is destroyed by the endonuclease. Multiple classes of RM systems have been characterised across both bacteria and archaea[53,54], providing innate immunity against a subset of phages. Recently-discovered antiviral defences, such as DISARM[54] (defence island system associated with restriction–modification) and Dnd[55] (DNA phosphorothioation) systems, function in a similar manner, respectively attacking foreign DNA that lacks methyl- or sulphur modification.

The degradation of harmful agents reaches astonishing complexity in CRISPR–Cas systems, which provide bacteria with adaptive immunity against phages whose genomic signatures have previously been encountered. These systems store fragments of foreign DNA in the bacterial genome, which then guide Cas restriction enzymes to degrade DNA in the cell that resembles that of past phage infections[56] or other mobile genetic elements[57]. The recently-discovered prokaryotic Argonaute (pAgo) proteins operate on a similar principle, providing guided DNA interference against harmful genetic elements including plasmids, transposons and phages[58].

[H2] Cellular defences

[H3] Membranes, capsules and extracellular vesicles. Most harmful agents must enter a cell before they can cause harm, and bacterial membranes are often pivotal in restricting this entry.

Indeed, the outer membrane of Gram-negative bacteria may have evolved in part to better protect cells from antimicrobial compounds[59]. The structures decorating a membrane are also crucial to barrier function: some structures (for example, transporters or surface polysaccharides) function as binding sites or entry points for phages and protein toxins, and bacteria that lack such structures, or have modified those structures, benefit from resistance. Other surface structures (for example, lipopolysaccharides[60] and curli fibres[61]) confer protection by occluding phage- or toxin-binding sites, or by armouring the cell against mechanical insult. For example, bacterial capsules, which are protective sheaths of exopolysaccharides, can armour cells against penetration by the T6SS[62,63]. Similarly, a layer of interlocking surface proteins, known as the S-layer[64], can protect bacteria from entry by *Bdellovibrio* bacteria[65], as can certain lipopolysaccharides[66]. Beyond their barrier role, membranes can perform additional defensive functions when shed as bubble-like extracellular vesicles[67]. As well as enhancing envelope stability (by removing misfolded or mislocalised envelope components)[67], vesicles can function as extracellular 'decoys', absorbing antibiotics, peptide toxins and phages, and carrying toxin-degrading enzymes[68]. Vesicle release is actively upregulated in response to envelope stress, and is thought to have intersecting roles in anti-phage and anti-toxin defence[68].

[H3] Efflux pumps. When the cell envelope fails to stop harmful molecules from entering, bacteria can instead force them back out. Efflux pumps are a diverse group of membrane transport proteins universal to bacteria, with a broad range of substrate specificities[69] and physiological functions[70]. In particular, they are an effective and fast-acting antibiotic resistance mechanism[71], sufficient in some cases to protect antibiotic-producing bacteria against their own toxins[72].

[H3] Motility. Using flagellae, type IV pili or other motility systems[73], bacteria can evade threats that would otherwise kill them. In planktonic environments, bacteria with sufficiently high swimming speeds (>30 μm s^{-1}) can avoid capture by protozoan predators, despite meeting them more often at high speeds[74]. Indeed, motility can be beneficial even if a bacterium cannot 'outrun' a threat: *Bdellovibrio* predators swim approximately twice as fast as *V. cholerae* prey cells[75], but the drag forces generated by prey motility impede predator attachment[66]. However, motility is not always a good defence: many phages bind to motility systems as part of their infection process[76], and movement can also spread phage within bacterial groups[77].

[H2] Multicellular defences

[H3] Biofilms. Clonal groups of bacteria often work together, collectively enduring threats which would kill single cells[78]. The most ubiquitous example of a multicellular defence in

bacteria is the formation of **biofilms [G].** Biofilms underlie a range of chronic infections, and often form in response to antibiotics and competition from other strains[79–81]. They can render bacteria extremely hard to kill, for multiple reasons. Diffusion limitation of solutes, such as oxygen or nutrients, means that many biofilms contain large numbers of slow-growing or dormant cells, which are more tolerant of toxins that target cell growth and division machinery than their fast-growing counterparts[82]. The outer regions of a biofilm can also protect cells deeper inside, collectively absorbing[83] and degrading[84] toxins and limiting their penetration into the community. Cells in biofilms also produce a slimy matrix of polysaccharides, proteins, DNA and other compounds: these surround cells and create an additional physical barrier that can inhibit the passage of antibiotics[85], block T6SS attacks[62,78] and screen cells from phages[61] and predators[86]. Matrix production can also function as an offensive strategy, which enables bacteria within the biofilm to spread out and smother competitors[87]. Matrix-trapped phage can even become weapons, protecting a biofilm from invasion by competing bacteria[88].

[H3] Phenotypic heterogeneity. Another **collective defence [G]** strategy displayed by bacteria is to maintain standing population variability in phenotype (for example, growth phase), such that not all individuals fare equally badly when conditions deteriorate. Such phenotypic heterogeneity is associated with clinical antibiotic tolerance[89], and is also a route through which bacteria resist toxins from competitors[90]. Sources of this variability include the gradients in nutrients and other solutes discussed above, which commonly occur in biofilms, and can drive differences in cell physiology across space[91]. However, phenotypic variation also emerges in the absence of environmental gradients, via stochastic mechanisms. A key example of this is the ability of bacteria to switch epigenetically to slow-growing antibiotic-tolerant 'persister' states[92], or to rapid growth modes that avoid antibiotic accumulation[90]. An evolutionary experiment showed that antibiotic treatment can select for *E. coli* point mutations that increase the rate of this switching, which results in high levels of multi-drug tolerance[93]. This result suggests that production of persister cells represents an evolved defence mechanism.

[H3] Counterattacks. Sometimes offence is the best defence — true to this maxim, many bacterial species launch en-masse **counterattacks [G]** to eliminate perceived threats[10]. Of course, counterattack strategies can be protective at the individual level too: environmental *V. cholerae* cells use the T6SS as an anti-grazer defence[94], whereas *Pseudomonas aeruginosa* cells respond to T6SS-mediated attacks by competitors with spatially coordinated T6SS firing[95,96]. However, for many secreted toxins, lethality is strongly dependent on producer cell density[97,98], making counterattacks more effective when undertaken collectively[99]. Some bacteria regulate toxin counterattacks via autoinduction: when toxin concentration and

production are connected in a positive feedback loop, a minor aggression may be met with disproportionate retaliation[83,100]. In some cases, mass-counterattacks lead to runaway conflict escalation and even mutual destruction[100,101].

[H3] Suicide. Saving nearby clonemates via self-sacrifice is another striking form of defence shown by bacteria. Active cell suicide is both collective and cooperative by definition, as it kills the individual while benefiting neighbouring cells. Many bacteria protect their kin from the spread of a phage infection using a strategy called abortive infection[102], whereby an infected cell pre-emptively triggers its own lysis, or growth arrest, before phage particle assembly is completed, thereby sparing kin from subsequent infection. Multiple anti-phage defences, including bacterial gasdermins[103], the CBASS system[104], and certain toxin–antitoxin[105] and CRISPR systems[106], function in this way; other recent discoveries (for example, RADAR[107], Theoris[108] and Zorya[109] systems) may behave likewise. Interestingly, cell suicide is also at the heart of some striking examples of counterattack: colicin toxins produced by *E. coli* are too large to pass through standard secretion apparatus, necessitating destructive cell lysis for their release. Other large protein weapons, such as eCISs (extracellular contractile injection systems)[110] and R tailocins[14], are similarly constrained. However, it was shown that only *E. coli* cells that have already sustained lethal damage undergo the lytic toxin release pathway, which reduces the effective costs of suicide[111]. The result can be a massive counterattack by the doomed cells, paralleling suicidal stinging by honeybees.

[H1] Competition sensing and defence regulation

Bacteria use some defensive structures by default; for example, the outer membrane is a permanent protective feature of Gram-negative bacteria[59]. However, many defences are not fixed and are instead **plastic responses [G]** to perceived threats. These responses are distinct from evolutionary responses (population changes in genotype), which we discuss in the next section. Critical to using plastic defences is the ability to infer that a threat is present or likely to occur, and bacteria use a range of information sources (cues) to achieve this[112] when acclimating to new and hostile environments (Figure 3).

[H2] Bacteria sense attacks through direct and indirect means.

First, many bacteria regulate defences by sensing attack signatures; that is, cues that result directly from a biotic threat. Physiological stress is a primary indicator that a focal bacterium could be under attack, and bacteria detect stress using a wide range of **stress responses [G]** [79,113]. These regulatory networks respond to diverse forms of stress, of both biotic and abiotic origins. However, there is strong evidence that bacteria differentiate between different stress cues, deploying anti-competitor defences only in response to stressors that are likely to stem

from a biotic threat (Figure 3). This behaviour is known as **competition sensing [G]** [79], and is thought to regulate a wide range of defences. The clearest evidence for competition sensing comes from the upregulation of antibacterial toxins, because in that case one can infer that the likely function of the response is to cope with competitors. For other defences, such as DNA repair systems, it is more challenging to tell if the response evolved primarily due to biotic or abiotic stressors. However, multiple of the major stress responses are known to be activated by biotic threats, which is consistent with their use in competition sensing[41,81].

Antibacterial weapons often target vital structures such as the cell envelope or chromosome (Figure 1). Damage to these components, sensed via specific stress response pathways[113], is frequently used to regulate counter-attacks and structure-specific repair pathways[79]. As cellular damage often results in the production of reactive oxygen species[41], many bacteria also use oxidative stress as a cue to produce toxins[79,114]. General stress responses can also be used to regulate defences: when attacked by T6SS-armed competitors, *Salmonella enterica* serovar *Typhimurium* activates various damage responses, including the general stress response, to induce biofilm formation and efflux pump expression[81]. In some cases, cellular perturbation is sensed without a canonical stress response: *P. aeruginosa* bacteria directly sense oncoming T6SS attacks through the resulting perturbations to its membranes, likely via the TagQRST pathway[95]. By sensing the specific location of these strikes, defenders gain valuable information on the position of the attacker cells, helping them to more effectively counter-attack with their own T6SS weaponry[115]. There is also evidence that competition sensing by *P. aeruginosa* is induced by the cytotoxins of *Staphylococcus aureus*, which is a key ecological competitor during infections[116].

Competition sensing, therefore, enables bacteria to infer the presence of competitors, and the efficient activation of defences and counter-attacks. There is growing evidence that stress responses can play analogous roles in sensing and responding to cell damage stemming from other biotic threats. Envelope stress responses are frequently triggered during phage infection: filamentous phages compromise *E. coli* membrane integrity during chronic infection, triggering the so-called ‘phage shock’ cascade, and activating membrane repair pathways[117]. Likewise, lytic phages stimulate phage shock proteins in *Lactococcus lactis*, which responds by altering its metabolism to restore loss of proton-motive force[118]. Certain toxin–antitoxin systems sense phage infection via canonical stress responses, or via transcriptional changes caused by infection[119]. In a similar vein, cellular damage can warn of predator activity. *Tetrahymena* ciliates engulf bacteria to feed on them, but this can activate the bacterial SOS response. When *Tetrahymena* eat enterohemorrhagic *E. coli,* the result is that the engulfed bacteria retaliate by suicidally releasing shiga toxins, killing the predator from within, and

protecting kin cells from the predator[120]. Shiga toxins are the causative agents of enterohemorrhagic diarrhoea[121], underscoring that anti-predator defences can be linked to human disease.

Cell damage is a reliable indicator of an urgent threat[79,112], but by the time a cell detects injury, it may already be too late for defensive action. For instance, *E. coli* cell invasion by *Bdellovibrio* predators prompts host upregulation of genes associated with osmotic, envelope and general stress responses, but these do not seem to confer any resistance to the predator[122]. In such cases, detecting alternative attack signatures, such as chemical cues that precede an attack, may provide an important alternative to damage sensing[79]. Through '**danger sensing [G]** '[123], bacteria intercept chemical signatures of the attacker: for example, peptidoglycan sheddings[124], or signal molecules (Figure 3). Some bacteria express receptors for quorum sensing molecules that they themselves do not produce[125], which enables them to 'eavesdrop' on the communications among competitor strains and thereby monitor their density[79,123]. Similarly, the perception of predator-associated chemical cues is widespread in planktonic microorganisms[126]; for instance, *Pseudomonas fluorescens* responds to diffusible cues produced by protozoan predators by producing membrane-disrupting biosurfactants that are toxic to protozoa[127]. Intriguingly, some bacteria are even capable of directly sensing attackers' toxins (for example, antimicrobial peptides[123] and □-lactam antibiotics[128]), and responding before the toxin takes its effect. In the sense that genetic material injected by phages is itself a harmful agent, anti-phage systems that detect foreign DNA (for example, CRISPR, restriction–modification and DISARM systems) fall into this sensing category.

When attacked, bacteria can also forewarn their kin of danger, priming defences in advance of physiological harm. When attacked by phage or antibiotics, *P. aeruginosa* cells produce a quinolone signal that repels other clonemates from the affected area[129]. Similarly: in response to neighbour infection, non-infected *Bacillus subtilis* cells can modify phage binding sites (cell wall teichoic acid polymers) on their surface, adding analyl groups that hinder phage binding[130]. Cell lysate factors, such as DNA and other mislocalised cytoplasmic molecules[131], often serve as danger cues for bacteria, eliciting toxin and exopolysaccharide production in kin cells. These cues are sensed via transduction pathways (for example, the Gac–Rsm and PhoPQ pathways in *P. aeruginosa* and other Gammaproteobacteria) that are often independent from classic response pathways[131]. As discussed, these enable cells to raise defences and launch counterattacks before they enter stress states[123,132].

[H2] Bacteria associate nutrient depletion with competition.

Short of direct threat, certain environmental changes can also imply the presence of competing organisms. Nutrient starvation may indicate **exploitation competition [G]** , driven by high numbers of clonemates, competitors or both[112] (Figure 3). Consistent with their use in competition sensing, bacteria use starvation stress pathways to regulate the production of anti-competitor toxins[79]. For example, the stringent response is a ubiquitous signalling cascade that is triggered by limitations to key resources, such as amino-acids, fatty acids, inorganic phosphate or iron[133]. As well as triggering cell cycle arrest and the cessation of growth, the stringent response upregulates the production of toxins across diverse bacterial species[134–136].

[H2] Bacteria use kin density to forecast threats.

A third important information source for defence regulation is **quorum sensing [G]** [112,137,138]. By monitoring the concentration of density cues (both canonical quorum sensing autoinducers and other 'quorum-related' cues[79]; for example, peptidoglycan fragments[124]) bacteria can sense high kin densities and prepare for an expected attack (Figure 3). Recent work demonstrated that CRISPR–Cas activity and adaptation is regulated via quorum sensing, such that antiviral defences are primed when bacteria are at high density, and most vulnerable to virulent phage[139]. Density sensing also informs whether bacterial groups have sufficient members for collective defences to be effective. Biofilm defences are frequently regulated using quorum sensing[137,140]; various bacterial species also use quorum sensing to control collective counterattacks, using antibiotics[141], bacteriocins[142] or T6SSs[143]. For instance, when at high cell density, *P. aeruginosa* produces the phenazine pyocyanin in a quorum sensing-dependent manner. Among a wealth of other potential functions, pyocyanin production was recently found to stimulate upregulation of multiple efflux pump systems, which means cells are better defended against a range of antibiotics[144].

[H1] Evolution of defences

How did bacteria acquire their impressive defensive functions? At a fundamental level, the evolution of biological functions ('adaptation' in evolutionary biology) is driven by natural selection acting on variation[145]. In bacteria, two key processes generate the variation upon which natural selection depends. Mutation, stemming from DNA replication error or chromosomal rearrangements[146], generates raw genetic sequence variation, and **horizontal gene transfer** (HGT) **[G]** adds further variation by mixing alleles and genes among different cells[147]. Phages, competitors and predators can then generate natural selection and favour bacterial variants with improved defences. In this section, we discuss how these processes enable the evolution of defensive traits, before examining how this impacts bacterial genomes (Figure 4).

[H2] Evolutionary processes.

[H3] Mutations and other genetic changes. Compared with larger organisms, mutational variation often arises quickly in bacteria because of their short generation times and large population sizes[148], which can enable the rapid emergence of protective phenotypes. Simple point mutations can drastically reduce toxin-binding affinities of their targets, generating resistance to antibiotics[149], bacteriocins[150] and phages[151] (Figure 4a). Minor changes in regulatory genes can also provide protection against harmful agents. For example, inactivation of a repressor gene (*ramR*) in *S. Typhimurium* results in over-expression of the AcrAB efflux pump, conferring resistance to diverse quinolones, phenicol, and tetracycline antibiotics[152]. Likewise, alterations to regulators of lipopolysaccharide[153] and cell wall synthesis[154] have been shown to generate resistance to bacteriocins, antibiotics and phages. Mutation rates can also increase in times of stress[155], or at low cell density[156], potentially accelerating defensive adaptation[157].

[H3] Horizontal gene transfer. Bacteria can also acquire new defensive genes from other microorganisms via conjugation, natural transformation and transduction[158] (Figure 4a). These HGT events have a central role in bacterial evolution[159], and seem to be particularly important for defence evolution[160]. Importantly, HGT can provide a suite of new genes to a recipient cell in a single step[159], which confers a complex protective phenotype much faster than would be possible through mutation alone. In parallel, HGT can rapidly generate novel and beneficial combinations of alleles via recombination[161]. HGT has facilitated the spread of defences against bacterial, viral and eukaryotic threats. Resistance to antibiotics is often conferred by plasmids[162] and integrative conjugative elements[163]. Other antibacterial weapons and their cognate defences, including bacteriocins[19], T6SS[51] and Cdi[164] systems, are frequently encoded on mobile elements, such that bacteria can gain both resistance and potentially counterattack capability through HGT. Many phage protection systems are also extensively shared via HGT[165–167]. Though less well-documented, anti-predator toxins can be acquired in the same manner: the biosynthetic operon for the toxin pyrrolnitrin seems to be mobile[168], and confers protection against protozoa to various Gram-negative bacteria[169].

[H3] Natural selection and genetic drift. Natural selection can act on the genetic variation generated by mutation and HGT whenever a threat affects survival and reproduction, and so bacterial fitness. In some situations, low population sizes can introduce stochastic changes in the frequency of a given genotype, which can limit defence evolution via genetic drift and related processes[170]. Nevertheless, the potential strength of natural selection for bacterial defences is made clear by evolutionary experiments with competitors, phage and predators,

where the rapid evolution of defences has been observed[154,171–173]. This potential is further underlined by the current antimicrobial resistance crisis: the widespread use of antimicrobials by humans has created concerted selection for drug-resistant bacteria, making previously treatable infections deadly.

However, even when a particular defence is under strong natural selection, it may not lead to the fixation of a given genotype. The utility of some defensive genes can diminish as they become more common (frequency-dependent selection[172]). For example, variability in O-antigen composition of a pathogen is thought to be driven by frequency-dependent selection for evasion of host immune cells[174], intestinal protozoa[175] or phages[176], as rarer genotypes can have an advantage if they are less likely to be recognised. In other cases, **pleiotropy [G]** can limit, or enhance, selection for defensive attributes[177]. Many defensive adaptations have secondary phenotypic effects that are subject to evolutionary trade-offs (antagonistic pleiotropy). For instance, bacteria that gain resistance to a lytic phage might suffer enhanced susceptibility to another[172]. Alternatively, resistance to one threat might also enhance protection to another (synergistic pleiotropy, also referred to as a 'trade-up')[177,178]. Moreover, even strong trade-offs can be insufficient to drive the loss of a defensive adaptation. Compensatory mutations can substantially reduce the fitness costs of defensive genes, which enables them to persist even in the absence of a threat[158]. This has worrying consequences for the long-term maintenance of antibiotic resistance genes: once a bacterium gains resistance, it may not easily lose it[179].

[H2] Evolutionary consequences.

[H3] Genomic organisation of defences. The evolution of defences can have major impacts on bacterial genomes. Across diverse environments and lifestyles, genomes are replete with genes that encode defensive functions[180]. These genes are often clustered together in specialised repositories (Figure 4b–d), each encoding protection against a particular class of threat. Perhaps best-known are bacterial 'defence islands': these mosaic-like chromosomal regions are enriched in diverse anti-viral defences, and have been the source of multiple recent defence system discoveries[54,58,109]. In addition to antiviral genes, bacteria retain clusters of toxin immunity and detoxification genes for use during anti-competitor warfare. Examples include the recently-discovered antagonism resistance (arc1-3) clusters in *P. aeruginosa*[132], and the orphan immunity gene libraries (dubbed 'acquired interbacterial defence' (AID) arrays) widely found among human gut *Bacteroides* species[50,51] (Figure 4b).

Some clusters acquire new defensive genes in a highly ordered manner. Many of the AID immunity genes seem to be actively captured via recombinases, which enables gut bacteria

to expand into niches occupied by aggressive competitors[51]. CRISPR spacer libraries can likewise be regarded as gene capture systems, which generate arrays of phage DNA templates that guard against future infections[56] (Figure 4c). Integrons, which are ancient DNA-scavenging machines that capture mobile gene cassettes[181], commonly confer antibiotic resistance, and are another example of active defence acquisition (Figure 4d). Multi-resistance integrons (MRIs) that contain up to eight resistance cassettes have been reported[182], and super-integrons with >200 cassettes are also known[183]. Integron gene expression is triggered by cellular stress, and bacteria also seem to alter the expression of different integron genes by shuffling their order[184].

Some defences are always found in a given species (that is; they form part of its core genome). Core defences include the outer membrane of Gram-negative bacteria (thought to be an adaptation to ancient antibiotic warfare[59]), some restriction–modification systems[185], and multi-drug efflux pumps[186]. However, many defence genes are found in the accessory genome, and are a major contributor to intraspecific variation among bacteria[187–189]. Indeed, the content of the accessory genome can be overwhelming defensive[160]: in certain marine bacteria, anti-phage systems represent >90% of all accessory genes[190].

[H3] The impact of selfish genes. The beneficial acquisition of new defensive capacities through HGT can occur as a by-product of the infectious actions of mobile genetic elements[159]. This can blur the lines of what can be considered a 'bacterial' defensive adaptation: a mobile element may be the primary recipient of the benefit of the defensive system[167]. Consider superinfection exclusion, whereby phage infection of a bacterium prevents similar phages from infecting the same cell. While this may benefit the bacterium, superinfection exclusion presumably evolved due to benefits to the infecting phage, which then avoids competing with other phages for the hosts' resources[191]. In a similar vein: some anti-phage or anti-plasmid systems may have first evolved not in bacterial chromosomes, but in mobile genetic elements, either as adaptations to fend off competing genetic parasites (using, for example, CRISPR and restriction-modification systems[167]), or as systems to ensure their own maintenance during host replication (for instance, some toxin–antitoxin modules[192]). Nevertheless, even if defence genes did not originate as bacterial adaptations, bacteria may still benefit from inter-parasite conflict, or come to integrate and exploit selfish genes for their own ends. For example, CRISPR–Cas systems are often now part of the bacterial chromosome, and are no longer under the direct control of mobile elements[193].

[H1] Overcoming bacterial defences

Bacterial defences have the potential to coevolve with the offensive strategies of their aggressors. A new defence mechanism can generate natural selection on attackers for **countermeasures [G]** , examples of which are shown in Figure 5. Countermeasures may precipitate an evolutionary arms race, whereby attackers and defenders become progressively better-adapted to defeat each other[194]. However, such escalation is only one possibility; coevolutionary dynamics can also be cyclical, which may facilitate the coexistence of many different types of attack and defence strategy[195]. Coevolution can also be short-lived if antagonists diverge to the point of non-interaction: for instance, if a phage switches host preference away from a focal bacterium[196]. Alternatively, a defender might simply develop such a strong defence that an attacker is tolerated[36] or driven to extinction[197]. Whichever the trajectory it takes, the coevolution of attack and defence, measure and countermeasure, seems to be a major driver of bacterial diversity[198].

[H2] Bacterial competitor countermeasures.

Consistent with the prevalence of inter-bacterial warfare[2,4,5], bacteria have numerous adaptations for thwarting the defences of competitors. One solution to the evolution of resistance is for an attacker to innovate new toxins; this selects for attackers with novel toxins, driving diversification of bacterial weapons[199,200]. Resistant targets may simply select for attackers that produce more toxin[173], or for those that secrete cocktails of multiple toxins (Figure 5a). Of 102 bacteriocin-producing faecal *E. coli* isolates surveyed in a study in 2006, the majority (58%) produced two or more different bacteriocins[201]; similarly, *P. aeruginosa* releases multiple tailocins and other bacteriocins simultaneously[202]. A diverse cocktail of toxins may also maintain lethal function over a wider range of environmental conditions, and can benefit from synergistic interactions between toxins[21]. Mirroring antibiotic combination therapy, toxin cocktails may also make resistance less likely to evolve in the first place[203] (Box 1).

A more sophisticated countermeasure is to directly inhibit a defensive mechanism, thereby negating resistance to a particular attack (Figure 5a). The adjuvant Clavulanic acid, which inhibits □-lactamase enzymes, functions in this way: the soil bacterium *Streptomyces clavuligerus* co-regulates clavulanic acid production with the synthesis of the antibiotic cephamycin C, to destroy □-lactamase-protected competitors[204]. A related approach is to deploy efflux pump inhibitors[205] that limit the ability of target bacteria to remove toxins from the cell – another adjuvant countermeasure used in combination with antibiotic therapy[206].

Attackers have also evolved ways of surmounting barriers to cell entry (Figure 5b). For example, some bacteria produce ‘Trojan Horse’ toxins called sideromycins[207], which comprise

an antibiotic covalently attached to a siderophore molecule. Siderophores are used by cells to scavenge iron and are imported via dedicated receptors, which enables sideromycins to enter the cell and deliver their antibiotic cargo via the same route[208]. Some bacterial weapons take a more direct route to toxin translocation: the bacterial T6SS physically punctures target cells, conveying toxins into the target cell without the need to rely upon specific surface receptors or transporter machinery. This direct approach to toxin delivery affords the T6SS a very broad range of target organisms, spanning both Gram-negative and Gram-positive bacteria, fungal cells and other eukaryotes[209]. Finally, attackers can thwart collective defences (Figure 5c), using proteases and surfactants to disperse biofilm-dwelling bacteria[210], and quorum-quenching molecules to disrupt intercellular signalling and collective responses, including biofilm formation[211]. Additionally, attackers can avoid mass retaliation by deploying 'silent' toxins that are poorly detected by stress responses, thereby suppressing alarm signalling[101].

[H2] Phage and predator countermeasures.

Phages have a well-described set of counter-adaptions that enable them to bypass bacterial defences[212]. These adaptations include counter-modification of phage tail fibres, enabling binding of modified cell surface receptors[213], and epigenetic modification of phage DNA to mimic the host DNA, thereby escaping degradation via restriction–modification systems[214]. Similarly, defence against restriction (Dar) proteins, injected into hosts by coliphage P1, mask the recognition sites used by restriction enzymes[215]. Some phages encode anti-CRISPR proteins that bind to and inhibit CRISPR–Cas complexes[216]; others boast tail sections with hydrolytic domains, which enables them to penetrate the thick polysaccharide capsules of host cells[217]. Phages also have evolved ways of bypassing bacterial abortive infection mechanisms, thus preventing hosts from interrupting construction of progeny phage[102]. For example, coliphage T4 encodes Dmd, an antitoxin 'mimic' that disarms suicide toxins during infection[218]. Finally, paralleling bacterial quorum sensing, some phages use their own 'arbitrium' peptide signal to assess local phage density, transitioning from lytic to lysogenic lifestyles when uninfected hosts become scarce[219]. While not a counter-measure *per se*, this example underlines the sophistication of the responses of phages to their hosts. Meanwhile: though less well-studied, predator adaptations to bacterial defences are also known[220]. These include countermeasures to overcome toxin production by prey: mirroring *P. aeruginosa,* the free-living amoeba *Acanthamoeba castellanii* has modified cytochrome oxidases, which enable it to tolerate prey-produced cyanide[169]. Some eukaryote predators may also be able to suppress toxin production by prey[169], including via quorum quenching mechanisms[221].

[H1] Conclusion

Bacteria have evolved a wide range of defensive adaptations that can make them difficult to kill. Knowledge of these defences has already driven technological revolutions in microbiology and beyond, providing researchers with new tools (restriction enzymes[53], CRISPR gene-editing[56,222] and DNAi/RNAi silencing[58]) and therapeutic approaches (novel antivirals[223], antimicrobials[224] and biotherapeutics[225]). In addition to these applications, defence systems are also central to understanding bacterial biology: they are deeply integrated into their core regulatory networks[79,81,123], and can determine which species will persist in a given environment[4,51,94,172]. Some defences protect only against a particular threat, but many are general and protect against a range of attacks[144,154,226]. Still others alter bacterial virulence[120,121,186], with the potential to exacerbate disease transmission and severity.

These are indeed exciting times for the study of bacterial defences. Spearheaded by bioinformatic[165] and high-throughput[227] approaches, the staggering diversity of bacteria has become clear and with this, the myriad ways they can defend themselves. The past 5 years alone have seen an explosion in the number of novel anti-phage systems identified in bacterial defence islands[104,109,228] (>50 since 2018), with many more likely awaiting discovery. The diversity and spread of these anti-phage systems highlights how little, in comparison, we know of anti-competitor and anti-predator defences. What might these same approaches teach us about bacterial adaptations against ever-present predator or competitor threats? Early signs are promising: as with the bountiful phage defence islands, anti-competitor defence genes also form clusters in bacterial genomes[51,132,184]; mining these might therefore reveal novel routes through which bacteria evade rivals' attacks.

As well as examining survival mechanisms, we must understand their broader impact within microbial communities, and the conditions and pathways that trigger them. A major current goal is to control bacteria and their communities, both ecologically and evolutionarily[3,229,230]. Replacing a pathogen in a community with a biotherapeutic strain[231], for example, will require us to understand both the attack and defence strategies of bacteria[5,10]. And whenever we attempt to eliminate bacteria, whether via antibiotics or one of the emerging alternatives, there is the potential for evolution[9]. As for antibiotic resistance evolution, therefore, the study of how bacterial defences evolve in nature and in the clinic is an important topic for the future.

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Author contributions

W.P.J.S. researched data for article. W.P.J.S., K.R.F., B.R.W., and C.D.N. contributed substantially to the discussion of content. W.P.J.S. and K.R.F. wrote the article. W.P.J.S., B.R.W., C.D.N., and K.R.F. reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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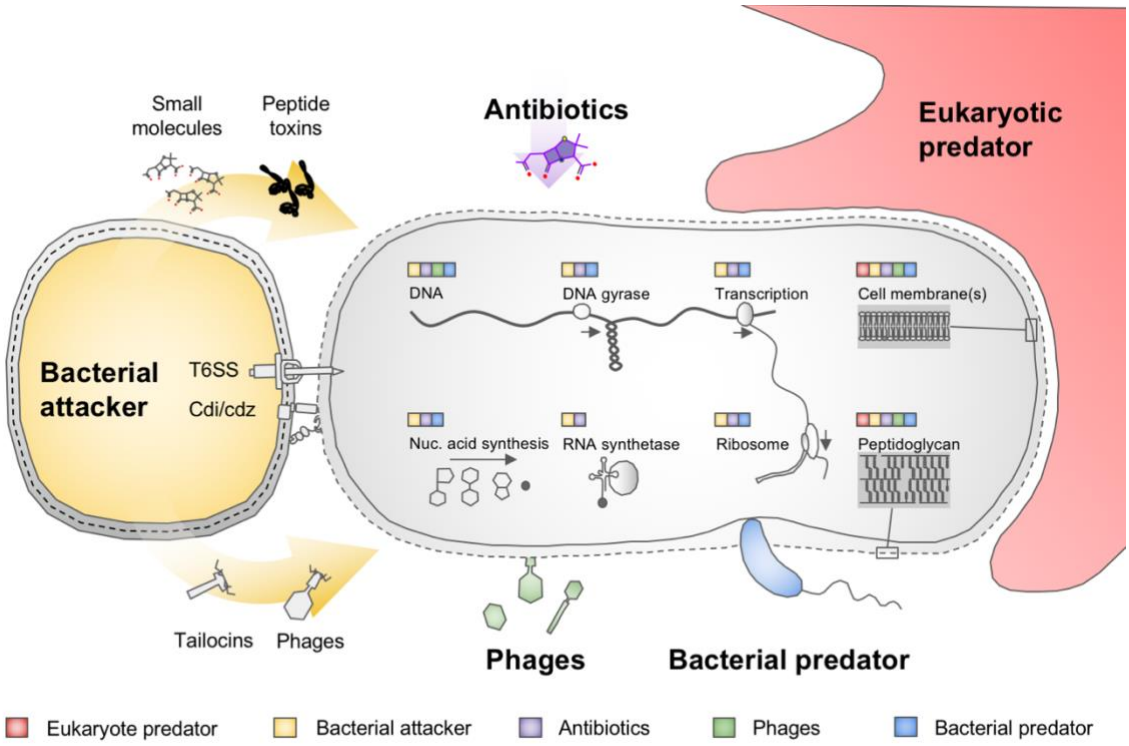

Figure 1. Bacteria face diverse threats from competitors, viruses and predators. Most attacks target select core cellular processes and functions of the bacterial target cell. Coloured squares indicate whether a given threat type typically acts on a particular target. Bacterial competitors antagonise a target bacterium via diverse mechanisms, including both contact-dependent weaponry (the type VI secretion system (T6SS); Cdi effectors) and diffusible weaponry (small molecules, peptide toxins, and tailocins). The majority of clinical antibiotics are also derived from bacteria and other microorganisms. Following infection of a bacterial cell, phages attack cell walls and membranes to release their progeny via cell lysis. Some bacterial predators, such as *Bdellovibrio* species and like organisms (BALOs), invade the host cell periplasm, injecting toxins that digest various cytoplasmic components. Many eukaryotic predators engulf and digest target bacteria whole in phagosome compartments.

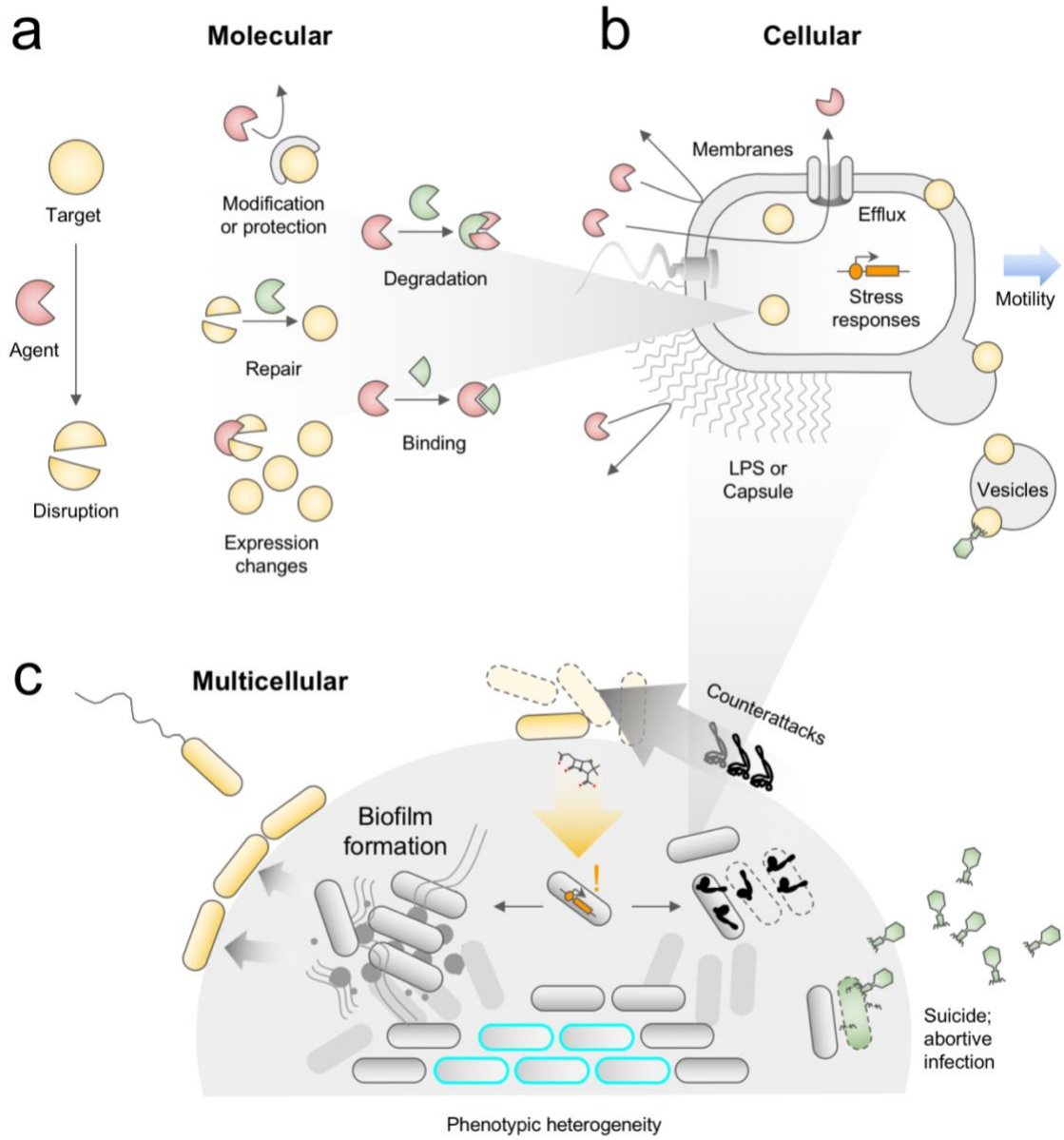

Figure 2. Bacteria have evolved multiple lines of defence against biotic threats. At both the individual and collective level, bacteria draw upon a plethora of defensive adaptations to escape harm. Defences are arranged according to the spatial scale at which they operate. **(a)** *Molecular level*: attacks by competitors, phages and predators are mediated by harmful agents (for example, toxins, enzymes and genetic elements), which disrupt cellular functions by interacting with diverse targets. Bacteria can mitigate disruption at a molecular level, by altering the target or compensating for its disruption, or by destroying or binding the harmful agent. **(b)** *Cellular level*: macromolecular barriers, including cell membranes, S-layers, lipopolysaccharide (LPS) or capsules, prevent harmful agents from entering a bacterial cell. Efflux pumps remove harmful molecules that overcome barriers, and motile bacteria can escape harmful environments by repositioning themselves. Secreted membrane vesicles can bind and inactivate toxins and phages. Stress responses and other regulatory pathways enable these defences to be activated in response to specific or general threat cues. **(c)** *Multicellular level*: bacteria also create collective barriers (production of extracellular polymeric substances (EPS); biofilm formation) or resistant subpopulations (phenotypic heterogeneity), launch en-masse counterattacks, and, in some circumstances (e.g. abortive infection), commit suicide to protect kin cells.

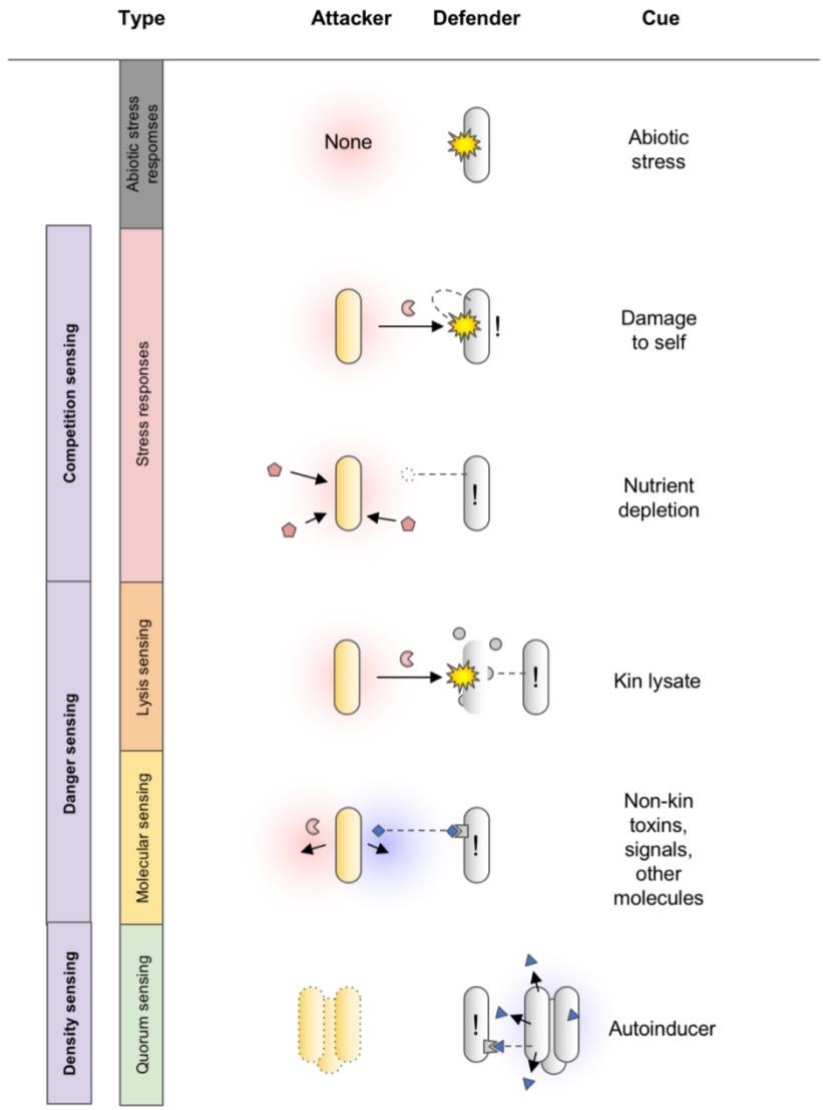

Figure 3. Bacteria mount defences in response to diverse cues. Examples are ordered according to the proximity of potential harm, and grouped according to type. Some cues emanate from direct harm to a focal cell (harm from abiotic stressors; nutrient depletion or attacks by competitors); bacteria identify and distinguish these cues via competition sensing, and respond defensively. Bacteria can also respond to attacks before they themselves are harmed, activating defences in response to danger cues (kin lysate, non-kin toxins, signals and other molecular attacker signatures). Bacteria also use autoinducer-mediated quorum sensing, and other density-sensing mechanisms, to raise defences in anticipation of attacks.

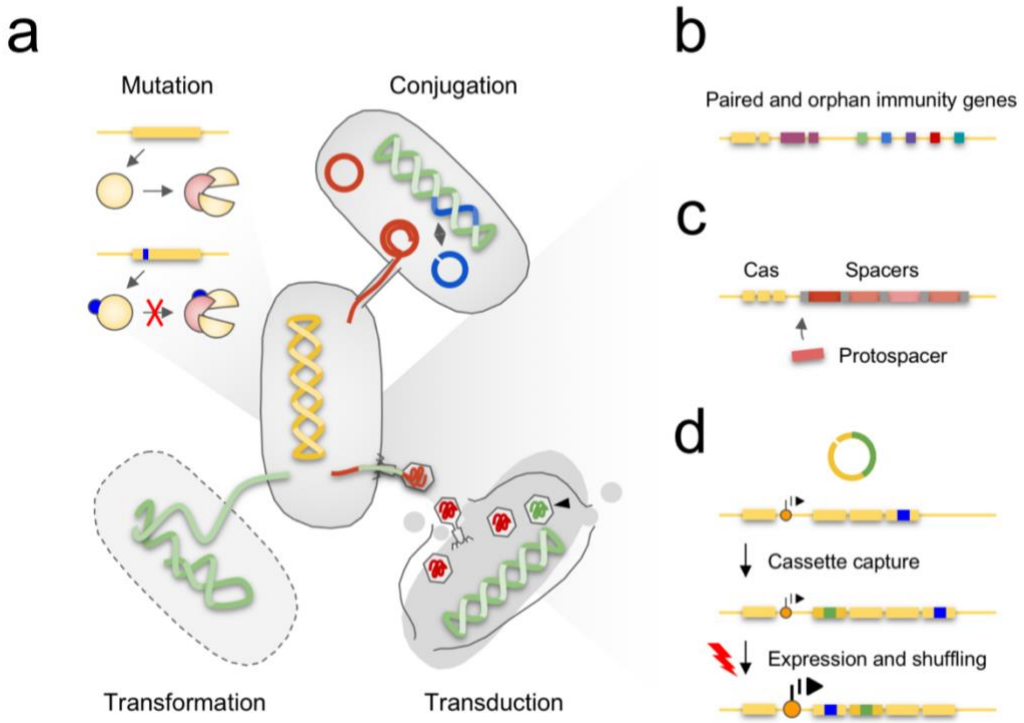

Figure 4: Bacteria innovate, acquire and accumulate defences. **(a)** Random mutations alter bacterial susceptibility to threats (for example, via modification of target structures), occasionally conferring survival benefits. Bacteria may also acquire new defence genes via horizontal gene transfer: conjugation, natural transformation and phage transduction. **(b)** Bacteria accumulate toxin-immunity pairs and orphan immunity genes in their genomes, protecting them against the cognate toxins of both kin and competitor cells. **(c)** CRISPR–Cas systems remember past infections by storing phage and plasmid DNA samples in spacer libraries. **(d)** Gene cassettes encoding antibiotic resistance and other defensive functions are captured by integrons via site-specific recombination. Stress cues (lightning bolt) stimulate expression of captured genes; stress-induced integrases also shuffle cassettes, resulting in diverse gene expression profiles within a population.

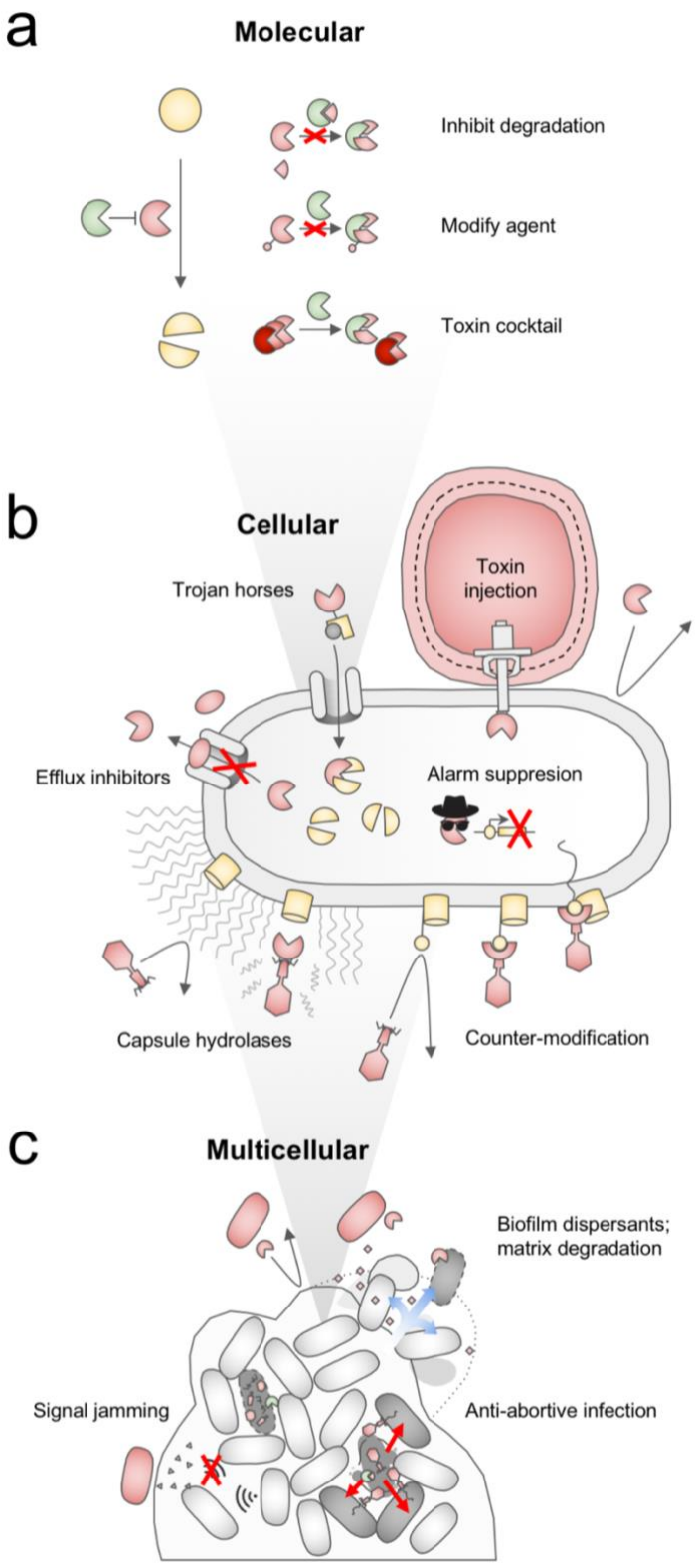

Figure 5: Counter-adaptations to bacterial defences by competitors, phage and predators. (a) Attackers prevent degradation of their toxins (or DNA, in the case of phages) using adjuvants to inhibit defence enzyme function, or by modifying toxin structure. Toxin cocktails may offer toxin synergy and delay resistance evolution. **(b)** Competitors bypass the membranes of their target cells using toxin injection systems (the type VI secretion system (T6SS)) or by disguising toxins as useful substrates ('Trojan horses'). Some toxins kill without

triggering key stress responses, suppressing defensive behaviour ('Alarm suppression'). Efflux pump inhibitors prevent expulsion of absorbed molecular toxins. Phages penetrate cell capsules using tail-mounted hydrolases, and adapt to alterations in host receptor structure via counter-modification or stochastic expression of receptor-binding proteins. **(c)** Competitors degrade biofilms using dispersants and matrix hydrolases, and inhibit response coordination using quorum quenching. Phages override collective immunity by bypassing abortive infection mechanisms, using hijacked or surrogate immunity proteins to disarm suicide systems.

Box 1. Clinical implications of ancient bacterial defences

The study of bacterial defences can inform current and future antibacterial therapeutics.

[bH1] Origins of drug resistance. Understanding where resistance genes come from can help to predict and restrict antibiotic resistance proliferation[232]. Environmental reservoirs harbour many old and diverse resistance genes[233]. For example, the methicillin-resistance genes found in methicillin-resistant *Staphylococcus aureus* (MRSA) seem to have first emerged in hedgehog-associated *Staphylococcus aureus,* as a protection against fungal □-lactam antibiotics[39]. More generally, toxin-mediated competition among environmental bacteria is widespread[234], and, along with phages and predators[41,61], can select for defences that increase virulence[120,121,186] or protect bacteria against multiple different threats[144,154,226]. Studying and surveying bacterial defences in environments with strong competition and conflict, therefore, may help to predict which resistance mechanisms are most likely to arise.

[bH1] New strategies against resistance. Many bacteria use antimicrobials to eliminate competitors[10], which suggests they are often able to overcome the defences of their targets. We might look to bacteria, therefore, for strategies that help to overcome drug resistance. Evidence supporting this idea comes from the use of adjuvant therapy: *Streptomyces clavuligerus* produces clavulanic acid, which inhibits □-lactamase-based resistance mechanisms[204]. This strategy forms the basis for *Augmentin*, a therapeutic that uses both a □-lactam antibiotic and clavulanic acid to combat □-lactamase-based resistance[235]. Another feature of bacterial attack strategies is that they commonly use multiple different toxins against competitors[10,201,236]. This contrasts with classic mono-therapy, which remains the clinical norm, but draws comparisons to a growing number of strategies that combine multiple antibiotics with the goal of limiting resistance evolution[237–239]. In addition, many bacterial toxins are polymorphic, with a modular structure that enables new variants to be readily innovated as resistance emerges[240]. Adopting modular designs when developing new antimicrobials could enable us to exploit this adaptability[241].

[bH1] Targeting defences. The defensive responses of bacteria[79,81,123] can increase virulence and protect against antimicrobial treatment, thereby exacerbating disease[81,242,243]. Directly targeting defensive mechanisms, therefore, has the potential to greatly improve treatment efficacy when performed in combination with antibiotics or other bactericidal treatments. Diverse bacteria respond to antibiotic treatment by forming biofilms, which are notoriously difficult to treat[80]. However, physical disruption of biofilm structures can increase bacterial exposure to antibiotics, sensitising recalcitrant infections[244]. Targeting defences also raises the possibility of treatments with a minimised risk of resistance evolution. Biofilm inhibitors can enhance antibiotic susceptibility while minimising resistance to the biofilm inhibitor, because resistant genotypes pay the fitness costs of EPS production[245]. A related defence-targeting strategy is to introduce strains of bacteria that do not contribute to collective defences ('cheat therapy')[246,247]. Where cheater strains can outcompete the original strain, they have the potential to undermine defences and improve treatment outcomes without strong natural selection for resistance evolution.

[bH1] Exploiting novel antimicrobials. Phages[248], predators[249] and competing bacteria[5,224] all have potential as alternative therapeutics for bacterial infections[225]. As we have discussed, however, bacteria have already evolved many defences against these threats. As with antibiotics, therefore, the rapid emergence of resistance in clinical settings seems to be likely[9,250]. But these alternative antimicrobials share a potential major advantage over antibiotics: being biological, they have the potential to coevolve with their targets, such that resistance in a target is circumvented by countermeasures in the attacker. Although this outcome is far from guaranteed (it requires, amongst other things, that the survival of the therapeutic depends on defeating the target pathogen), it raises the possibility that evolution can be directed to overcome pathogen resistance as it emerges. Moreover, by combining therapies, one can exert contrasting selective pressures on pathogens, which may limit resistance evolution more than via antibiotic therapy alone[177,251,252].

Glossary of terms

Competitor

Another type of bacteria that competes with a focal bacterium for resources. Often this will be a genetically similar, but non-identical bacterium (for example, a different strain), as similar bacteria are most likely to have overlapping resource needs. Genetically identical organisms compete in an ecological sense, but not in an evolutionary sense (as they have the same evolutionary interests). In this Review, we use the term in the former sense.

Bacteriophage (phage)

A virus that infects bacteria.

Predator

An organism that consumes another for food, killing it in the process.

Defence mechanisms

Traits that evolved, at least in part, to protect an organism against a threat. This term is often used in the context of bacterial defences against viral threats, but in this Review, we expand it to encompass protection against competitors and predators.

Biotherapeutic

Medicine that is derived from (and often incorporating) biological entities. Phages are a potential biotherapeutic for treating bacterial infections.

Preadaptations

Evolutionary adaptation which serves a different purpose from the one for which it first evolved. For instance, many modern efflux pumps function to remove antibiotics from bacterial cells, but homologous structures likely served different functions (e.g. metabolite export) in ancestral strains.

Stressors

Changes in environmental or physiological conditions that perturb cell homeostasis.

Weaponry

Cellular systems that evolved, at least in part, to harm other organisms.

Parasitism

An evolutionary relationship between two organisms, in which one benefits at the expense of the other. In contrast to predators, parasites are generally smaller than and physically associated with the organisms they exploit.

Mutualism

A mutually beneficial evolutionary relationship between two organisms; that is, one in which the fitness of the two parties are both improved by the presence of the other.

Agents

Substances (particularly toxins and injected viral DNA) that, through interaction with targets, produce harm to a bacterial cell.

Biofilms

Densely-packed cell groups that can contain billions or trillions of cells, enveloped by secreted extracellular matrix.

Collective defence

Any defensive behaviour that becomes more effective when many individuals engage in it. Collective defences benefit the social partners of a focal bacterium, but do not always evolve for this reason.

Counterattacks

Aggressions in response to aggression (apparent or actual).

Plastic responses

'Programmed' alterations to bacterial phenotype in response to environmental change. Plasticity does *not* result from genetic change (though it may be genetically encoded).

Stress responses

A set of regulatory pathways found in bacteria, which alter gene expression and cell physiology in response to harmful environmental changes and help the bacteria to survive stress.

Competition sensing

The bacterial behaviour of discerning and responding to stress cues associated with competitor activity, often via stress responses. This is often used to regulate defences, especially counterattacks.

Danger sensing

Conceptually similar to competition sensing, but pertaining to cues other than those resulting from direct harm to a focal cell.

Exploitation competition

Mutually harmful interactions between bacteria, stemming from competition for contested resources (for example, space or nutrients). Contrasts with interference competition, where harm is inflicted directly via weaponry or other means.

Quorum sensing

A widespread density-sensing mechanism found in bacteria and other microbes. Bacteria probe their effective density by secreting small molecules (autoinducers), which stimulate their own production. High autoinducer concentrations then become a proxy for high cell density or for restrictive spatial constraints that limit autoinducer diffusion. Quorum sensing is often used to regulate costly traits whose benefits depend on collective action.

Horizontal gene transfer (HGT)

The flow of genetic information between two organisms, other than that which occurs via reproduction (vertical gene transfer).

Pleiotropy

Phenomenon whereby one gene simultaneously affects multiple traits. Through pleiotropy, a defensive adaptation may affect the phenotype of a bacterium in unexpected ways (for example, reducing its fitness in the absence of a threat).

Table of content:

In this Review, Smith, Foster and colleagues explore the protective strategies of bacteria, including the mechanisms, evolution and clinical implications of these ancient defences. They also review the countermeasures that attackers have evolved to overcome bacterial defences.