ANTIBIOTIC ALTERNATIVES — OPINION

Targeting virulence: can we make evolution-proof drugs?

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Abstract | Antivirulence drugs are a new type of therapeutic drug that target virulence factors, potentially revitalising the drug-development pipeline with new targets. As antivirulence drugs disarm the pathogen, rather than kill or halt pathogen growth, it has been hypothesized that they will generate much weaker selection for resistance than traditional antibiotics. However, recent studies have shown that mechanisms of resistance to antivirulence drugs exist, seemingly damaging the 'evolution-proof' claim. In this Opinion article, we highlight a crucial distinction between whether resistance can emerge and whether it will spread to a high frequency under drug selection. We argue that selection for resistance can be reduced, or even reversed, using appropriate combinations of target and treatment environment, opening a path towards the development of evolutionarily robust novel therapeutics.

It is well established that our current practices of antibiotic use are unsustainable owing to the spread of antibiotic-resistant pathogens[1]. Resistance mechanisms are readily acquired both by *de novo* mutation[2] and by horizontal gene transfer from environmental reservoirs[3,4]. Viable resistance mechanisms have even been shown for therapeutics such as vancomycin and cationic antimicrobial peptides, for which resistance was once thought to be impossible[4,5].

If an antibiotic kills or inhibits the growth of sensitive strains, this will enable any resistant strains to grow in a competitor-free environment, creating strong selection for antibiotic resistance mechanisms[6]. Although resistance is often initially 'costly' to the pathogen, secondary mutations that ameliorate this cost quickly spread so that the frequency of resistance does not decline when antibiotic use is reduced[7]. For example, mutations in *rspL* (which encodes ribosomal protein S12) that confer streptomycin resistance in *Escherichia coli* impose costs by slowing peptide elongation[8]. However, secondary mutations in *rpsD* and *rpsE* (which encode ribosomal proteins S4 and S5, respectively) increase the rate of elongation, removing the cost of resistance[8,9]. The rapid spread of resistance means that the clinical lifespans of antibiotics are short, which reduces profits, and therefore incentives for the development of novel antibiotics, thus compounding the issue of resistance[10].

So, what can be done when the very action of antibiotics strongly selects for resistance? Rather than kill or halt bacterial growth, one emerging strategy is to 'disarm' pathogens[11,12] by directly targeting virulence using antivirulence drugs (BOX 1). As antivirulence drugs are not designed to directly harm their targets, several papers have argued that they will have little effect on the fitness (that is, the net growth rate) of the pathogen in the host[11,12] and therefore approach the ideal of an 'evolution-proof' drug that does not impose selection for resistance. Resistance to antibiotics is commonly defined and quantified as the recovery of population growth following antibiotic exposure[4]. However, as we show below, there is often a considerable disconnect between bacterial growth and the expression of virulence factors (FIG. 1), and therefore, a definition of resistance that is expressed purely in terms of growth recovery will not suffice for resistance to antivirulence drugs. Therefore, in this Opinion article, we define resistance to an antivirulence drug as the recovery of virulence factor expression following antivirulence drug treatment.

On first examination, the hypothesis that antivirulence drugs are evolution-proof clearly seems to be false, as resistance has already been reported in several cases. Resistant strains have been isolated in clinical settings[13,14] and have been generated in laboratory systems[14–16]. For example, the inhibitory effect of the salicylidene acylhydrazide drug B81-2 (BOX 1) on type IV secretion system formation was diminished in several mutants that were identified by directed mutagenesis of the target protein VirB8, showing that mechanisms of resistance are available to selection[16]. This and the other examples that are discussed below have led to suggestions that resistance will hinder the clinical efficacy of antivirulence drugs[17,18]. However, the existence of mechanisms of resistance does not necessarily mean that this resistance will spread and become a clinical problem[19].

In this Opinion article, we highlight a crucial distinction between whether potential mechanisms of resistance exist (a question of mechanism) and whether potential mechanisms of resistance will spread to a high frequency in treated populations (a question of selection). The observed ubiquity of resistance mechanisms in natural populations[13,14,17,20] suggests that it is the question of selection that is most crucial, as it is selection that governs the persistence and spread of any potential resistance mechanism. Given the inevitability of resistance mechanisms, will they spread in the event of the widespread use of antivirulence drugs? What can we do to mitigate the spread of resistance to antivirulence drugs? To understand these questions, we must first consider the consequences of virulence-factor expression for pathogen fitness or, more colloquially, ask: what is virulence for?

Why be virulent?

The evolution of virulence (that is, pathogen-induced host damage) is a major puzzle in evolutionary biology and has generated a range of responses to the underlying theoretical question: why harm the source of your livelihood — your host[21]? The dominant hypothesis states that virulence is an unavoidable cost or side effect of growing within a host and transmitting to the next host, and is maintained as the result of a trade-off between the costs of host pathology and the benefits of transmission to a new host[21,22]. Other hypotheses highlight the importance of selection in non-disease settings, where alternative functions of virulence factors can coincidentally select for virulence factor-induced damage to human hosts[23,24] (FIG. 1; and discussed in the following section).

We argue that uncovering the selective forces that maintain the carriage and expression of a virulence factor is vital to understanding the selective pressures that affect resistance to an antivirulence drug targeting that virulence factor. The identification of virulence factors classically involves a simple screen for non-essential genes that

Box 1 | **How can we target virulence?**

Virulence factors are molecular determinants of virulence; they are pathogen components that are non-essential to *in vitro* growth in rich media but cause increased virulence during infection of a host[25]. Virulence factors are the key target of antivirulence drugs (including antibodies and enzymes that are not small molecules). Several antivirulence drugs and their targets are listed below (for more exhaustive lists see REFS 12,94).

- Bicyclic 2-pyridones bind to the PapC and FimH chaperones, which prevents the interaction of the chaperone–pilus subunit complex with the usher, inhibiting pilus formation in *Escherichia coli*[32].
- Virstatin inhibits the expression of cholera toxin and the toxin co-regulated pilus in *Vibrio cholerae*[15].
- 2-imino-5-arylidene thiazolidinone inhibits type II and type III secretion systems in a wide range of Gram-negative pathogens, probably owing to an effect on the conserved secretin protein that is involved in both processes[91].
- B81-2 is a salicylidene acylhydrazide molecule that impedes VirB8 dimerization in *Brucella abortus* and thus inhibits type IV secretion[16]. Similar salicylidene acylhydrazide compounds inhibit type III secretion in several other pathogens[95].
- Urtoxazumab is one of many antitoxin antibodies; it is in clinical trials as an inhibitor of Shiga toxin function in enterohaemorrhagic *E. coli*[87,96].
- Phosphonosulphonates inhibit dehydrosqualene synthase (CrtM), preventing the biosynthesis of staphyloxanthin, which is a golden pigment that protects *Staphylococcus aureus* from reactive oxygen species[42]. Phosphonosulphonates are one of several antivirulence drugs that are repurposed from existing drugs, which reduces development time and costs.
- AiiA enzyme is a lactonase that was isolated from *Bacillus* species; it degrades the lactone bond of acyl homoserine lactone (AHL) molecules, which are used as quorum-sensing signals[97].
- BuT DADMe-ImmA is a transition-state analogue that inhibits the 5′-methylthioadenosine nucleosidase (MtaN) enzyme, preventing the synthesis of quorum-sensing signals in *V. cholerae* and *E. coli*[74].
- C-30, which is a derivative of natural furanone compounds, targets the LasR receptor in *Pseudomonas aeruginosa*[98]; it is one of many inhibitors that target the signal–receptor complex.

are predictive of damage in a model host system[25] (FIG. 1a). Evidence for virulence factors being 'non-essential' is inevitably found in rich *in vitro* growth media and is often simply the ability of a mutant to grow to a density that can be used in further assays[26], which differs greatly from the *in vivo* conditions that govern selection for resistance to antivirulence drugs. In this Opinion article, we focus on whether the targeted virulence factor provides a selective advantage to the target pathogen at the *in vivo* site of treatment. In BOX 2 and FIG. 2, we outline a series of predictions for the evolutionary robustness of different antivirulence strategies, which are expanded on below.

Non-beneficial virulence factors

The first scenario is the simplest: the virulence factor has no benefit at the site of infection (that is, the site of colonization and damage in the focal host), just as virulence factors commonly have no benefit in rich media *in vitro* (FIG. 1a). If a virulence factor confers no benefits to a pathogen at the site of infection, then targeting this virulence factor at the site of infection will not impose any within-host selection for resistance. Resistance could even be selected against, as treatment enables the sensitive bacteria to avoid the metabolic costs of inappropriate virulence factor expression and potentially reduced transmission from an ill host (BOX 2; FIG. 2). Although the logic is clear, the empirical question of whether there are virulence factors that offer no fitness benefit to a pathogen at the site of infection is more open to debate.

Coincidental virulence factors. The best candidates for non-beneficial virulence factors are found in opportunistic pathogens that normally exploit distinct environments (for example, non-human environments or commensal compartments within human hosts[24]), with virulence factors that are the products of coincidental selection in these environments[23,24] (FIG. 1b). Potential candidates can be found among the extraintestinal pathogenic *E. coli* (ExPEC) strains (such as uropathogenic and meningitis-associated *E. coli*). ExPEC strains are opportunistic pathogens that are frequently isolated from healthy intestinal microbiota but also cause various diseases in distinct extraintestinal sites, such as the brain or urinary tract, which are associated with poor transmission compared with the intestines[27].

ExPEC-associated virulence factors, including adhesins (such as P pili) and iron-aquisition factors (such as yersiniabactin)[28,29], are associated with persistence as a commensal microorganism in the intestines. Outer membrane protein A (OmpA) and lipopolysaccharide have also been shown to be beneficial for interactions with amoebae in the environment[30]. Although these factors are associated with virulence, in an extraintestinal mouse model they conferred no measurable benefits in the presence of various biological stressors[29]. Furthermore, phylogenetic analyses suggest that coincidental selection among commensal microorganisms in the intestines — and not direct selection for virulence outside the intestines — is responsible for the maintenance of these virulence factors[31]. The biogenesis of P pili in ExPEC strains is targeted by bicyclic 2-pyridones[32] (BOX 1); however, the selective consequences of bicyclic 2-pyridones will depend on the environment in which they are used. If treatment specifically targets the urinary tract, we would predict that resistance would not be selected for but that pathology would be reduced. However, in the more likely case of systemic treatment (including both the urinary tract and the intestines), resistance may be selected for in the intestines, where P pili are reported to confer a selective advantage.

Although various virulence factors have been hypothesized to be selected for outside the human host[23,33], the list of candidate 'locally non-beneficial' virulence factors is currently very short. We think that this is mostly due to a lack of research focus on mapping the costs and benefits of virulence-factor expression to pathogens at the site of infection (for exceptions, see the plant pathogen literature[34]). We suggest that pathogen fitness is an overlooked quantity; future work should seek to identify the fitness costs and benefits of virulence-factor expression in relevant host environments.

Beneficial virulence factors

One of the key attributes of antivirulence drugs is that they transform pathogenic populations into a less virulent state rather than clearing pathogens directly, meaning that long courses of antivirulence drugs may be required to maintain a state of reduced virulence. However, several antivirulence drugs have been shown to aid clearance. Furanone inhibitors of quorum sensing (BOX 1) increase immune or antibiotic-associated clearance of *Pseudomonas aeruginosa*, owing to the effects of quorum sensing on

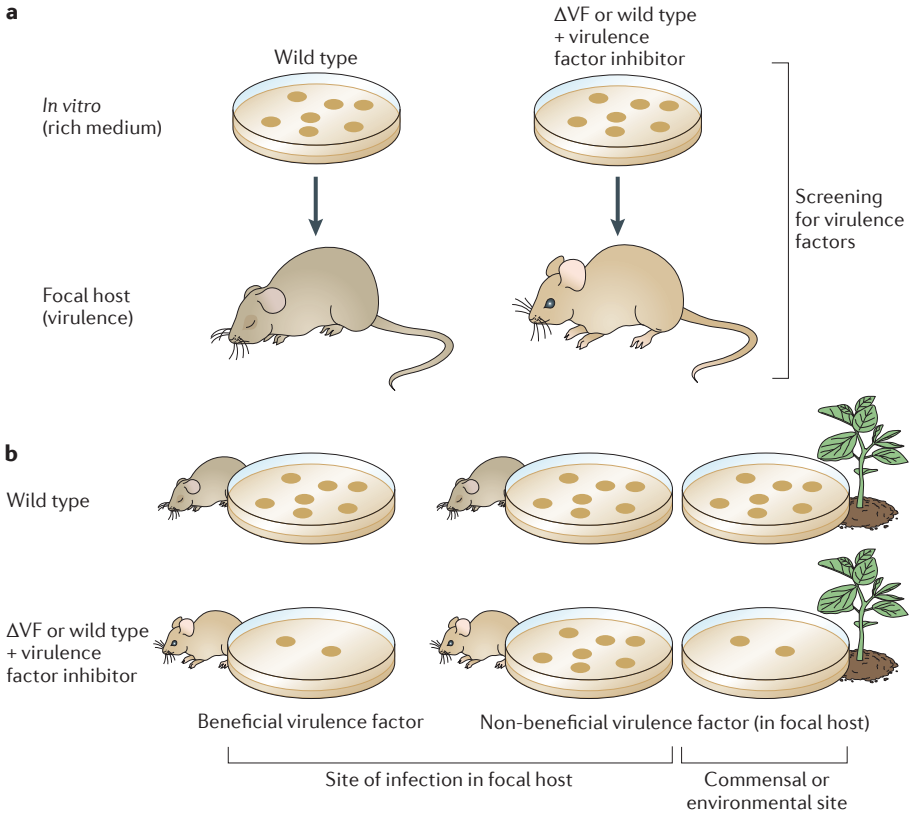


Figure 1 | **The effects of virulence factors on fitness and virulence in different environments.** **a** | Virulence factors are commonly defined as molecular determinants of virulence; they are pathogen components that are non-essential to *in vitro* growth in rich media but cause increased virulence during infection of a host[25]. Importantly, this definition of virulence factors makes no predictions about the consequences of virulence-factor expression for pathogen fitness in the host. The figure shows that a mutant strain in which the virulence factor has been deleted (ΔVF) can have identical fitness to a wild-type strain in rich medium *in vitro* but causes less pathology in a focal host. **b** | The effects of virulence-factor expression on pathogen fitness can be variable, particularly between sites of infection and commensal or environmental sites. The number of colonies represents pathogen fitness (that is, net growth) in the specified environment. If an inhibitor targets a beneficial virulence factor that aids growth at the site of infection, pathogen fitness will be reduced, causing selection for resistance to the inhibitor. If an inhibitor targets a non-beneficial virulence factor that does not aid growth at the site of infection, there will be no effect on pathogen fitness and no selection for resistance at the site of infection.

immune modulation and biofilm formation, respectively[35,36]. Although this renders antivirulence treatment potentially more attractive (particularly as a combination therapy with traditional antibiotics), it also implies that some of the virulence factors that are inhibited by furanone compounds must be beneficial to the pathogen in the host, at least in the context of an intact immune system and/or concurrent antibiotic treatment.

Several virulence factors have now been shown to confer a range of benefits within the host, in addition to the protective roles that are described above. These benefits include providing access to limiting resources[37,38] and enhancing interspecific competitive ability[39,40]. For example, blocking the expression of cholera toxin and the toxin co-regulated pilus in *Vibrio cholerae* using virstatin (BOX 1) has a negligible effect on growth *in vitro* but markedly decreases colonization of an infant mouse model, which indicates that these virulence factors increase fitness within the host[15]. Coupling between virulence-factor expression and pathogen fitness means that antivirulence drug treatment will be detrimental to pathogens. In this case, a resistant mutant will recover fitness, leading to selection for resistance, at least within the host[41] (BOX 2; FIG. 2). In accordance with this expectation, a resistant mutant that has a point mutation in the target of virstatin (*toxT*) outcompeted a susceptible strain in a treated host[15].

Species and environment specificity. Unlike antibiotics, the targets of antivirulence drugs are only likely to be beneficial in specific environments, which may be within or outside the site of infection[29], or indeed, outside the host entirely[33] (FIG. 1b). In addition, the targets of antivirulence drugs are often specific to certain pathogen species (known as narrow-spectrum drugs). Therefore, populations that are outside crucial sites at which a beneficial virulence factor is expressed are unlikely to undergo selection for resistance.

As an example of why the environmental specificity of antivirulence drugs is advantageous, consider the treatment of *Staphylococcus aureus* with phosphonosulphonates[42] (BOX 1). Phosphonosulphonates inhibit CrtM — an enzyme that is responsible for the biosynthesis of staphyloxanthin, which is a golden pigment that protects *S. aureus* from reactive oxygen species (ROS). Susceptible *S. aureus* cells are not directly harmed by the drug, but will be killed by ROS at the site of infection. Phosphonosulphonates will therefore impose strong selection for resistance at the site of infection (even though death is caused by the immune system and not directly by the drug). However, in its commensal lifestyle, *S. aureus* is exposed to relatively minor ROS challenge, and, as a result, *crtM* expression has no effect on nasal colonization[42]. Therefore, populations at the commensal site will not be under strong selection for resistance. This specificity can be viewed as an extension of the principle of narrow-spectrum antibiotics to include environmental specificity as well as species specificity. By restricting the population from which resistance can be selected (compared with antibiotics), environmental specificity will similarly slow the evolution of resistance (by restricting mutational supply[43] and exposure to selection[44]), even for virulence factors that are tightly coupled to fitness at the site of infection (BOX 2).

Even for virulence factors that are strongly beneficial within the site of infection, note that the epidemiological spread of resistance mechanisms that restore virulence-factor expression can be mostly, or completely, halted if the infection site is an epidemiological 'dead end', with transmission instead coming from commensal or environmental populations[45,46].

Collectively beneficial virulence factors

Within the broad class of beneficial virulence factors, an important distinction must be made between virulence factors that

Box 2 | **Key predictions on the direction of selection for resistant strains**

Non-beneficial virulence factors

- Prediction 1: antivirulence drugs will select against resistant strains when the targeted virulence factor confers no benefits to the pathogen at the site of treatment.

Beneficial virulence factors

- Prediction 2a: antivirulence drugs will select for resistant strains when the targeted virulence factor confers direct benefits to the pathogen at the site of treatment.
- Prediction 2b: antivirulence drugs will generate weaker selection for resistance if the target virulence factor is conditionally beneficial and/or conditionally expressed.

Collectively beneficial virulence factors

- Prediction 3a: antivirulence drugs will select against resistant strains when the targeted virulence factor confers collective benefits to a well-mixed population.
- Prediction 3b: antivirulence drugs will select for resistant strains when the targeted virulence factor confers collective benefits to a sufficiently structured population.

Quorum sensing-controlled virulence factors

- Prediction 4: antivirulence drugs that reduce the supply of quorum-sensing signals (for example, signal-degrading enzymes) will generate weak selection for resistance in well-mixed populations.

confer an immediate and private benefit to the (focal) bacterium that expresses the trait (for example, adhesins) and collectively beneficial virulence factors that confer a benefit to a group or neighbourhood of bacteria (for example, secreted siderophores, enzymes and toxins). Many virulence factors belong to the second 'cooperative' category[47], which is characterized by the secretion of costly molecules that scavenge, digest or liberate resources that promote growth[48–51]. From a social-evolution perspective, these secretions are characterized as 'public goods' — which are costly individual contributions to a collectively beneficial enterprise[52]. Theoretical work has shown that targeting collectively beneficial virulence factors can greatly reduce selection for resistance, as public goods can be exploited by neighbours[53].

Exploitation of social behaviours. A widely corroborated result of social-evolution theory is that cooperative behaviours are vulnerable to local exploitation by cheats that do not carry out the cooperative behaviour. For example, in *P. aeruginosa*, non-producers (that is, cheats) of the secreted siderophore pyoverdin avoid paying the metabolic costs of pyoverdin production but are still able to use siderophores that are produced by their cooperative neighbours[54]. As a result, cheats increase in frequency in a well-mixed environment (featuring random interactions — for example, a shaken flask)[52,54,55] and within hosts in animal-infection models[51,56], where this also reduces virulence[51].

The local advantage of cheats over cooperators translates into the prediction that antivirulence drugs targeting collectively beneficial virulence factors will select against resistance in a well-mixed environment[53]. If a drug inhibits a collectively beneficial virulence factor, the susceptible population become phenotypic cheats[57]. These cheats will then socially exploit any resistant individual that can produce the collectively beneficial virulence factor, leading to selection against resistance in mixed populations[57] (BOX 2; FIG. 2). Consistent with this prediction, treating *P. aeruginosa* with gallium (and thus quenching extracellular pyoverdin) consistently inhibited growth over 12 days of experimental evolution, whereas inhibition rapidly failed in parallel 12 day treatments using conventional antibiotics, owing to the evolution of resistance[58].

The effects of structure. The evolutionary robustness of targeting collectively beneficial virulence factors has one important caveat. Cooperative behaviours can be under positive selection if the population is sufficiently structured, as genes that promote cooperation will then preferentially help gene copies in neighbouring cells[54,59–61]. Therefore, within-host structuring is likely to select for resistant mutants that maintain the expression of collectively beneficial virulence factors, as the benefits of cooperative investments by resistant individuals will preferentially benefit clonally related (that is, resistant) neighbours (BOX 2; FIG. 2). There is growing evidence for within-host structuring (that is, genetic segregation) in several host–pathogen systems, from systemic *Salmonella enterica* infections in mice[62] to *P. aeruginosa* lung infections in patients with cystic fibrosis[63]. At a more local scale, biofilms are a common and

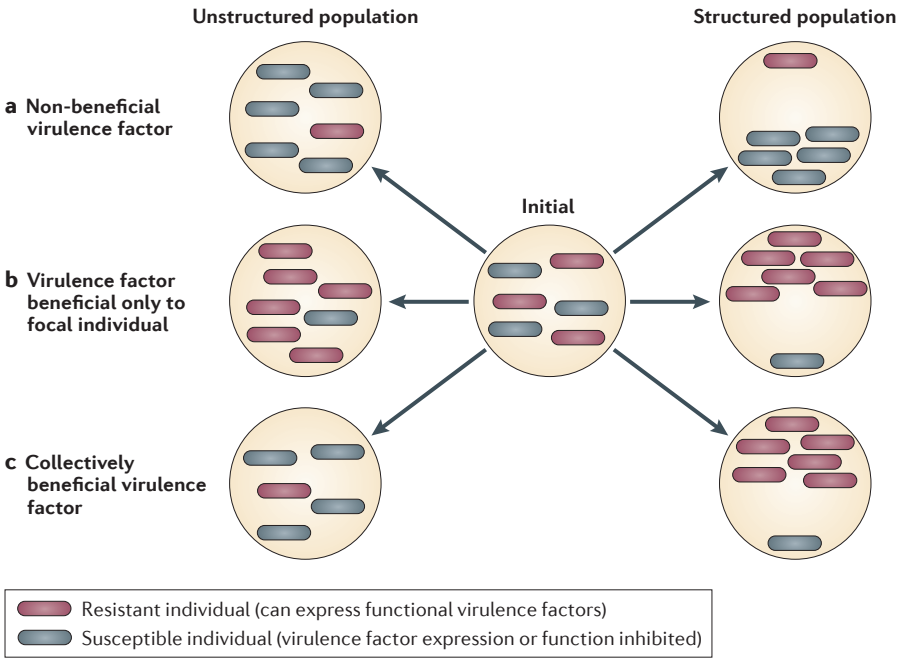


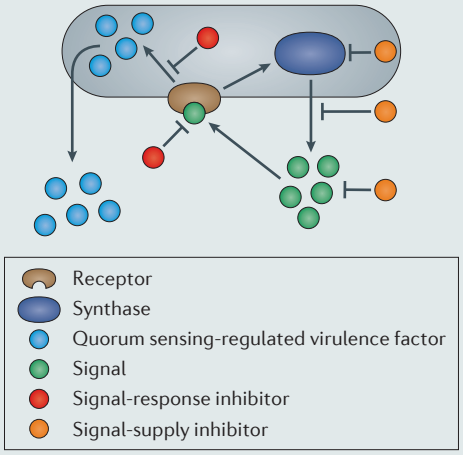
Figure 2 | **Predicted selection on resistance to antivirulence therapeutics.** The direction of selection on resistance mechanisms is predicted to vary as a function of local benefits of virulence-factor expression and population structure. **a** | The cost of expressing non-benefical virulence factors selects against resistance, regardless of the population structure. **b** | The benefits of expressing individually beneficial virulence factors select for resistance, regardless of the population structure. **c** | The benefits of collectively beneficial virulence-factor expression can be exploited by susceptible individuals, but only if the population is unstructured.

Box 3 | **Quorum sensing as a regulator of virulence and how we can target it**

Quorum sensing is a cell–cell communication system that controls many phenotypes, including virulence, in many bacterial pathogens, such as *Erwinia carotovora*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Vibrio cholerae* (for a relevant review see REF. 99). Quorum sensing contributes to virulence by regulating many virulence factors, including a disproportionate number of secreted, collective virulence factors[100] (for example, proteases, lectins, toxins and biofilm polymers). This has spurred interest in quorum-sensing inhibitors (also known as quorum quenchers) as antivirulence drugs. Several quorum-sensing inhibitors have been shown to reduce virulence and to aid the clearance of pathogens in both animal and plant models of infection[35,97,101,102].

The specific quorum-sensing network architecture varies among species, but the key steps of signal supply and signal response are constant, as in any communication system[99,103]. We propose that this creates two functional classes of quorum-sensing inhibitors (see the figure): signal-supply inhibitors (orange) and signal-response inhibitors (red), depending on whether a drug inhibits the function of the signaller or the receiver.

Signal production can be chemically complemented by other individuals in the population, whereas signal response cannot; this creates different selection pressures for resistance to signal-supply inhibitors and signal-response inhibitors. Signal-response inhibitors are exemplified by antagonistic receptor-binding drugs, including signal-molecule analogues, such as peptide inhibitors of the *agr* system in *S. aureus*[101] or mimics of acyl homoserine lactone (AHL) signals that are used by Gram-negative bacteria[104]. Response inhibitors include compounds that interfere with the responses to the received signal, such as cinnemaldehyde inhibitors of *V. cholerae* quorum sensing[105]. Signal-supply inhibitors can inhibit the production[74] (in the case of But-DADMe-ImmA) and, theoretically, the export[106] of signal, or they can inactivate signals in the environment, by degrading them (in the case of AiiA)[97] or by binding to them (in the case of AHL-[107] and peptide signal-specific antibodies[75]).



problematic feature of many bacterial infections[64] and are characterized by considerable genetic structuring[65].

Quorum sensing-controlled virulence

Quorum sensing is a cell–cell signalling behaviour that has received a lot of attention as a potential therapeutic target, as it often controls many virulence factors (BOX 3). We highlight the distinction between signal-response inhibitors (that is, inhibitors that impair the ability of individual cells to respond to signal molecules) and signal-supply inhibitors (that is, inhibitors that impair the production and/or persistence of signals in the environment), as we predict that these two approaches will present different risks of the evolution of resistance (BOX 3; FIG. 3).

Resistance to quorum-sensing inhibitors. The relatively large research focus on quorum-sensing inhibitors has led to some of the best characterized examples of resistance mechanisms to antivirulence drugs[17]. Resistance to furanone competitive inhibitors by overexpression of the MexAB–OprM efflux pump can be selected for *in vitro* in *P. aeruginosa*, and similar resistance mechanisms have been found in clinical isolates[14]. The isolation of quorum sensing-inhibitor resistance from clinical isolates is particularly concerning and highlights the risks that are posed by cross-resistance to quorum-sensing inhibitors, which is driven by antibiotic selection for broad-activity resistance mechanisms, such as efflux pumps[14].

Overexpression of the *traR* quorum-sensing receptor in *Agrobacterium tumefaciens* reduced the antagonistic capabilities of compounds that inhibited signal binding in a wild-type strain[66]. A study that carried out mutagenesis of the *luxR* quorum-sensing receptor of *Vibrio fischeri* in an *E. coli* reporter strain documented mutations that reduce antagonistic binding of several competitive inhibitors to the LuxR receptor[67]. This study also revealed that, as competitive inhibitors have a similar structure to the signal, resistance to these antagonists may also reduce sensitivity to the native signal, which constrains the number of mutations that lead to effective resistance[67,68]. A similar constraint would also occur if a change in signal structure caused resistance[68]. Signal alteration has not been documented as a response to quorum-sensing inhibition, but there is natural variation in the peptide signals that are used by the *agr* locus in *S. aureus* and other staphylococci[69]. There is also high variability in signal-production levels in many strains that have quorum-sensing systems[17], and strains of *V. cholerae* with constitutive activation of quorum sensing-regulated genes[70] will be insensitive to quorum-sensing inhibition (if it occurs upstream of constitutive quorum-sensing activation[71]). Similarly, if individual virulence factors escape quorum-sensing regulation[72], then virulence-factor expression will be unaffected by quorum-sensing inhibitors. Resistance may also occur by direct inactivation of the quorum-sensing inhibitor, but this has not been documented.

As discussed previously, the selective pressures that affect resistance will determine the fate of the resistance mechanisms that arise. In TABLE 1, we summarize our predictions about the selection of resistance as a function of the benefits of quorum sensing-controlled genes, within-host structure and the mechanism of quorum-sensing inhibition.

Signal-response inhibitors. Signal-response inhibitors make susceptible cells signal-blind, reducing the production of quorum sensing-regulated virulence factors. Therefore, signal-response inhibitors will impose similar selective forces to those that have been described for antivirulence drugs that directly inhibit virulence-factor expression (FIG. 2), and selection is dependent on the costs and benefits of the regulated virulence factors in the treatment environment[73].

If quorum sensing-regulated virulence factors are collectively beneficial, inhibitors of signal response render susceptible individuals phenotypic cheats. For example, genetic knockouts of *P. aeruginosa* show that signal-blind cheats are able to exploit a protease that is produced by signal-responsive individuals during well-mixed growth *in vitro* and in animal models[51], in which the secreted protease is required for growth[57,60] (TABLE 1). As described in FIG. 2, this conclusion will change if the population is structured and resistant mutants can group together to form cooperative patches.

By contrast, if the quorum sensing-regulated virulence factors only benefit the

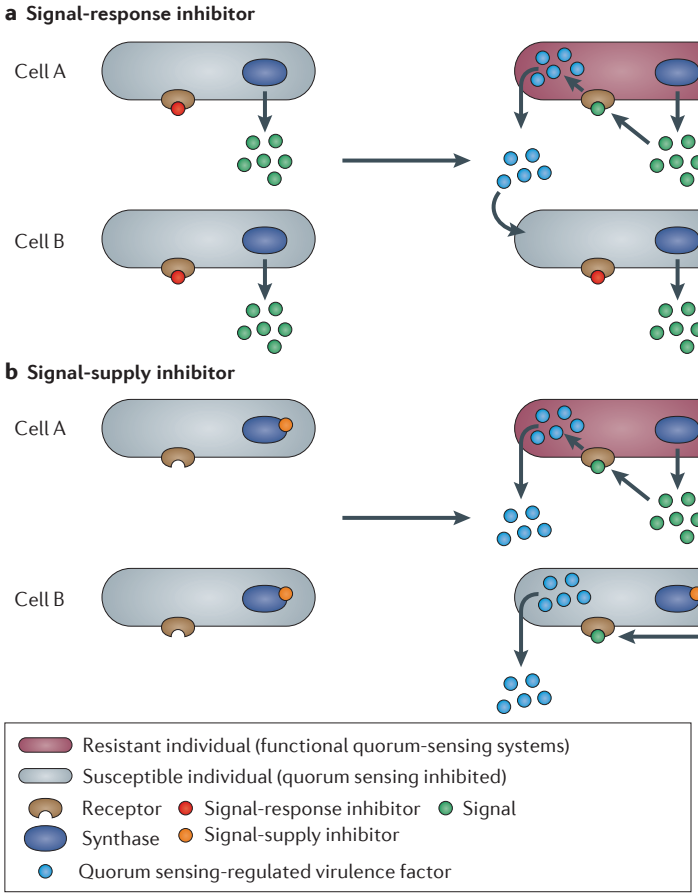


Figure 3 | **The mechanistic target of the quorum-sensing inhibitor influences the strength of selection for resistance.** **a** | If the inhibitor targets signal response (for example, by receptor blocking), only a resistant mutant can sense the signal and produce virulence factors; collectively beneficial virulence factors can be exploited by neighbours. **b** | If the inhibitor targets signal supply (for example, signal-cleaving enzymes), only resistant mutants will produce signal, inducing virulence-factor production in neighbours. For signal-supply inhibitors, the costs of virulence-factor production are shared and resistant individuals are neither favoured nor disfavoured compared with neighbouring susceptible pathogens.

individual that expresses them, they cannot be exploited, regardless of structuring, and resistance will always be positively selected[14,57]. Furanone inhibitors of quorum sensing can select for resistance in *P. aeruginosa* grown on adenosine even in a well-mixed environment, as quorum sensing-dependent adenosine catabolism is intracellular (that is, private) and therefore cannot be socially exploited by neighbouring cells[14].

Signal-supply inhibitors. Inhibitors of signal supply (which target either signal production or environmental persistence via signal-degrading enzymes) will reduce signal levels in susceptible populations[74], therefore attenuating the expression of quorum sensing-controlled virulence factors[75]. If resistance mechanisms arise, then active signal in the environment will be produced only by resistant individuals but will be accessible to all individuals at equal (initially low) concentrations in a mixed environment. All individuals will express quorum sensing-controlled virulence factors to an equal extent (if at all), meaning that the benefits and costs of the virulence factors will not affect selection for resistance, so resistance may be neutral and subject to genetic drift. For example, genetic knockouts of *P. aeruginosa* that are unable to synthesize signal do not outcompete strains that can synthesize signal when competed in a well-mixed environment in which protease is required for growth[76]. If individuals are resistant because they express more signal than susceptible bacteria (rather than signal being insensitive to degradation), the cost of signal production in nutrient-poor environments may still select against resistance. By contrast, if a population is structured, signal will be preferentially detected by resistant individuals, meaning that only these cells will produce and benefit from virulence factors, selecting for resistance if quorum sensing has any benefit in the environment (BOX 2; TABLE 1).

Multiple targets. Quorum sensing influences the expression of a large proportion of the genome (approximately 5–10% for *P. aeruginosa*[72,77]), including multiple virulence factors. This broad-based influence on virulence-factor expression is a major part of the attraction of quorum-sensing inhibition; however, it also raises the concern that such a large perturbation of cell function will promote selection for resistance[18]. We argue that, despite the large expression footprint of quorum-sensing inhibition, selection for resistance is not inevitable and is environmentally determined. Given that approximately 90% of the quorum-sensing regulon is upregulated in response to signal[78], resistance to quorum-sensing inhibition will incur a substantial cost in simple environments in which the quorum-sensing regulon is redundant[60], driving selection for sensitivity. When one, or a few, quorum sensing-controlled traits confer individual or collective advantages, these benefits must be titrated against the simultaneously incurred costs of expression of other, redundant traits. In a defined environment, conferring both individual and collective advantages to quorum sensing (specifically, protein plus adenosine media), the individual benefit of quorum sensing-mediated adenosine catabolism was generally sufficient to drive selection for quorum sensing (and by inference, resistance to quorum-sensing inhibition), overcoming the costs of redundant gene expression as well as the social costs of collective protein degradation[73]. The complex and highly interactive nature of quorum-sensing regulation in *P. aeruginosa* also introduces the prospect of more nuanced strategies of interference with quorum sensing. Recent work has shown that a combination of partial receptor antagonism and agonism produces the most effective net reduction of crucial virulence phenotypes[79].

Changes in intrinsic virulence

We have discussed the selective fate of mutants that are resistant to antivirulence drugs (that is, mutants that are able to express the targeted virulence factors in the presence of the drug) but are otherwise identical to their susceptible ancestor. Resistance is an important factor in the potential evolution of a pathogen in response to antivirulence drugs, but it is not the only way in which an evolving pathogen might respond.

Table 1 | **Predicted direction of within-host selection for resistance to quorum-sensing inhibitors***

Benefit of quorum sensing-regulated virulence factors	Signal response		Signal supply	
	Unstructured	Structured	Unstructured	Structured
No benefit	–	–	0	–
Benefit to focal individual only	+	+	0	+
Collective benefit	–	+	0	+

*Resistance mechanisms are predicted to increase (+), decrease (–) or drift (0) (that is, there is no deterministic increase or decrease) in frequency as a function of inhibition mechanism, population structuring and resource environment.

In the following section, we briefly discuss how pathogens may recover their fitness by altering their intrinsic (that is, drug-free) virulence-factor expression[80], as this is one of the most important of these effects.

Increased virulence. The most worrying examples are those in which the use of antivirulence drugs may select for higher intrinsic pathogen virulence, as this would be particularly detrimental to untreated patients who are not protected by the drug. Theory suggests that interventions that limit the virulent exploitation of a host (such as antivirulence drugs) can select for higher intrinsic levels of virulence by relaxing the constraint of host death on transmission[81]. Köhler *et al.* found that, by blocking costly collective virulence-factor expression in *P. aeruginosa*, quorum-sensing inhibition (using the signal-synthesis inhibitor azithromycin) reduced within-host selection for avirulent mutants (that is, cheats) and maintained more virulent wild-type genotypes[82]. Lastly, it is possible that interference with regulatory processes may increase virulence by selecting for the constitutive expression of regulated virulence factors[70].

Reduced virulence. Antivirulence drugs may also select for reduced virulence, as has been proposed for antitoxin vaccines, which inhibit the function of toxins once they have been produced[83]. In the presence of the toxin inhibitor, the toxin has no function (and imposes only a metabolic cost), so the loss of the virulence factor confers no cost to the pathogen in the treated host but reduces the metabolic costs of producing a virulence factor. Therefore, virulence factor-negative strains are under positive selection[83]. In support of this, an antitoxin vaccine that was targeted against diphtheria toxin (which is a metabolically costly phage-encoded toxin in *Corynebacterium diphtheriae*) led to a return to a commensal state and a decrease in toxin-positive strains[84]. However, pathogens that become resistant to the antitoxin vaccine and re-express a beneficial virulence factor will be more fit than virulence factor-negative strains, which may explain why this result has not been replicated[85]. Antitoxin drugs may also be overcome by overexpressing the toxin, which would also increase virulence in untreated hosts[85]. It is imperative that these conflicting outcomes are reconciled, as several antitoxin therapeutic antibodies are already in clinical trials[86,87].

Although phages are not antivirulence drugs (as they kill pathogens), it is interesting that certain phage therapies may select for avirulence. Phages co-evolve with bacterial pathogens, so often the only long-term bacterial resistance strategy is to lose the receptor for phage entry; if the receptor is a virulence factor, the outcome will be avirulence[88,89]. There would still be strong selection to express a modified receptor if it is beneficial; however, unlike antitoxin drugs, mutations that cause overexpression of the virulence factor would increase susceptibility to the phage and would be selected against.

Conclusions

There is real potential for the development of new and effective antivirulence drugs, thanks to improved screening methodologies using genetically modified strains[90], drugs that target processes associated with virulence in several pathogenic species[91,92] and the positive results of antivirulence drugs in clinical trials[86,87]. But what are the potential lifetimes of antivirulence drugs and how can we extend their effective use in the face of bacterial evolution? We have argued that, although the existence of mechanisms of resistance to these novel drugs is inevitable (and several have already been observed[14–16,18,66,67]), their rise in frequency under the action of drug selection is not inevitable and can even be reversed for particular combinations of virulence-factor target and treatment environment.

Unlike traditional antibiotics, for which resistance is always advantageous to the pathogen (for an intriguing exception see REF. 93), the selective picture for antivirulence drugs is more nuanced, and resistance is potentially costly even in the presence of drugs. We have outlined a series of predictions (BOX 2) on the direction of selection for resistant mutants as a function of the match between the virulence factor target and the infection environment of the pathogen (FIG. 2; TABLE 1). Our predictions suggest that a truly 'evolution-proof' combination of virulence factor target and treatment environment — in which the drug treatment consistently selects against resistance — is possible[58] (BOX 2), in particular for cases in which the targeted virulence factor damages the host but confers no benefit to the pathogen. However, we caution that the benefits of virulence-factor expression are, in general, poorly understood and are often indirectly mediated by effects on resistance to immune- or antibiotic-mediated clearance[35,36]. We suggest that, by a careful integration of molecular and evolutionary microbiology, real progress can be made in the design and effective use of more evolutionarily robust novel drugs, both alone and in combination with existing traditional therapeutic drugs. Further progress in understanding and managing the evolutionary risks of antivirulence drugs is currently limited by our lack of data on the costs and benefits of virulence-factor expression during infection. We strongly encourage more work in this direction.

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1. Davies, J. & Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* **74**, 417–433 (2010).
2. Toprak, E. *et al.* Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. *Nature Genet.* **44**, 101–105 (2012).
3. D'Costa, V. M. *et al.* Antibiotic resistance is ancient. *Nature* **477**, 457–461 (2011).
4. Wright, G. D. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature Rev. Microbiol.* **5**, 175–186 (2007).
5. Habets, M. G. J. L. & Brockhurst, M. A. Therapeutic antimicrobial peptides may compromise natural immunity. *Biol. Lett.* **8**, 416–418 (2012).

6. Lipsitch, M. & Samore, M. H. Antimicrobial use and antimicrobial resistance: a population perspective. *Emerg. Infect. Dis.* **8**, 347–354 (2002).
7. Andersson, D. I. & Hughes, D. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature Rev. Microbiol.* **8**, 260–271 (2010).
8. Schrag, S. J. & Perrot, V. Reducing antibiotic resistance. *Nature* **381**, 120–121 (1996).
9. Levin, B. R., Perrot, V. & Walker, N. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* **154**, 985–997 (2000).
10. Coates, A. R. M., Halls, G. & Hu, Y. Novel classes of antibiotics or more of the same? *Br. J. Pharmacol.* **163**, 184–194 (2011).
11. Clatworthy, A. E., Pierson, E. & Hung, D. T. Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chem. Biol.* **3**, 541–548 (2007).
12. Rasko, D. A. & Sperandio, V. Anti-virulence strategies to combat bacteria-mediated disease. *Nature Rev. Drug Discov.* **9**, 117–128 (2010).
13. García-Contreras, R. *et al.* Resistance to the quorum-quenching compounds brominated furanone C-30 and 5-fluorouracil in *Pseudomonas aeruginosa* clinical isolates. *Pathog. Dis.* **68**, 8–11 (2013).
14. Maeda, T. *et al.* Quorum quenching quandary: resistance to antivirulence compounds. *ISME J.* **6**, 493–501 (2012).
15. Hung, D. T., Shakhnovich, E. A., Pierson, E. & Mekalanos, J. J. Small-molecule inhibitor of *Vibrio cholerae* virulence and intestinal colonization. *Science* **310**, 670–674 (2005).
16. Smith, M. A. *et al.* Identification of the binding site of *Brucella virB8* interaction inhibitors. *Chem. Biol.* **19**, 1041–1048 (2012).
17. Defoirdt, T., Boon, N. & Bossier, P. Can bacteria evolve resistance to quorum sensing disruption? *PLoS Pathog.* **6**, e1000989 (2010).
18. García-Contreras, R., Maeda, T. & Wood, T. K. Resistance to quorum quenching compounds. *Appl. Environ. Microbiol.* **79**, 6840–6846 (2013).
19. Read, A. F., Day, T. & Huijben, S. The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proc. Natl Acad. Sci. USA* **108**, 10871–10877 (2011).
20. D'Costa, V. M., McGrann, K. M., Hughes, D. W. & Wright, G. D. Sampling the antibiotic resistome. *Science* **311**, 374–377 (2006).
21. Alizon, S., Hurford, A., Mideo, N. & Van Baalen, M. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J. Evol. Biol.* **22**, 245–259 (2009).
22. Anderson, R. M. & May, R. M. Coevolution of hosts and parasites. *Parasitology* **85**, 411–426 (1982).
23. Levin, B. R. & Eden, C. S. Selection and evolution of virulence in bacteria — an ecuminical excursion and modest suggestion. *Parasitol.* **100**, S103–S115 (1990).
24. Brown, S. P., Cornforth, D. M. & Mideo, N. Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. *Trends Microbiol.* **20**, 336–342 (2012).
25. Silverstein, S. C. & Steinberg, T. H. in *Microbiology* 485–505 (J. B. Lippincott, 1990).
26. Bae, T. *et al. Staphylococcus aureus* virulence genes identified by *Bursa aurealis* mutagenesis and nematode killing. *Proc. Natl Acad. Sci. USA* **101**, 12312–12317 (2004).
27. Köhler, C.-D. & Dobrindt, U. What defines extraintestinal pathogenic *Escherichia coli*? *Int. J. Med. Microbiol.* **301**, 642–647 (2011).
28. Nowrouzian, F. L., Adlerberth, I. & Wold, A. E. Enhanced persistence in the colonic microbiota of *Escherichia coli* strains belonging to phylogenetic group B2: role of virulence factors and adherence to colonic cells. *Microbes Infect.* **8**, 834–840 (2006).
29. Diard, M. *et al.* Pathogenicity-associated islands in extraintestinal pathogenic *Escherichia coli* are fitness elements involved in intestinal colonization. *J. Bacteriol.* **192**, 4885–4893 (2010).
30. Alsam, S. *et al. Escherichia coli* interactions with *Acanthamoeba*: a symbiosis with environmental and clinical implications. *J. Med. Microbiol.* **55**, 689–694 (2006).
31. Gall, T. L. *et al.* Extraintestinal virulence is a coincidental by-product of commensalism in B2 phylogenetic group *Escherichia coli* strains. *Mol. Biol. Evol.* **24**, 2373–2384 (2007).
32. Pinkner, J. S. *et al.* Rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria. *Proc. Natl Acad. Sci. USA* **103**, 17897–17902 (2006).
33. Steinberg, K. M. & Levin, B. R. Grazing protozoa and the evolution of the *Escherichia coli* O157:H7 Shiga toxin-encoding prophage. *Proc. Biol. Sci.* **274**, 1921–1929 (2007).
34. Montarry, J., Hamelin, F. M., Glais, I., Corbi, R. & Andrivon, D. Fitness costs associated with unnecessary virulence factors and life history traits: evolutionary insights from the potato late blight pathogen *Phytophthora infestans*. *BMC Evol. Biol.* **10**, 283 (2010).
35. Wu, H. *et al.* Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. *J. Antimicrob. Chemother.* **53**, 1054–1061 (2004).
36. Christensen, L. D. *et al.* Synergistic antibacterial efficacy of early combination treatment with tobramycin and quorum-sensing inhibitors against *Pseudomonas aeruginosa* in an intraperitoneal foreign-body infection mouse model. *J. Antimicrob. Chemother.* **67**, 1198–1206 (2012).
37. Oogai, Y. *et al.* Expression of virulence factors by *Staphylococcus aureus* grown in serum. *Appl. Environ. Microbiol.* **77**, 8097–8105 (2011).
38. Genco, C. A. & Dixon, D. W. Emerging strategies in microbial haem capture. *Mol. Microbiol.* **39**, 1–11 (2001).
39. Stecher, B. *et al. Salmonella enterica* serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS Biol.* **5**, 2177–2189 (2007).
40. Lysenko, E. S., Lijek, R. S., Brown, S. P. & Weiser, J. N. Within-host competition drives selection for the capsule virulence determinant of *Streptococcus pneumoniae*. *Curr. Biol.* **20**, 1222–1226 (2010).
41. Levin, B. R. & Bull, J. J. Short-sighted evolution and the virulence of pathogenic microorganisms. *Trends Microbiol.* **2**, 76–81 (1994).
42. Liu, C.-I. *et al.* A cholesterol biosynthesis inhibitor blocks *Staphylococcus aureus* virulence. *Science* **319**, 1391–1394 (2008).
43. Hall, A. R., Griffiths, V. F., MacLean, R. C. & Colegrave, N. Mutational neighbourhood and mutation supply rate constrain adaptation in *Pseudomonas aeruginosa*. *Proc.Biol. Sci.* **277**, 643–650 (2010).
44. Dyken, J. D. V. & Wade, M. J. The genetic signature of conditional expression. *Genetics* **184**, 557–570 (2010).
45. Sokurenko, E. V., Gomulkiewicz, R. & Dykhuizen, D. E. Source-sink dynamics of virulence evolution. *Nature Rev. Microbiol.* **4**, 548–555 (2006).
46. Meyers, L. A., Levin, B. R., Richardson, A. R. & Stojiljkovic, I. Epidemiology, hypermutation, within-host evolution and the virulence of *Neisseria meningitidis*. *Proc. Biol. Sci.* **270**, 1667–1677 (2003).
47. Nogueira, T., Touchon, M. & Rocha, E. P. C. Rapid evolution of the sequences and gene repertoires of secreted proteins in bacteria. *PLoS ONE* **7**, e49403 (2012).
48. Brown, S. P., Hochberg, M. E. & Grenfell, B. T. Does multiple infection select for raised virulence? *Trends Microbiol.* **10**, 401–405 (2002).
49. Buckling, A. *et al.* Siderophore-mediated cooperation and virulence in *Pseudomonas aeruginosa*. *FEMS Microbiol. Ecol.* **62**, 135–141 (2007).
50. Nogueira, T. *et al.* Horizontal gene transfer of the secretome drives the evolution of bacterial cooperation and virulence. *Curr. Biol.* **19**, 1683–1691 (2009).
51. Rumbaugh, K. P. *et al.* Quorum sensing and the social evolution of bacterial virulence. *Curr. Biol.* **19**, 341–345 (2009).
52. West, S. A., Diggle, S. P., Buckling, A., Gardner, A. & Griffins, A. S. The social lives of microbes. *Annu. Rev. Ecol. Evol. Systemat.* **38**, 53–77 (2007).
53. Andrê, J. & Godelle, B. Multicellular organization in bacteria as a target for drug therapy. *Ecol. Lett.* **8**, 800–810 (2005).
54. Griffin, A. S., West, S. A. & Buckling, A. Cooperation and competition in pathogenic bacteria. *Nature* **430**, 1024–1027 (2004).
55. Brown, S. P., West, S. A., Diggle, S. P. & Griffin, A. S. Social evolution in micro-organisms and a Trojan horse approach to medical intervention strategies. *Philos. Trans. R. Soc.Lond. B. Biol. Sci.* **364**, 3157–3168 (2009).
56. Harrison, F., Browning, L. E., Vos, M. & Buckling, A. Cooperation and virulence in acute *Pseudomonas aeruginosa* infections. *BMC Biol.* **4** (2006).
57. Mellbye, B. & Schuster, M. The sociomicrobiology of antivirulence drug resistance: a proof of concept. *mBio* **2**, e00131-11 (2011).
58. Ross-Gillespie, A., Weigert, M., Brown, S. P. & Kümmerli, R. Gallium-mediated siderophore quenching as an evolutionarily robust antibacterial treatment. *Evol. Med. Public Health* **1**, 18–29 (2014).
59. Hamilton, W. Genetical evolution of social behaviour I. *J. Theor. Biol.* **7**, 1–16 (1964).
60. Diggle, S. P., Griffin, A. S., Campbell, G. S. & West, S. A. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* **450**, 411–417 (2007).
61. Kümmerli, R., Griffin, A. S., West, S. A., Buckling, A. & Harrison, F. Viscous medium promotes cooperation in the pathogenic bacterium *Pseudomonas aeruginosa*. *Proc. Biol. Sci.* **276**, 3531–3538 (2009).
62. Grant, A. J. *et al.* Modelling within-host spatiotemporal dynamics of invasive bacterial disease. *PLoS Biol.* **6**, 757–770 (2008).
63. Willner, D. *et al.* Spatial distribution of microbial communities in the cystic fibrosis lung. *ISME J.* **6**, 471–474 (2012).
64. Costerton, J. W., Stewart, P. S. & Greenberg, E. P. Bacterial biofilms: a common cause of persistent infections. *Science* **284**, 1318–1322 (1999).
65. Kreft, J. U. Biofilms promote altruism. *Microbiology* **150**, 2751–2760 (2004).
66. Zhu, J. *et al.* Analogs of the autoinducer 3-oxooctanoyl-homoserine lactone strongly inhibit activity of the traR protein of *Agrobacterium tumefaciens*. *J. Bacteriol.* **180**, 5398–5405 (1998).
67. Koch, B. *et al.* The LuxR receptor: the sites of interaction with quorum-sensing signals and inhibitors. *Microbiology* **151**, 3589–3602 (2005).
68. Eldar, A. Social conflict drives the evolutionary divergence of quorum sensing. *Proc. Natl Acad. Sci. USA* **108**, 13635–13640 (2011).
69. Wright, J. S. *et al.* The *agr* radiation: an early event in the evolution of staphylococci. *J. Bacteriol.* **187**, 5585–5594 (2005).
70. Joelsson, A., Liu, Z. & Zhu, J. Genetic and phenotypic diversity of quorum-sensing systems in clinical and environmental isolates of *Vibrio cholerae*. *Infect. Immun.* **74**, 1141–1147 (2006).
71. Defoirdt, T. *et al.* The natural furanone (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone disrupts quorum sensing-regulated gene expression in *Vibrio harveyi* by decreasing the DNA-binding activity of the transcriptional regulator protein luxR. *Environ. Microbiol.* **9**, 2486–2495 (2007).
72. Chugani, S. *et al.* Strain-dependent diversity in the *Pseudomonas aeruginosa* quorum-sensing regulon. *Proc. Natl Acad. Sci. USA* **109**, E2823–E2831 (2012).
73. Dandekar, A. A., Chugani, S. & Greenberg, E. P. Bacterial quorum sensing and metabolic incentives to cooperate. *Science* **338**, 264–266 (2012).
74. Schramm, V. L. *et al.* Transition state analogues in quorum sensing and SAM recycling. *Nucleic Acids Symp. Ser.* **52**, 75–76 (2008).
75. Park, J. *et al.* Infection control by antibody disruption of bacterial quorum sensing signaling. *Chem. Biol.* **14**, 1119–1127 (2007).
76. Wilder, C. N., Diggle, S. P. & Schuster, M. Cooperation and cheating in *Pseudomonas aeruginosa*: the roles of the *las*, *rhl* and *pqs* quorum-sensing systems. *ISME J.* **5**, 1332–1343 (2011).
77. Schuster, M. & Greenberg, E. P. A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. *Int. J. Med. Microbiol.* **296**, 73–81 (2006).
78. Schuster, M., Lostroh, C., Ogi, T. & Greenberg, E. P. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J. Bacteriol.* **185**, 2066–2079 (2003).
79. O'Loughlin, C. T. *et al.* A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. *Proc. Natl Acad. Sci. USA* **110**, 17981–17986 (2013).
80. Vale, P. F., Fenton, A. & Brown, S. P. Limiting damage during infection: lessons from infection tolerance for novel therapeutics. *PLoS Biol.* **12**, e1001769 (2014).
81. Gandon, S., Mackinnon, M. J., Nee, S. & Read, A. F. Imperfect vaccines and the evolution of pathogen virulence. *Nature* **414**, 751–756 (2001).
82. Köhler, T., Perron, G. G., Buckling, A. & van Delden, C. Quorum sensing inhibition selects for virulence and cooperation in *Pseudomonas aeruginosa*. *PLoS Pathog.* **6**, e1000883 (2010).
83. Soubeyrand, B. & Plotkin, S. A. Microbial evolution — antitoxin vaccines and pathogen virulence. *Nature* **417**, 609–610 (2002).
84. Pappenheimer, A. in *Bacterial Vaccines* (Ed. Germanier, R.) 1–36 (Academic Press, 1984).

85. Gandon, S. & Day, T. Evidences of parasite evolution after vaccination. *Vaccine* **26**, (Suppl. 3), C4–C7 (2008).
86. Lowy, I. *et al.* Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N. Engl. J. Med.* **362**, 197–205 (2010).
87. López, E. L. *et al.* Safety and pharmacokinetics of urtoxazumab, a humanized monoclonal antibody, against Shiga-like toxin 2 in healthy adults and in pediatric patients infected with Shiga-like toxin-producing *Escherichia coli*. *Antimicrob. Agents Chemother.* **54**, 239–243 (2010).
88. Filippov, A. A. *et al.* Bacteriophage-resistant mutants in *Yersinia pestis*: identification of phage receptors and attenuation for mice. *PLoS ONE* **6**, e25486 (2011).
89. Smith, H. W., Huggins, M. B. & Shaw, K. M. The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *J. Gen. Microbiol.* **133**, 1111–1126 (1987).
90. Rasmussen, T. B. *et al.* Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J. Bacteriol.* **187**, 1799–1814 (2005).
91. Felise, H. B. *et al.* An inhibitor of Gram-negative bacterial virulence protein secretion. *Cell Host Microbe* **4**, 325–336 (2008).
92. Hong, K.-W., Koh, C.-L., Sam, C.-K., Yin, W.-F. & Chan, K.-G. Quorum quenching revisited — from signal decays to signalling confusion. *Sensors* **12**, 4661–4696 (2012).
93. Chait, R., Craney, A. & Kishony, R. Antibiotic interactions that select against resistance. *Nature* **446**, 668–671 (2007).
94. Fernebro, J. Fighting bacterial infections — future treatment options. *Drug Resist. Updat.* **14**, 125–139 (2011).
95. Wang, D. *et al.* Identification of bacterial target proteins for the salicylidene acylhydrazide class of virulence-blocking compounds. *J. Biol. Chem.* **286**, 29922–29931 (2011).
96. Yamagami, S. *et al.* Efficacy of postinfection treatment with anti-shiga toxin (stx) 2 humanized monoclonal antibody tma-15 in mice lethally challenged with stx-producing *Escherichia coli*. *J. Infect. Dis.* **184**, 738–742 (2001).
97. Dong, Y. H. *et al.* Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. *Nature* **411**, 813–817 (2001).
98. Hentzer, M. *et al.* Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology* **148**, 87–102 (2002).
99. Rutherford, S. T. & Bassler, B. L. Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb. Perspect. Med.* **2**, a012427 (2012).
100. Gilbert, K. B., Kim, T. H., Gupta, R., Greenberg, E. P. & Schuster, M. Global position analysis of the *Pseudomonas aeruginosa* quorum-sensing transcription factor LasR. *Mol. Microbiol.* **73**, 1072–1085 (2009).
101. Wright, J. S., Jin, R. & Novick, R. P. Transient interference with staphylococcal quorum sensing blocks abscess formation. *Proc. Natl Acad. Sci. USA* **102**, 1691–1696 (2005).
102. Papaioannou, E. *et al.* Quorum-quenching acylase reduces the virulence of *Pseudomonas aeruginosa* in a *Caenorhabditis elegans* infection model. *Antimicrob. Agents Chemother.* **53**, 4891–4897 (2009).
103. Smith, J. M. & Harper, D. *Animal Signals.* (OUP Oxford, 2003).
104. Ishida, T. *et al.* Inhibition of quorum sensing in *Pseudomonas aeruginosa* by *N*-acyl cyclopentylamides. *Appl. Environ. Microbiol.* **73**, 3183–3188 (2007).
105. Brackman, G. *et al.* Structure–activity relationship of cinnamaldehyde analogs as inhibitors of ai-2 based quorum sensing and their effect on virulence of *Vibrio* spp. *PLoS ONE* **6**, e16084 (2011).
106. Ni, N., Li, M., Wang, J. & Wang, B. Inhibitors and antagonists of bacterial quorum sensing. *Med. Res. Rev.* **29**, 65–124 (2009).
107. Kaufmann, G. F. *et al.* Antibody interference with *N*-acyl homoserine lactone-mediated bacterial quorum sensing. *J. Am. Chem. Soc.* **128**, 2802–2803 (2006).

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Competing interests statement

The authors declare no competing interests.