REVIEW
Shi and Zarkan, *Microbiology* 2022;168:001266
DOI 10.1099/mic.0.001266

Bacterial survivors: evaluating the mechanisms of antibiotic persistence

Xiaoyi Shi[1] and Ashraf Zarkan[2,*]

Abstract

Bacteria withstand antibiotic onslaughts by employing a variety of strategies, one of which is persistence. Persistence occurs in a bacterial population where a subpopulation of cells (persisters) survives antibiotic treatment and can regrow in a drug-free environment. Persisters may cause the recalcitrance of infectious diseases and can be a stepping stone to antibiotic resistance, so understanding persistence mechanisms is critical for therapeutic applications. However, current understanding of persistence is pervaded by paradoxes that stymie research progress, and many aspects of this cellular state remain elusive. In this review, we summarize the putative persister mechanisms, including toxin–antitoxin modules, quorum sensing, indole signalling and epigenetics, as well as the reasons behind the inconsistent body of evidence. We highlight present limitations in the field and underscore a clinical context that is frequently neglected, in the hope of supporting future researchers in examining clinically important persister mechanisms.

INTRODUCTION

Less than a century after the first major antibiotic, penicillin, was discovered in 1928 and put into mainstream manufacturing and usage in the 1940s, humans are losing the battle against antibiotic resistance: in 2019, it was estimated that there were 4.95 million deaths associated with antibiotic resistance, including 1.27 million deaths directly attributable to antibiotic resistance [1]. Alarmingly, serious pathogens that are resistant to virtually all presently available antibiotics highlight the gravity of the dwindling antibiotic pipeline [2]. Furthermore, a 2014 review chaired by Jim O'Neill and supported by the UK Government reported that the worldwide cost of antibiotic-resistant bacterial infections will be approximately 100 trillion USD over the next 35 years, imposing a significant burden on the global healthcare system [3]. Although antibiotic resistance is widely regarded as the primary cause of poor treatment outcomes, persistence has been linked to treatment failure for a number of important pathogenic bacteria including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Escherichia coli* [4].

Antibiotic persistence was first named by Joseph Bigger in 1944 when he discovered a subpopulation of *Staphylococcus pyogenes* that survived a penicillin treatment [5]. In contrast to antibiotic-resistant bacteria, persisters cannot multiply but can survive at bactericidal antibiotic concentrations. One of the hallmarks of persistence is a biphasic killing curve: the death of the bulk of the population is represented by an immediate, swift reduction in bacterial counts, followed by a subsequent phase with considerably slower kinetics that represents the slow killing of persisters [6]. When the antibiotics are removed, persisters recover to form a sensitive population [7]. The ability to survive bactericidal antibiotic concentrations and resuscitate after the drug is removed distinguishes persisters from other states such as 'tolerance' or a 'viable but non-culturable cell (VBNC)', two concepts sometimes misapplied to describe persistence [7].

Persister formation has been identified in most bacterial species, and persistence has been described as a stepping stone to antibiotic resistance [4, 8]. Given the prevalence of persisters and their clinical importance, it is critical for researchers to decipher the mechanisms behind persister development. To date, various mechanisms, including toxin–antitoxin (TA) modules, quorum

Received 02 September 2022; Accepted 28 October 2022; Published 06 December 2022
Author affiliations: [1]Cambridge Centre for International Research, Cambridge CB4 0PZ, UK; [2]Department of Genetics, University of Cambridge, Cambridge CB2 3EH, UK.
***Correspondence:** Ashraf Zarkan, maa77@cam.ac.uk
Keywords: persister; toxin/antitoxins; quorum sensing; indole signalling; antibiotics; stress response.
Abbreviations: 2-AA, 2-aminoacetophenone; AHL, acyl-homoserine lactone; CSP, competence stimulating peptide; DosP, direct oxygen sensing phosphodiesterase; IHF, integration host factor; 3-oxo-C6-HSL, 3-oxo-C6-homoserine lactone; 3-oxo-C12-HSL, N-3-oxododecanoyl homoserine lactone; PCD, programmed cell death; PCN, pyocyanin; QS, quorum sensing; SR, stringent response; TA, toxin–antitoxin; TMP-SMX, trimethoprim-sulfamethoxazole; VBNC, viable but non-culturable.

Impact statement

Microbial persisters can cause recurrent infections and pave the way for the subsequent development of antibiotic resistance, resulting in a significant clinical burden worldwide. Elucidating the mechanisms behind persistence is therefore critical to the development of anti-persister medications. Numerous putative persister mechanisms previously discovered are riddled with inconsistencies, owing to unstandardized persister assays and the constrained applicability of each mechanism. Therefore, it is critical to investigate the validity and relevance of each mechanism. In this review, we summarize major findings in persister mechanisms, including toxin–antitoxin (TA) modules, quorum sensing, indole and epigenetics, identifying major gaps in our current understanding and critical factors that have been less well-addressed in previous studies. Additionally, a table summarizing the persister assay conditions utilized by key TA-mediated persistence studies was established with the goal of improving the uniformity and reproducibility of persister assays across different laboratories. Finally, we underline the necessity of studying persister formation with clinical contexts in mind, intending to direct future researchers to investigate persister mechanisms in more clinically applicable ways so that they may facilitate future breakthroughs in the field.

sensing and indole signalling, have been linked with persister development [9–11]. Additionally, epigenetics, albeit less researched, has been associated with persister formation [12]. The disagreements surrounding currently proposed mechanisms suggest that there is unlikely to be a single pathway that explains persister development across all species and conditions. Indeed, a recent review on *M. tuberculosis* concluded that 'not all antibiotic persisters are created equal' [13]. In this literature review, we first describe the mechanisms of persister formation and identify gaps in previous research. Then, we evaluate the most recent findings in epigenetics-mediated persistence and probe the nature of persistence from a stochastic versus a deterministic standpoint. Finally, we highlight the importance of contextualizing persister research with clinical outcomes.

TA MODULES: THE MASTER REGULATORS?

TA modules are ubiquitous bacterial gene loci composed of a toxin and a cognate antitoxin. These modules are primarily involved in physiological processes such as plasmid maintenance, abortive infection and persistence [14]. While plasmid TA modules are involved in plasmid stabilization and cell viability, chromosomal TA modules have been associated with persistence and biofilm formation [14]. Moreover, TA modules can reach an astoundingly high number in some pathogenic bacteria. For instance, 88 putative TA systems have been identified in *M. tuberculosis* [15], contrary to the presence of only five TA modules in *Mycobacterium smegmatis* [16], highlighting the importance of elucidating TA's role in mediating virulence and pathogenesis.

To date, TA modules have been classified into eight classes based on the type of interaction between the toxin and the antitoxin [14]. (i) Type I: the antisense RNA antitoxin counteracts the toxin mRNA. (ii) Type II: the antitoxin protein counteracts the toxin protein. (iii) Type III: the RNA toxin counteracts the toxin protein. (iv) Type IV: the antitoxin inhibits the toxin's activity by interacting with the toxin's target. (v) Type V: the enzyme antitoxin degrades the toxin mRNA. (vi) Type VI: the antitoxin acts as a proteolytic adaptor protein that facilitates the protease-mediated degradation of the toxin. (vii) Type VII: the enzyme antitoxin enzymatically modifies the toxin via a transient interaction. (viii) Type VIII: the antisense RNA antitoxin counteracts the small mRNA toxin.

Notably, both Type I and Type II TA modules have been associated with persister formation [9, 17]. It is commonly assumed that TA modules promote persistence via the stringent response (SR) or the SOS response under stress conditions including starvation and antibiotic treatments (Fig. 1a) [9, 18], although there is mounting evidence suggesting that TA's principal function may be phage defence [19]. The classifications of toxins associated with persistence based on protein sequence homology and their respective cellular targets are illustrated in Fig. 1(b) In this section, we will discuss findings in TA-mediated persistence and evaluate their validity.

Type II TA modules and persister formation

The HipA family

HipBA is the first TA module to have been associated with persister formation [20], and this correlation was first highlighted after a *hipA7* (high persistence) allele survived successive ampicillin treatments in *E. coli* [21]. Notably, *hipA7* mutants are observed in both pathogenic and commensal *E. coli* strains and induce a high-persistence phenotype in clinical isolates and bladder cells [22]. *HipBA* upregulation in *Klebsiella pneumoniae* upon antibiotic challenges hints at a possible correlation between *hipBA* and persistence [23, 24]. Additionally, HipA ectopically expressed via expression plasmids increases persistence [25, 26]. However, it is important to note that overexpression experiments of toxins via plasmids have been criticised as inadequate for the evaluation of TA's role in persistence [9]. It is mainly because overexpression of other proteins unrelated to TA systems also increases persistence [27]. Moreover, although a Δ*hipA* strain was reported to show reduced persistence, the deletion was later determined

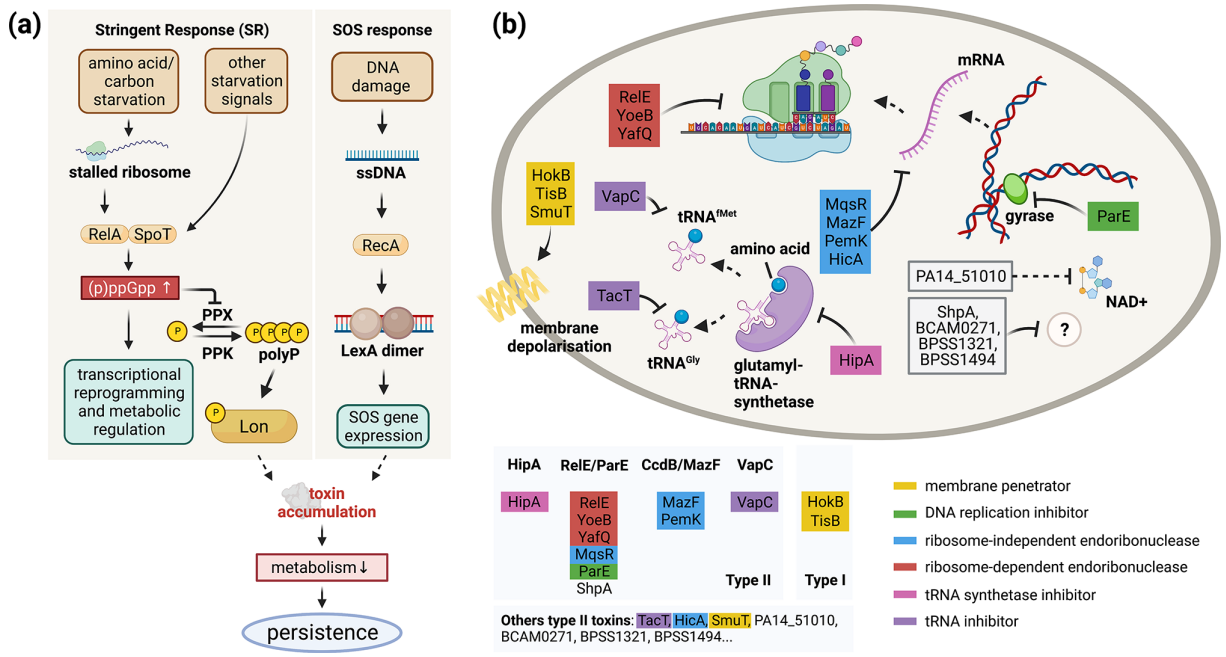

Fig. 1. Responses (a) and classification (b) of TA-mediated persistence. (a) The stringent response (SR) and the SOS response. In the SR, stalled ribosomes in the absence of amino acids stimulate RelA [195], while other starvation signals (e.g. phosphate, iron, fatty acid) stimulate SpoT [196], leading to the production of the alarmone (p)ppGpp, reprogramming many metabolic processes [195]. It has been proposed that an increase in (p)ppGpp production inhibits exopolyphosphatase (PPX), causing polyphosphate (polyP) accumulation, which activates proteases such as Lon that preferentially degrades antitoxins, thus releasing the toxins [14]. In the SOS response, DNA damage, which can be caused by antibiotics such as fluoroquinolones, results in the mass of ssDNA activating RecA, promoting LexA self-cleavage [195], and subsequently activating the SOS genes, which induces TA modules such as the *tisB/istR* Type I locus [14]. Toxin activation and accumulation via SR or SOS are thought to result in decreased metabolic activity, eventually leading to persistence. (b) Classification of TA-mediated persistence. Based on sequence homology, Type II toxins involved in persister formation are classified into several families, including the HipA, RelE/ParE, CcB/MazF and VapC families [197], while TisB and HokB are two of the best-characterized Type I toxins. Toxins inhibit metabolic processes in a variety of ways. Some impair translation via the inhibition of tRNA synthetase (magenta), tRNA (violet), ribosome-dependent RNA cleavage (red) and ribosome-independent RNA cleavage (blue), while some cause membrane depolarization (yellow) and inhibition of DNA replication (green) [17, 198–203]. Recently, many new toxins related to persistence have been identified, including TacT, HicA, SmuT, PA14_51010, BCAM0271, BPSS1321 and BPSS1494 [57, 61, 62, 198, 204]. Their respective cellular targets are shown in the figure, while some remain elusive and hence are indicated by a question mark.

to extend into a critical *dif* region important for chromosome partitioning [28, 29]. Notably, a precise *hipA* deletion does not exhibit the phenotype observed in the unclean deletion strain [29]. Furthermore, *hipA7*'s high persistence was reported to depend on inoculum age and antibiotics [30]. Collectively, these studies suggest that *hipBA* is unlikely to be the universal key regulator of persistence. Additionally, *hipBA*, but not *relBE, vapBC* or *mazEF*, was suggested to rely on SR, albeit the conclusion cannot be generalized to antibiotics other than ampicillin and ciprofloxacin [31, 32]. Notably, in *Caulobacter crescentus,* although the SR regulator SpoT is required for HipA-mediated persistence in the stationary phase, persister cells can arise in the absence of *hipBA* or SpoT [33], highlighting the possibility of different persister-producing routes in *C. cresentus*.

The RelE/ParE family

RelBE

RelBE was demonstrated to be essential for *M. tuberculosis* persistence and upregulated in *K. pneumoniae* and *S. aureus* upon antibiotic challenge [14, 23, 24, 34, 35]. In *E. coli*, RelE overexpression enhances persistence towards all the antibiotics tested except mitomycin C [28]. RelBE's effect on persister formation might be independent of SR because RelE-induced persistence was not significantly impaired by *relA* deletion [32]. However, Δ*relBE* (as well as Δ*mazEF* and Δ*dinJ-yafQ*) was suggested to not affect persistence in *E. coli* [28]. This incongruence might owe to a difference in the bacterial growth phase (stationary phase for Δ*relBE* but exponential phase for overexpression strains), antibiotics and concentration, and the redundancy of TA systems [28, 32]. Similarly, Δ3TA (*mazEF* and two *relBE* homologues) does not affect persistence in *S. aureus* [36].

ParDE

Enterobacteriaceae ParE overexpression in *E. coli* significantly enhances persistence, albeit its persister-forming role in the natural host remains uncertain [37].

MqsRA

MqsR overexpression induces persistence probably without (p)ppGpp, and while Δ*mqsR* or Δ*mqsRA* shows reduced persistence, Δ*mqsR*'s phenotype varies with inoculum age [30, 32, 33]. Interestingly, Δ*mqsR*'s phenotype was observed in *E. coli* K12 BW25113 but not MG1655 [38, 39], suggesting the potential impact of genotype on persistence. Notably, the study on BW25113 concentrated the culture before persister assays, which might have impacted the bacteria's growth condition given that the inoculum's growth condition impacts persistence [30, 38]. Intriguingly, it has been indicated recently that MqsA is transcriptionally uncoupled from MqsR, allowing for a steady MqsA production, and no fluctuation in MqsA expression was observed under stress, questioning MqsRA's role in the stress response [40].

YefM-yoeB

Δ*yoeB* and Δ*yefM-yoeB* show reduced persistence in *S. aureus* and *Edwardsiella piscicida*, and it was observed that polyP and (p)ppGpp do not affect YefM degradation during serine starvation, yet this conclusion cannot be generalized to other situations [41–43].

DinJ-yafQ

In planktonic culture, YafQ overexpression increases persistence without (p)ppGpp, but Δ*yafQ* does not show significantly reduced persistence, and Δ*dinJ* shows only a slightly larger persister fraction [44–46]. In these studies, different antibiotics, growth phases and genotypes were chosen [44–46], which might have caused the incongruence. In biofilms, Δ*yafQ* displays reduced persistence, and YafQ overexpression induces persistence, although these results were only observed when treated with certain antibiotics [45]. However, Δ4TA (*symER, tisAB-istR, dinJ-yafQ, yafNO*) does not produce a different persistence profile in starved biofilms [47]; still, this conclusion might not be extrapolated to stress-free biofilms.

ShpAB

Hyper-persistent *shp* mutants isolated from *Salmonella enterica* serovar Typhimurium were indicated to be Lon-dependent but RelA-independent [48]. Moreover, an *shpAB* knockout mutant displays a reduced rate of persister formation in a murine model of typhoid fever [49]. In contrast, a deletion mutant deficient in three TA modules, including *shpAB*, had no effect on virulence and persistence in a murine model [50].

The CcdB/MazF family

MazEF

MazF upregulation increases persistence, but the authors pretreated the bacteria overnight pre-culture with ampicillin, which might have induced persisters prior to the persister assay, and hence the finding should be evaluated with prudence [51]. Moreover, it has been indicated that MazEF is independent of (p)ppGpp [32, 44]. In *S. aureus*, Δ*mazF* represses persistence in biofilms and some planktonic cultures, and *mazEF* is upregulated in both *S. aureus* and *K. pneumoniae* upon antibiotic treatments [24, 34, 35, 52].

PemIK

PemIK was characterized in the *K. pneumoniae* pCA24N plasmid essential for persister formation [53]. However, multiple genes other than *relBE* are encoded by pCA24N, so this study fails to establish a direct correlation between *pemIK* and persistence [53].

The VapC family

VapB mutations were selected after successive ampicillin treatments, and *vapBC* upregulation has been observed in *K. pneumoniae* upon antibiotic challenges [8, 23, 24]. Notably, *vapBC* is the most abundant TA module in *M. tuberculosis*, and Δ*vapC12* shows reduced cholesterol-mediated persistence required for survival inside macrophages [54]. Additionally, VapC21 overexpression increases persistence in a foreign host lacking *vapBC21*, which is counteracted by the cognate antitoxin VapB21 and the non-cognate VapB32 [54]. However, Δ*vapC21* showed a negligible effect on *M. tuberculosis* persistence [54]. Hence, a compelling correlation between *vapBC21* and persistence is absent. Still, VapBC21's capacity to interact with a non-cognate antitoxin warrants more research into the complex cross-talk between TA homologues.

Other Type II TA modules and their correlation with persister formation

Other Type II toxins include TacT, HicA, PA14_51010, SmuT, BCAM0271, BPSS1321 and BPSS1494. TacT overexpression increases persistence, and Δ*hicAB* shows reduced persistence [55, 56]. Although most Type II TA modules target key steps in translation or DNA replication, PA14_51010 overexpression induces persistence via lowering the intracellular NAD^+ concentration [57]. Moreover, a functional tripartite TA system *smuATR* module has been shown to mediate persistence via membrane permeabilization [58]. Due to the diversity of the TA modules, toxin identification might sometimes be confounded. For instance, PasT, long considered to be a persistence-associated Type II toxin [59], was recently identified as a mitochondrial protein homologue [60]. Therefore, although BCAM0271, BPSS1321 and BPSS1494 were identified as toxins via bioinformatics and have been

linked to persistence [61, 62], *in silico* characterization of toxins should be interpreted cautiously to authenticate the toxins in the first place.

Type I TA modules and persister formation

Two Type I TA modules, *hokB/sokB* and *tisB/istR*, have been implicated in persister formation [17, 63]. It has been indicated that the conserved GTPase Obg, implicated in ribosome biogenesis, DNA replication and stress responses, mediates persistence via activating the transcription of the toxin *hokB* in a (p)ppGpp-dependent but polyP-independent way [63]. In the case of *tisB/istR*, a *tisB/istR* mutant Δ1–41 Δ*istR* (ΔΔ; deletion of the inhibitory *tisB* 5′ untranslated region (UTR) structure and the antitoxin-coding *istR-1*) has been characterized as a high-persistence mutant, which, unlike the WT, was later proved to be uncoupled from SOS [64, 65]. Notably, strong TisB production by ΔΔ has been suggested to inhibit the SOS response, indicating that the SOS response is non-essential for ΔΔ [66]. Surprisingly, compared with the WT, ΔΔ's persister level was ~15-fold lower towards mitomycin C [66], suggesting that high-persistence phenotypes are sometimes conditional. Based on this discovery, it is reasonable to postulate that bacteria may hedge their bets by possessing many genotypes for generating persisters under different environments. Recently, a new model where TisB functions as a persistence-stabilizing factor that plays a role at later time points were proposed, based on the critical observations of a strong delay in TisB-related events, such as the decline in cellular ATP level after 4–6 h of ciprofloxacin treatment [67]. This shift in perspective may drive future studies toward persistence-stabilizing factors rather than rapid persistence inducers, offering insight into additional functions of TA modules in mediating persistence. Still, since *tisB/istR* is conserved in pathogenic *E. coli* and *Enterobacteriaceae* [68], *tisB/istR* should be studied in species other than the *E. coli* lab strain to get further clinical insights.

COUNTERARGUMENTS REGARDING TA'S KEY ROLE IN PERSISTENCE

It has long been assumed that TA modules mediate persistence via the classical SR pathway (Figs 1a and 2). However, there is accumulating evidence that TAs might not be a universal regulator of persistence. It has been suggested that simultaneous deletion of multiple TAs in *E. coli* and *Salmonella enterica* serovar Typhimurium shows no disadvantage in persistence [43, 69–71]. TA redundancy aside, many other studies cast doubts on the TA-mediated persistence paradigm, questioning essentially every step of this pathway (Fig. 2).

First, (p)ppGpp's function in the pathway is unclear. PolyP production was proposed to depend on the transcription factor DksA instead of (p)ppGpp and, notably, previous studies reporting (p)ppGpp-dependent polyP production utilized Δ*relA*Δ*spoT* strains containing unsuspected stringent mutations of RNA polymerase that explain the decreased polyP accumulation [72]. Similarly, polyP production does not depend on (p)ppGpp in *C. crecentus* or *P. aeruginosa,* and persister formation is independent of (p)ppGpp in both *Salmonella enterica* serovar Typhimurium and *M. smegmatis* [71, 73–75].

Second, the roles of PPX, PPK, and polyP remain ambiguous. Δ*ppkx* does not affect persistence [76]. Moreover, there is no observed correlation between *ppk* gene expression and polyP production, though a reporter fusion construct was employed, which might not reflect the native *ppk* transcript [72]. Additionally, polyP appeared to inhibit Lon *in vitro* instead of activating it [77].

Third, it is unclear whether TA upregulation and Lon-mediated degradation of antitoxin lead to toxin accumulation. A Δ*lon* mutant does not show reduced ampicillin persistence, and the decreased ciprofloxacin persistence of Δ*lon* results from unchecked SulA production instead of hindered antitoxin degradation [44, 78]. Moreover, there is mounting evidence suggesting that toxins are not liberated by preferential antitoxin degradation, since not all antitoxins are unstructured and hence are probably not favoured by proteases [79]. Additionally, the high affinity between toxin and antitoxin renders antitoxin degradation unlikely, while most research studied unbound toxins and misattributed the lability to bound toxins [79]. Furthermore, toxins might be activated by *de novo* synthesis via post-transcriptional approaches instead of being liberated, such as the preferential degradation of the antitoxin mRNA in the GhoST module [80]. In line with this assumption, it was recently reported that synonymous mutations in the *ccdA* antitoxin gene affect bacterial growth, which is caused by the change in the relative translation efficiencies of the antitoxin CcdA and the toxin CcdB, leading to a change in the toxin:antitoxin ratio but not toxin/antitoxin binding [81]. Similarly, TA upregulation during stress does not cause toxin liberation; in fact, antitoxins are never depleted and are much more abundant than toxins [70]. Collectively, these findings indicate that the classical paradigm of antitoxin degradation by Lon is not well established, and TA upregulation is not equivalent to toxin activation.

TA SYSTEMS: WHY SO MANY? WHAT FOR?

Given the ambiguity surrounding TA's involvement in persistence, the TA field should presumably answer the question proposed in 2010 from an evolutionary perspective: TA systems, why so many? What for? [82].

There is accumulating data suggesting that TA modules have evolved as a phage defence system, a 26-year paradigm initially discovered in a plasmid *hok/sok* module and recently reviewed [83, 84]. To begin with, the phage defence phenomenon has been observed in at least four types of TA in a variety of ways, including abortive infection and CRISPR-Cas safeguard [85–88].

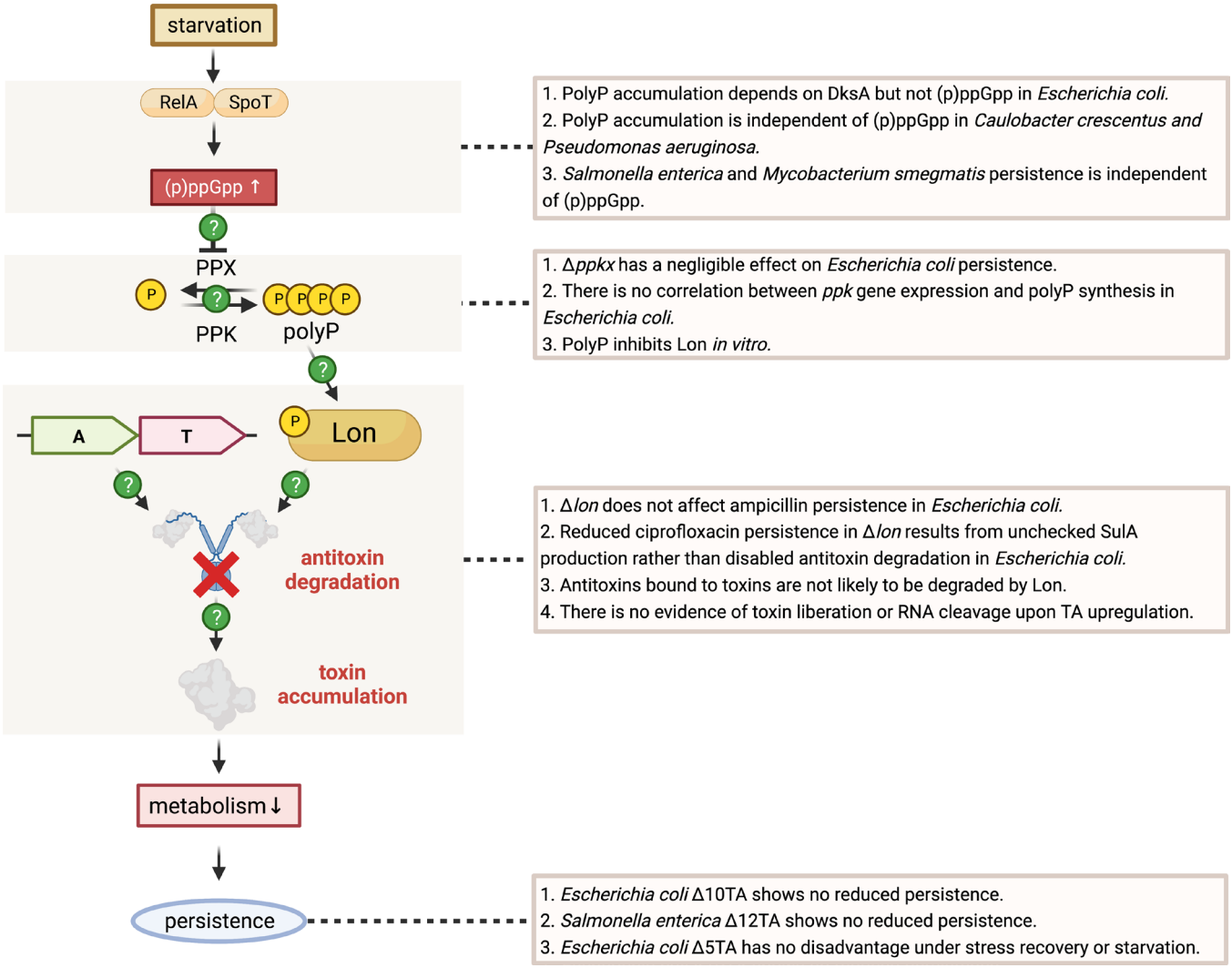

Fig. 2. Controversies over the TA-mediated persistence paradigm. The classical model unifying (p)ppGpp, polyP, Lon, TA and persistence is illustrated (left). Counterarguments opposed to the model are listed (right). Green question marks indicate controversial steps in the pathway (left).

Notably, the phage defence role of the MqsRA system has recently been established [89]. Moreover, TA has been suggested to be one of the ancestors of the CRISPR-Cas system [90]. Additionally, TA has left behind a wealth of co-evolutionary footprints documenting the phage–host arms race. First, many phages have evolved antitoxins as a defence mechanism [91–93]. Notably, it was proposed that the T7 phage produces a Lon protease inhibitor that prevents bacterial toxin liberation [94], but given the controversies over Lon-mediated toxin activation, this phage defence route should be further examined. Second, a TA homologue exists in prophage as a modulator of phage production [95]. Third, an intriguing evolutionary trade-off was observed in the phage-defence *toxIN*: incomplete blockage of host transcription allows for phage propagation but activates the *toxIN* phage defence [96]. Additionally, an evolutionary experiment indicated that T4 phage can rapidly evolve to inhibit the ToxN toxin by acquiring segmental amplifications of the inhibitor gene [97]. Fourth, it has been indicated recently that phages benefit from an orphan toxin MazF in a phage-inducible chromosomal island to compete with other phages for the same bacterial host [98].

In light of the various evolutionary traces, it is tempting to speculate that TA's core purpose is phage defence rather than persistence. As a result, while the two functions are not incompatible, persistence may merely be a by-product of this phage defence mechanism, which explains why certain TA mutations have been identified as putative persistence mutations through evolutionary experiments but are unlikely to be key regulators of persistence.

LIMITATIONS OF RESEARCH ON TA-MEDIATED PERSISTENCE

Overexpression limitations, phage-contaminated cell lines and unstandardized persister assay parameters have greatly complicated TA research. Furthermore, although some studies report a good transcriptome–proteome correlation [99], a number of studies have proved it to be poor in multiple species [100–102], and hence studies probing the transcription of TA modules may not effectively represent the change in toxin or antitoxin concentration. Moreover, deletion studies are also hard to convincingly correlate TA with persistence due to redundancy and unclean deletions created inadvertently, often only causing a minor change in persistence. Furthermore, because most studies have been done *in vitro* on *E. coli* lab strains, more research on other bacterial species and animal models should be conducted to generalize TA's involvement in persistence to a range of conditions. Future research on TA should perhaps primarily work on persister assay standardization; here, a table documenting persister assay parameters of major studies on TA-mediated persistence is presented (Table 1). It is worth noting that some articles included in the table used the terms 'antibiotic tolerance' and 'antibiotic persistence' interchangeably but actually researched persistence rather than tolerance, an ambiguity resolved in a consensus statement released in 2019 [7].

SIGNALLING MOLECULES (QS/INDOLE) MEDIATE PERSISTER FORMATION

Quorum sensing (QS) is a synchronized population-wide response that was first reported in the 1990s when it was observed that the Gram-negative *Vibrio fischeri,* later reclassified as *Aliivibrio fischeri* [103], bioluminates exclusively upon symbiosis with marine creatures but not in a planktonic condition [104]. It was then discovered that the signal 3-oxo-C6-homoserine lactone (3-oxo-C6-HSL) generated by the *luxR/luxI* locus mediates bioluminescence in *V. fischeri* [104]. This finding was followed by subsequent discoveries of LuxR/LuxI systems in more than 70 Gram-negative species [105]. In *P. aeruginosa*, the two LuxR/LuxI systems (Las and Rhl) are intimately connected to the *Pseudomonas* quinolone signal (PQS) system [106], which regulates the generation of virulence factors including pyocyanin (PCN; also denoted as PYO in some papers) [107]. Notably, the three QS systems (Rhl, Las, PQS) in *P. aeruginosa* are interconnected and organized in a complicated network, though the advantage of such an organizational structure is not entirely clear [106].

In Gram-positive bacteria, QS via competence stimulating peptide (CSP) is associated with natural competence, the physiological state that enables bacteria to absorb exogenous ('naked') DNAs and integrate them into the genome [108]. This phenomenon was discovered in the Gram-positive *Streptococcus pneumoniae* in the 1920s [109], yet the precise nature of the secreted competence factor and its mechanism remained enigmatic until the mid-1990s [110]. After the identification of the CSP, the CSP-mediated QS circuitry has been researched extensively in *Streptococcus pneumoniae* [108]. Additionally, *Streptococcus pneumoniae* not only utilizes CSP to mediate competence but also virulence factor production and biofilm formation [111].

At the beginning of the 21st century, the expanding discoveries of putative QS molecules were followed by difficulties in distinguishing actual QS molecules from leaky metabolites [112]. One of the controversial signals is indole, an organic compound found in microbiota in a variety of habitats [113]. In an attempt to clarify such ambiguities, Winser *et al.* developed four qualifying criteria for QS molecules [112]: a QS molecule must (i) be produced at a specific stage, (ii) accumulate externally and be recognized by a receptor, (iii) cause a concerted response after its accumulation reaches a key threshold, and (iv) the response should extend beyond detoxification. Although indole was first supposed to be a QS signal, it was subsequently deemed to violate the key QS criteria due to challenges in establishing its cellular receptor and its distinctive pulse signalling observed in *E. coli* [11].

QS systems and indole have been linked with persister formation [10]. The LuxR/LuxI systems that produce acyl-homoserine lactone (AHL), the PQS precursor 2-amino-acetophenone (2-AA) and PCN have been implicated in persister formation in *P. aeruginosa* [10]. Furthermore, CSP has been indicated to regulate *Streptococcus mutans* persister formation [114], and indole-mediated persistence is widely investigated in *E. coli* and other species [11]. In this section, we will introduce the findings in QS and indole-mediated persistence, and the mechanisms proposed thus far are illustrated in Fig. 3.

The *P. aeruginosa* QS network mediates persister formation

In the *P. aeruginosa* QS network, PCN, HSL and 2-AA are implicated in persister formation. PCN is an oxidative phenazine compound speculated to be a terminal signalling factor in *P. aeruginosa* that promotes persister formation [115, 116]. Moreover, oxidative stress has been identified as a major contributor to PCN cytoxicity [117], and paraquat also induces persister formation in *P. aeruginosa* [116]. However, the SoxR protein, thought to be activated by PCN and implicated in the oxidative stress response [115], was not required for PCN-mediated persister formation [116]. Furthermore, H_2O_2 and phenazine-1-carboxylic acid (PCA), two oxidative agents, do not increase persister formation [116], indicating that PCN-mediated persister development may rely on pathways other than the key oxidative stress regulators such as SoxR and OxyR. Instead, it may rely on a PCN-specific response. It has been indicated that in addition to oxidative stress responses, PCN substantially induces efflux systems [118], and given that enhanced efflux activities are associated with persister formation in *E. coli* [119], the relationship between efflux systems and PCN-mediated persister production might be worthy of investigation. Still, it is important to note that PCN (2 mM) was added exogenously in this study [116], and a low level of PCN (0.2 mM) was not able to induce persister formation [116]. Moreover,

Table 1. Major studies in TA-mediated persistence and the parameters of persister assays

TA type	Strain	Growth phase	Antibiotics and concn	Antibiotic treatment time	Antibiotic treatment medium	Recovery condition	Results	Conclusion	Ref.
hipBA	*Escherichia coli* K12 MG1655	OD_{600}~0.1	AMP 100 µg ml^{-1}	ON	LB-AMP plate	Penicillinase spray/24 h incubation	HipA or HipA7 overexpression increases persistence	HipA promotes persistence	[26]
	E. coli K12 MG1655	OD_{600} 0.3–0.5	AMP 100 µg ml^{-1}	ON	LB-AMP plate	Penicillinase spray/ON incubation	Δ*relA*Δ*spoT* eliminates *hipA7* phenotype; RelA overexpression restores high persistence in Δ*hipA7* Δ*relA*	*HipA7* is (p)ppGpp-dependent	[31]
	E. coli BL21 (DE3)	Not indicated	AMP 100 µg ml^{-1}, CIP 100 µg ml^{-1}	3–4 h	Not indicated	Not indicated	HipA overexpression leads to multi-drug persistence	HipA promotes persistence	[25]
	E. coli K12 and KL312	~2×10^{9} c.f.u./ biofilm	CIP 5 µg ml^{-1}, MMC 5 µg ml^{-1}	1 day	LB broth	Vortexed in LB/diluted/plated	Δ*hipA*Δdif shows reduced persistence, but not Δ*hipA*	*HipBA*'s role in persistence cannot be confirmed	[28, 29]
	Caulobacter crescentus NA1000	OD_{600} 0.2–0.4 or ~1.0	STR, KAN, VAN (10–500×MIC)	3 days	PYE broth	Diluted in PYE/1 week of incubation	Δ*hipBA* shows reduced persistence, which is restored by HipBA overexpression; SR is required for stationary phase but not exponential phase	HipA promotes persistence via multiple pathways	[33]
hipBA, mqsRA	*E. coli* K12 MG1655	stationary phase	AMP 200 µg ml^{-1}, AMK 25 µg ml^{-1}	3 h	LB broth	Diluted in PBS/plated/ON incubation	*HipA7* and Δ*mqsR*'s phenotype depends on inoculum age; amikacin kills cells from all ages	*HipA7* and Δ*mqsR*'s phenotype is conditional	[30]
hipBA, relBE, vapBC, phd/doc, mazEF	*Klebsiella pneumoniae* Kp9T, Kp21T	18-h-old biofilm	CST (50×MIC)	1 day	LB broth	Diluted/plated/24 h incubation	All TAs except *phd/doc* are upregulated in biofilm persisters	The studied TAs might mediate persistence	[24]
hipBA, relBE, yafNO, mazEF	*E. coli* K12 MG1655	30 min induction after OD_{600} ~0.25	CIP 2 µg ml^{-1}	4 h	LB broth	Centrifuged/resuspended in LB/diluted/ plated	HipA overexpression increases (p)ppGpp accumulation and fails to induce persistence in Δ*relA*; *mazEF*, *yafNO* and *relBE* are independent of (p)ppGpp	Only HipA-induced persistence is dependent on (p)ppGpp	[32]*
mazEF, relBE, dinJ-yafQ	*E. coli* K12 MG1655	10^{7} c.f.u. ml^{-1} or stationary phase	AMP 50 µg ml^{-1}, CTX 100 µg ml^{-1}, OFX 5 µg ml^{-1}, MMC 10 µg ml^{-1}, TOB 25 µg ml^{-1}	3 h	LB broth	Washed/diluted/plated	RelE overexpression increases persistence to all the antibiotics expect MMC; Δ*relBE*, Δ*mazEF* or Δ*dinJ-yafQ* has no effect on persistence	RelE promotes persistence	[28]
relBE	*Mycobacterium tuberculosis* ATCC 27294	OD_{650} 0.01–0.02	RIF 4 µg ml^{-1}	1 week	7H9 broth	Centrifuged/wahsed/diluted in 7H9/plated	RelE overexpression promotes persistence, while Δ*relE* shows reduced persistence	RelE promotes persistence	[14]
mazEF, relBE	*Staphylococcus aureus* N315 and 5 clinical MRSA isolates	OD_{600}~0.4	CIP 5 µg ml^{-1}	1 day	BHI broth	Washed in PBS/diluted/plated	*MazF* and *relE* are upregulated	*MazEF* and *relBE* might mediate persistence	[34]
mazEF, reBE, sprFG	*S. aureus* MRSA and MSSA	48-h-old biofilm	VAN (40×MIC), GEN (4×MIC), CIP (50×MIC)	3 h	TSB broth	Washed in PBS/resuspended/vortexed/ diluted/plated	*MazF*, *relE* and *sprG* are upregulated	*MazEF*, *reBE* and *sprFG* might mediate persistence	[35]

Continued

Table 1. Continued

TA type	Strain	Growth phase	Antibiotics and concn	Antibiotic treatment time	Antibiotic treatment medium	Recovery condition	Results	Conclusion	Ref.
parDE	*E. coli* J53	3 h induction after OD_{600} ~0.5	CIP (78×MIC), GEN (64×MIC), CTX (64×MIC)	5 h	Supplemented M9 broth	Diluted/plated	Overexpression of *Enterobacteriaceae* ParE induces SOS response genes and increases persistence in *E. coli*	*ParDE* might mediate persistence	[37]
dinJ-yafQ, mqsRA, mazEF, GhoST	*E. coli* K12 MG1655	OD_{600} ~0.8 or 2 h induction after OD_{600} ~0.2	CIP 5 µg ml^{-1}, AMP 100 µg ml^{-1}	3 h	LB broth	Centrifuged/resuspended in saline/drop assay	Overexpression of all the studied toxins leads to increased persistence in Δ*relA*Δ*spoT*	*dinJ-yafQ*, *mqsRA*, *mazEF* and *GhoST* are (p)ppGpp-independent	[44]
mqsRA	*E. coli* K12 BW25113	OD_{600} ~0.5 or 2 h induction after OD_{600} ~0.5 (all adjusted to 1)	AMP 100 µg ml^{-1}	2 h	LB broth	Diluted in saline/plated	Δ*mqsR* or Δ*mqsRA* shows reduced persistence; MqsR overexpression increases persistence	MqsR promotes persistence	[38]
	E. coli K12 MG1655	Mid-exponential phase (~ 5×10^7 cells ml^{-1})	CTX 100 µg ml^{-1}, MMC 10 µg ml^{-1}, OFX 5 µg ml^{-1}, TOB 20 µg ml^{-1}	3 h	LB broth	Diluted in PBS/plated	MqsR overexpression increases persistence, while Δ*mqsR* does not show reduced persistence	MqsR promotes persistence	[39]
yefM-yoeB	*Edwardsiella tarda* TX01	OD_{600}~0.5 then diluted with LB to 10^7 cells	CHL (50×MIC)	5 h	LB broth	Washed in PBS/diluted/plated	ΔyoeB and ΔyefM-yoeB show reduced persistence	*YefM-yoeB* mediates persistence	[42]
	E. coli K12 MG1655	OD_{600}~0.5	AMP 100 µg ml^{-1}, CIP 1 µg ml^{-1}, CHL 100 µg ml^{-1}, KAN 50 µg ml^{-1}, ERY 100 µg ml^{-1}	4 h	LB broth	Centrifuged/diluted/plated	Δ*relA*Δ*spoT* and Δ*ppk*Δ*ppx* show an upregulation of *yefM-yoeB* during amino acid starvation	*yefM-yoeB* is independent of polyP and (p)ppGpp	[43]
	S. aureus JE2 and Newman	24-h-old biofilm	CFZ, VAN (10×MIC)	2 days	TSB broth	Washed/sonicated/plated	Δ*yoeB* shows reduced persistence in a strain- and antibiotic- dependent manner	*YefM-yoeB* might mediate persistence	[41]
		0.5 McFarland turbidity then diluted to 10^7 c.f.u.	CFZ, VAN (10×MIC)	1 day	TSB broth	Not indicated			[41]
dinJ-yafQ	*E. coli* K12 BW25113	Overnight culture diluted to OD_{600} ~0.4	CIP 1 µg ml^{-1}	4 h	LB broth	Washed/centrifuged/resuspended in PBS/plated	Δ*yafQ* and Δ*dinJ* show slightly larger persister fractions than WT	*DinJ-yafQ*'s role in antibiotic persistence is unclear	[46]
		10^5–10^6 c.f.u./peg	CFZ 0.25–256 µg ml^{-1}, TOB 0.03125–32 µg ml^{-1}, DOX 0.03125–32 µg ml^{-1}, RIF 0.25–256 µg ml^{-1}	1 day	LB broth	Sonicated/diluted in saline/plated	Δ*yafQ* decreases biofilm persistence towards CFZ and TOB but not DOX or RIF; YafQ overexpression increases biofilm persistence to CFZ and TOB	YafQ promotes biofilm persistence	[45]
		Early stationary phase (ON growth)	CFZ 0.5–512 µg ml^{-1}, TOB 0.0625–64 µg ml^{-1}	1 day	LB broth	Centrifuged/resuspended/plated	Δ*yafQ* has no impact on planktonic persistence	YafQ is unlikely to mediate planktonic persistence	[45]

Continued

Table 1. Continued

TA type	Strain	Growth phase	Antibiotics and concn	Antibiotic treatment time	Antibiotic treatment medium	Recovery condition	Results	Conclusion	Ref.
shpAB	*Salmonella enterica* serovar Typhimurium LT2	Immediately after preculture dilution	AMP 100 μg ml^{-1}	ON	LB-AMP plate	Penicillinase spray/ON incubation	The *shp* mutant displays a multi-drug hyper-persistence phenotype, which is Lon-dependent but RelA-independent (when challenged with ampicilin)	*ShpAB* mediates persistence	[48]
		1 h incubation	OFX 5 μg ml^{-1}, PIP 200 μg ml^{-1}, CHL 20 μg ml^{-1}	4 h 30 min	LB broth	Centrifuged/resuspended in LB/plated			[48]
symER, tisB-istR, dinJ-yafQ, yafNO	*E. coli* K12 MG1655	24-h-old biofilm	OFX 5 μg ml^{-1}	1 day	M63B1 minimal broth	Not indicated	Δ4TA has no impact on persistence in biofilms upon leucine starvation	The studied TAs are not essential for persistence in starved biofilm	[47]
mazEF	*E. coli* K12 BW25113	1 h induction after OD_{600} ~0.2	CIP 0.4 μg ml^{-1}, CTX 100 μg ml^{-1}, MMC 10 μg ml^{-1}, TOB 25 μg ml^{-1}, STR 25 μg ml^{-1}	4 h	LB broth	Washed/diluted/plated	MazF (E24A) overexpression increases persistence, which is dependent on Lon and ClpP but independent of RecA	MazF mediates drug persistence dependent on Lon and ClpP	[51]
	S. aureus SH1000, Newman, and JE2	0.5 McFarland turbidity	CFZ, VAN (10×MIC)	one d	TSB broth	plated	Δ*mazF* decreases persistence only in strains with increasing doubling rate (JE2 and SH1000)	*MazEF* mediates planktonic persistence	[52]
		4-day-old biofilm	CFZ, VAN (10×MIC)	3 days	TSB broth	Washed/removed/sonicated/plated	Δ*mazF* decreases biofilm persistence in JE2, Newman, and SH1000	*MazEF* mediates biofilm persistence	[52]
pemIK	*K. pneumoniae* ST846-OXA48	OD_{600}~0.6	IPM (50×MIC), CHX (10×MIC)	2 days	LN-LB broth	Diluted/plated	Removing the *pemIK*-containing OXA48CA plasmid eradicates bacterial recovery after treatments	*PemIK* might mediate persistence	[53]
VapBC	*M. tuberculosis* Erdman	Diluted to OD_{600} ~0.2 following induction	AMK 0.78 μM, STR 0.2 μM, EMB 1.56 μM	12 h	7H9 broth	Diluted/plated	VapC21 overexpression increases persistence, which is counteracted by the cognate antitoxin VapB21 and the non-cognate VapB32; *vapC21* deletion has no impact on persistence	*VapBC* mediates persistence and crosstalks exist between *vapBC* homologues	[54]
tacAT	*S. enterica* serovar Typhimurium 12023	1 h induction after OD_{600} ~0.05	CFX 100 μg ml^{-1}	4 h	M9 minimal broth	Washed/plated	TacT overexpression increases persistence	TacT promotes persistence	[198]
hicAB	*Burkholderia pseudomallei* K96243	Early stationary	3 h induction then standardized to 2×10^8 cells ml^{-1}	1 d 6 h	LB broth	Washed/diluted/plated	Δ*hicAB* displays reduced persistence towards CIP but not CAZ; HicA overexpression increases persistence	*HicAB* mediates persister formation	[204]
PA14_51020/ PA14_51010	*Pseudomonas aeruginosa* PA14	OD_{600} 0.8–1.0	CIP 0.5 μg ml^{-1}, TOB 5 μg ml^{-1}	8 h	LB broth	Diluted/plated	PA14_51010 overexpression or Δ*PA14_51020* increases persistence; PA14_51010 overexpression reduces NAD^+, which is counteracted by the NAD^+ overproduction	The studied TA mediates persistence by affecting NAD^+ production	[57]

Continued

Table 1. Continued

TA type	Strain	Growth phase	Antibiotics and concn	Antibiotic treatment time	Antibiotic treatment medium	Recovery condition	Results	Conclusion	Ref.
smuATR	*Streptococcus mutans* UA159	Immediately after preculture dilution	OFX 20 μg ml^{-1}, OXA 2 μg ml^{-1}, CTX 2 μg ml^{-1}, VAN 20 μg ml^{-1}	1 d	THYE broth	Diluted/plated	Δ*smuATR* does not affect persistence; the CSP-inducible persistence phenotype was eliminated for Δ*smuATR*	*SmuATR* mediates CSP-induced persistence	[58]
	Streptococcus pyogenes M4	Immediately after preculture dilution	ampicillin 10 μg ml^{-1}	5 h	THYE broth	Diluted/plated	SmuAT overexpression increases	*SmuAT* induces persistence	[58]
pasTI	*E. coli* MG1655, CFT073	~2×10^8 c.f.u. ml^{-1}	CIP 2 μg ml^{-1}, CFX 200 μg ml^{-1}	5 h	LB broth	Centrifuged/resuspended/diluted/plated	Δ*pasTI* shows reduced persistence in CFT073 but not MG1655	*PasTI* mediates persistence	[59]
BCAM0272/ BCAM0271	*Burkholderia cenocepacia* J2315 (LMG16656)	24-h-old biofilm	TOB or CIP in a concentration of 4×MIC	1 day	LB broth	Vortexed/sonicated/plated	BCAM0271 overexpression increases persistence	*BCAM0272/BCAM0271* mediates persistence	[61]
`BPSS1321, BPSL1494	*E. coli* S17	Adjusted to 5×10^8 c.f.u. ml^{-1}	LVX (10×MIC)	1 day	LB broth or supplemented RPMI	Diluted/plated	Δ*BPSS1321* and Δ*BPSL1494* reduces persistence in LB but not RPMI	BPSS1321 and BPSL1494 might promote persistence	[62]
	Burkholderia thailandensis	2 h induction after OD_{600} ~0.2 then normalized to the same concentration	LVX (10× or 5×MIC)	1 day	Supplemented M9 broth	Not indicated	BPSS1321 (but not BPSL1494) overexpression promotes persistence	BPSS1321 might promote persistence	[62]
tisB/istR	*E. coli* K12 MG1655	Exponential phase	CIP 1 μg ml^{-1}, AMP 50 μg ml^{-1}, STR 25 μg ml^{-1}, CMS 10 μg ml^{-1}	3 h	MHB broth	Not indicated	Δ*tisB/istR* shows reduced persistence to ciprofloxacin, and TisB overexpression leads to muti-drug persistence	*TisB/istR* mediates persistence	[205]
		100 min incubation after preculture dilution	AMP 200 μg ml^{-1}, CIP (100×MIC)	6 h	MHB broth	Diluted/plated on LA plates	Δ1-41Δ*istR* (ΔΔ) displays high persistence	Regulatory RNAs of *tisB/istR* are implicated in persistence	[65]
		OD_{600}~0.4	CIP (100×MIC), MMC (4×MIC)	6 h	LB broth	Diluted in saline/plated on supplemented LB	Although ΔΔ displays higher persistence than WT towards CIP, WT displays better survival than ΔΔ upon MMC treatment	ΔΔ's phenotype is conditional	[66]
hokB/sokB	*E. coli* BW25113	Immediately after preculture dilution	CAZ 50 mg ml^{-1}, OFX 5 mg ml^{-1}, TOB 100 mg ml^{-1}	5 h	LB broth	Diluted in 10 mM $MgSO_4$/plated	Obg mediates persistence by activating *hokB* transcription in a (p)ppGpp-dependent but polyP-independent way	*HokB/sokB* mediates persistence	[63]

*This paper was retracted after the authors discovered contaminations caused by inadvertent lysogenization with the bacteriophage Φ80; notably, while fig. 3, fig. 4c and d, fig. 5c and d, fig. S2, fig. S3, and fig. S4C and D. were reported to be affected by Φ80, the data cited in this table come from fig. 1, which is unaffected by the contamination.

AMK, amikacin; AMP, ampicillin; BHI, brain heart infusion; CAZ, ceftazidime; CFZ, cefazolin; CHL, chloramphenicol; CHX, chlorhexidine; CIP, ciprofloxacin; CMS, colistin methane sulfonate; CST, colistin; CTX, cefotaxime; DOX, doxycycline; EMB, ethambutol; ERY, erythromycin; GEN, gentamicin; IPM, imipenem; KAN, kanamycin; LA, Luria agar; LB, Luria-Bertani; LN-LB, low-nutrient Luria-Bertani; LVX, levofloxacin; MHB, Mueller-Hinton broth; MMC, mitomycin C; OFX, ofloxacin; ON, overnight; OXA, oxacillin; PBS, phosphate buffered saline; PIP, piperacillin; PYE, peptone yeast extract; RIF, rifampicin; RPMI, Roswell Park Memorial Institute; STR, streptomycin; THYE, Todd-Hewitt broth supplemented with 0.3% (w/v) yeast extract; TOB, tobramycin; TSB, tryptic soy broth; VAN, vancomycin.

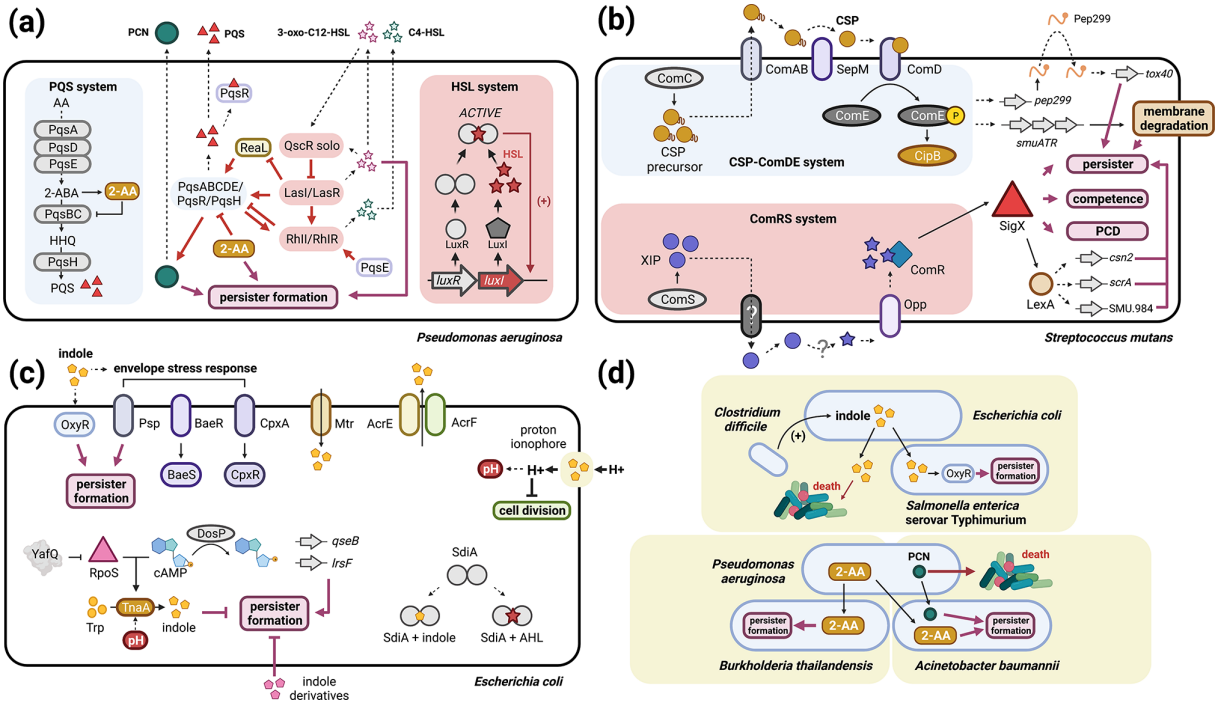

Fig. 3. Quorum sensing (QS) and indole mediate persister formation. (a) The *Pseudomonas* quinolone signal (PQS) and HSL systems in *Pseudomonas aeruginosa*. The *P. aeruginosa* QS systems include the PQS system and two HSL-producing systems, LasI/LasR and RhlI/RhlR. PQS biosynthesis is indicated in the blue box, and PQS is recognized by the PqsR receptor [206]. AA, anthranilic acid; 2-ABA, 2-amino benzoyl acetate; HHQ, 2-heptyl-4-(1H)-quinolone; 2-AA, 2-amino-acetophenone. In the HSL system paradigm (red box), a LuxI protein homologue synthesizes the HSL signal, and a LuxR protein homologue binds the HSL signal as a receptor. LasI/LasR and RhlI/RhlR produce and recognize *N*-3-oxododecanoyl homoserine lactone (3-oxo-C12-HSL) and *N*-butanoyl-L-homoserine lactone (C4-HSL), respectively [207], and a QscR orphan receptor devoid of a corresponding LasI homologue recognizes several signals including 3-oxo-C12-HSL [208]. Additionally, PqsE is essential for RhlR-dependent QS [209], and the small RNA ReaL is also embedded in the network [210]. The interactions within the *P. aeruginosa* QS system are indicated in red arrows [115, 209–213]. In this network, 3-oxo-C12-HSL, 2-AA and PCN have been suggested to promote persister formation in *P. aeruginosa*. (b) The CSP-ComDE (blue) and ComRS (red) system in *Streptococcus mutans*. In the CSP-ComDE system, CSP is produced, exported, modified and sensed, eventually upregulating CipB production; in the ComRS system, the double-tryptophan-containing signal peptide XIP is produced, sensed outside the cell and imported, eventually inducing the σ factor SigX. SigX activation leads to three divergent outcomes, persister formation, competence or programmed cell death (PCD) [114]. CSP-mediated persistence has been associated with the ComRS system, the tripartite TA module *smuATR* and the LexA regulator in the SOS response [58, 214]. Recently, it has been suggested that the signalling peptide Pep299 participates in CSP-mediated persistence via activating a Type II toxin gene *tox40* [130]. (c) Indole and QS regulators in *E. coli*. Indole is synthesized by tryptophanase (TnaA) from tryptophan (Trp). Although indole has been linked to a range of physiological changes, many key questions remain unanswered, including the authenticity of its putative cellular receptor (the LuxR solo SdiA) and membrane transporters (AcrEF and Mtr), as well as whether it triggers or inhibits persister development [70]. Considering that indole elicits a response in three envelope stress responses (Psp, BaeSR, CpxRA) and its high concentration in the cell membrane (50–100 mM), indole's main target may be the cell membrane [11]. Interestingly, cellular pH both regulates and is influenced by indole synthesis, and regulation of cellular pH by indole production has been indicated to be a distinctive trait of *E. coli* persisters [139–141]. Notably, indole leads to persister formation via at least three routes (OxyR and Psp, yafQ, and DosP) [135, 144, 146]. Additionally, two QS regulators, *qseB* and *lrsF*, are associated with *E. coli* persister formation [215]. (d) Signalling molecules might mediate persister formation in bacterial co-culture. In bacterial co-culture, bacteria might utilize signalling molecules (e.g. indole, 2-AA, PCN) to help their allies persist and colonize the host.

only PCN added to log-phase cells promoted persister formation [116], whereas PCN is usually produced during stationary phase [115], questioning whether PCN alone can induce persister formation under physiological conditions.

Besides PCN, 3-oxo-C12-HSL also significantly increases persister formation in *P. aeruginosa* [116]. Interestingly, while a low level of PCN (0.2 mM) alone does not induce persister formation, PCN increases persister numbers when accompanied by a low concentration of 3-oxo-C12-HSL [116], suggesting that persister formation might be mediated by 3-oxo-C12-HSL instead of PCN at this concentration. Moreover, 2-AA, a precursor of PQS, promotes persister formation by downregulating the transcription of genes involved in the translational capacity [120], an effect similar to Type II TA modules, suggesting that translation inhibition may be a general mechanism of persister formation. Corroborating this finding, 2-AA production inhibition resulting from PqsR antagonism reduces persister numbers, and 2-AA allows *P. aeruginosa* to persist *in vivo* [121]. Notably, three studies investigating the effect of the QS inhibitor BF8 on persister development were quoted to show the impact of QS inhibition on

persister formation [10]. However, these studies revealed that although BF8 inhibits persister formation, such an effect is not solely due to QS inhibition, and BF8 at least might have other cellular targets [122–124].

Given the intricate interplay of *P. aeruginosa* QS systems and the participation of at least three signals in persister generation, future research should focus on determining how several signals might work synergistically to facilitate persister formation in this QS network. Moreover, a link has been made between (p)ppGpp and the QS network in *P. aeruginosa* based on the observation that (p)ppGpp modulates the AHL-producing Rhl and Las system as well as the PQS system [125]. Considering that (p)ppGpp has long been implicated in persister formation [126], (p)ppGpp's involvement in QS-mediated persistence in *P. aeruginosa* may warrant additional research.

Streptococcus mutans competence stimulating peptide (CSP) mediates persister formation

CSP is considered an 'alarmone' because it stimulates competence [114], a stress response promoting DNA repair, and activates stress response genes such as the bacterial heat shock regulons. Therefore, it should not be surprising that CSP also mediates antibiotic persistence, which is a strategy that helps to combat antibiotic stress. In fact, three possible outcomes have been proposed for CSP signalling in *Streptococcus mutans*: competence, programmed cell death (PCD) and persister formation [114]. A complete signalling pathway encompassing ComDE, ComRS and the σ factor SigX is required for CSP-mediated persistence [127]. Moreover, a non-canonical SOS pathway involving LexA and LexA-regulated genes (*csn2*, *scrA* and SMU.984) was shown to affect CSP-mediated persister formation towards different antibiotics including some that do not cause DNA damage [127], hinting that CSP might mediate persistence to a variety of antibiotic actions as a general 'alarmone'. Interestingly, the tripartite TA module *smuATR* was shown to be essential for CSP-mediated persister formation by permeabilizing the cell membrane [58]. However, 2 µM CSP was added to the culture [58], whereas 1 µM has been indicated as the physiological condition [128], and hence it is unknown whether this pathway also applies to this physiological concentration. Additionally, it was speculated that CipB could insert into the cell membrane and form pores, decreasing the proton motive force and consequently the cellular ATP level, causing persister formation similar to the action of Type I TA modules [129]. Recently, a DNA damage-inducible gene *pep299* specifically activated in the persister population was suggested to code for an intraspecies signalling peptide Pep299. Moreover, Pep299 is required for CSP-mediated persistence in *Streptococcus mutans* via activation of the *tox40* gene (a Type II toxin gene) in the CSP-induced persister population [130].

These data suggest that CSP can lead to persister production via several routes, but no model unifies all of these processes. Moreover, how ComDE and ComRS are connected on a molecular level remains unknown and is greatly complicated by the medium-dependent response: the XIP response can be observed in a chemically defined medium rich in amino acids and lacking peptides but is hardly observed in peptide-rich media containing peptone, presumably because XIP will have to compete with these peptides to enter the cell via the Opp peptide transporter [131]. Therefore, future studies should select media with appropriate compositions. Notably, the *Streptococcus pneumoniae* genome encodes a *blpRH* locus paralogous to *comCDE*, which regulates bacteriocin production, and *Streptococcus mutans comCDE* shares a higher degree of homology to the *blp* locus than the *comCDE* locus in *Streptococcus pneumoniae* [131]. Moreover, the ComCDE system in *Streptococcus pneumoniae* has been speculated to participate in antibiotic stress tolerance [132]. Collectively, these findings imply a possible correlation between *Streptococcus pneumoniae blpRH* and persistence awaiting further confirmation.

Indole mediates persister formation in *E. coli* and other species

Indole is an intra-species, inter-species and inter-kingdom aromatic signalling compound produced by over 85 bacterial species [133]. It is involved in various biological processes including spore formation, drug resistance, virulence, plasmid stability and biofilm formation [11, 133].

Two modes of indole signalling have been characterized: 'persistent' and 'pulse', and the latter denotes the transient yet strong rise (~60 mM) in intracellular indole during the transition to stationary phase [134]. Adding an extra layer of complexity, two distinctive functions of indole have been proposed: stress response and stress insurance [11]. As stress insurance, indole increases persistence via OxyR and the phage shock response pathway to 'inoculate' bacteria from antibiotics, and this increase was supported by a later study [135, 136]. Indole pulse signalling has been shown to generate *E. coli* quinolone persisters by targeting DNA gyrase, and indole production was not increased in response to the antibiotic stress [137]. In *Pseudomonas fluorescens*, exogenous indole that could come from the plant rhizosphere enhances persister formation via modulating efflux systems and protect this rhizobacterium from multiple antibiotics [138]. As a proton ionophore, indole inhibits *E. coli* cell division at a high concentration (3–5 mM) and regulates *E. coli* pH via pulse signalling [139, 140]. Notably, it has been demonstrated that indole production is essential for maintaining a distinctive lower pH in *E. coli* persisters before ampicillin treatment [141], suggesting that indole's regulation of pH level might also account for a form of stress insurance.

Indole can also be part of the central stress response involving RpoS. Notably, depending on the experimental setup, RpoS deletion can either increase or inhibit persistence [142, 143]. Two RpoS-regulated mechanisms by which indole decreases persister formation have been proposed: (i) the Type II toxin YafQ inhibits RpoS, while RpoS activates TnaA – hence, YafQ decreases indole

production, thus increasing persistence [144]; and (ii) direct oxygen sensing phosphodiesterase (DosP), which has been suggested to be upregulated by RpoS [145], degrades cAMP, which lowers indole levels and increases persistence [146]. Notably, the first study utilized overexpression studies to establish YafQ's role in persister formation, and hence the result should be interpreted cautiously [144]. In *Vibrio cholerae*, indole is part of the stress response: cellular indole level rises in response to a sub-inhibitory level of aminoglycoside, activating RaiA-mediated persistence [147]. Notably, halogenated indole derivatives could potently inhibit *E. coli* and *Staphylococcus aureus* persisters [136].

Collectively, these findings suggest that indole may either operate as insurance that safeguards bacteria from future stress by boosting persistence, or it can be part of a stress response. Indole promotes persistence in certain circumstances but inhibits persistence in others. These seemingly contradictory findings illustrate the enigmatic activity of indole, which changes substantially depending on the species under investigation and relevant experimental conditions.

QS mediates persister formation in other bacteria

QS-regulated phenol-soluble modulins decrease persister formation in *Staphylococcus aureus* [148]. Moreover, the *Legionella* QS (Lqs) system controls persistence in *Legionella pneumophila* biofilms [149]. These findings suggest the possibility that signalling molecules other than AHL, CSP and indole might mediate persister development, highlighting the need to verify the role of QS in various species.

Co-existing in a community: signals as shields and swords in microbial warfare

Considering that QS molecules and indole regulate persistence and influence community-level behaviours, it is tempting to speculate that in bacterial co-cultures, these molecules might participate in microbial warfare via mediating persistence in an interspecies manner.

This is especially true for indole, which only controls a few genes of *E. coli* but is considered to greatly influence its neighbours as a 'manipulative' interspecies and interkingdom bacterial signal [150, 151]. Remarkably, it has been suggested that the gut pathogen *Clostridium difficile* manipulates *E. coli* and other indole-producing bacteria to produce a high concentration of indole that can be used to inhibit the growth of most gut-protective bacteria [152]. This might alter the colonization resistance in the gut microbiome, thus allowing *C. difficile* to persist [152]. Additionally, indole produced by *E. coli* promotes *Salmonella enterica* serovar Typhimurium persister formation mainly via the oxidative stress response [153]. However, *E. coli* competes with *P. aeruginosa* by upregulating indole production, thus inhibiting PCN production and AHL-regulated virulence factors in *P. aeruginosa* [154]. Considering that PCN and AHL mediate persistence in *P. aeruginosa,* these hostile actions of *E. coli* indole might decrease *P. aeruginosa*'s capacity to form persisters and hence diminish its advantage, although this is yet to be confirmed. Moreover, indole's quorum quenching (QQ) ability, such as interfering with QS regulator folding and upregulating QQ enzymes, highlights its potential in obstructing competitors' QS systems and decreasing persistence [155, 156].

Similarly, while PCN has antimicrobial effects on species including *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis* [157], it facilitates persister formation in *Acinetobacter baumannii* [158]. Moreover, 2-AA produced by *P. aeruginosa* increases persister formation in both *Burkholderia thailandensis* and *A. baumannii* [120]. Notably, although *E. coli* does not produce AHL signals, it has a LuxR solo receptor SdiA [11]. SdiA is highly promiscuous, responding to AHL signals with multiple chain lengths and, controversially, to indole [11, 159]. Since *E. coli* does not synthesize AHL signals, it has been suggested that SdiA's function might be eavesdropping in a bacterial co-culture [160]. Moreover, AHL-SdiA has been associated with decreased biofilm formation [161], and SdiA deletion increases motility [162]. Collectively, these findings suggest that SdiA activation inhibits biofilm formation [160]. Considering that biofilm formation provides a safe haven for persister formation [163], it is plausible that SdiA might be manipulated by AHL-producing bacteria to inhibit *E. coli* persister formation, with AHL acting as a weapon. However, this relationship has yet to be confirmed.

These findings imply that, aside from protecting bacteria themselves against environmental stressors, one of the primary goals of these signals in bacterial communities might be to protect comrades while eradicating undesirable neighbours by modulating persister development, and thus facilitating host colonization in a competitive microbiome. As a result, in addition to studying how QS signals and indole drive persister development in isolated colonies, future research may also explore how a network of interspecies signals might mediate persistence and function as both shields and swords in microbial warfare.

THE MISSING LINK: BRIDGING PERSISTER MECHANISMS WITH ANTIBIOTIC ACTIONS

Given the variety of antibiotic mechanisms, different classes of antibiotics are likely to orchestrate persistence via different pathways. Hence, a link should be made between the specific antibiotics used and the stress responses (e.g. SR, SOS) that regulate signalling-mediated persistence in order to hopefully bridge different mechanisms regulated by the same stress responses. In many situations, antibiotics were not selected in accordance with the particular pathway under investigation. For example, despite various attempts to link TA modules to the SR, a response primarily activated by stalled ribosomes, quinolones (DNA

gyrase inhibitors) and beta-lactam antibiotics (cell wall synthesis inhibitors) have been widely used, antibiotics that might not be very relevant in this particular context [31, 32, 44, 71, 75]. As a result, knowing the mechanism of action of various antibiotics is essential when designing persister assays to obtain a valid conclusion on the persister development pathway under inquiry. Protein synthesis inhibitors such as aminoglycosides can be utilized to investigate SR's role in modulating persistence, while DNA gyrase inhibitors can be employed to investigate mechanisms regulated by the SOS response. It is important to note that a persister mechanism directed towards a single class of antibiotics cannot be generalized to others.

In the case of signalling molecules, it has been indicated that CSP-inducible ofloxacin-persister formation is affected by the LexA pathway, presumably differently from the SOS-like response pathway in streptococci [127], and indole has been shown to mediate quinolone-persister formation by specifically targeting the GyrA subunit of DNA gyrase [137], although this pathway's relationship to the SOS response is unclear. Still, future research should probe other antibiotic-specific and QS/indole-mediated persister mechanisms.

BEYOND THE GENETIC CODE: EPIGENETICS AS A POTENTIAL PERSISTER MECHANISM

Epigenetics is defined as the alterations in the gene expression profile of a cell that are not caused by changes in the DNA sequence [164], and one of the best characterized bacterial epigenetic signals is DNA adenine methylation [12]. Since persistence is a non-genetic single-cell heterogeneity [7], it is tempting to speculate that persistence may not be purely a genetically wired strategy (e.g. TA, QS) but may also be controlled by the epigenome. Interestingly, a computational model suggests that epigenetics is associated with several reported persistence properties [164]. Moreover, it was suggested that epigenetic inheritance modulated by the transcription factor YdcI might explain a potential *E. coli* persister mechanism [165]. Additionally, it was shown that DNA adenine methylation might be involved in uropathogenic *E. coli* persister formation [166]. Given recent discoveries linking epigenetics to the generation of antibiotic resistance and a recent hypothesis combining bacterial memory of persistence and epigenetics [12, 167, 168], the epigenome may also serve as a repository for persistence strategies, speculation that merits more exploration in the future.

IS PERSISTER FORMATION STOCHASTIC, DETERMINISTIC OR BOTH?

Persisters have long been classified into two types: some encounter stress signals (e.g. stationary-phase starvations) and enter dormancy (Type I or 'triggered persistence'), while others 'stochastically' enter a non-growing state without outside stimuli (Type II or 'spontaneous persistence') [169]. However, the vagueness of such a description raises questions such as how 'stochasticity' contributes to persister formation and the range of applicability of the two persister types.

'Stochasticity,' or 'gene expression noise', is now defined as the stochastic events at the level of transcription and translation [170]. To date, stochasticity in several genetic circuits has been considered to be behind *E. coli* persister formation, including those producing HipBA [171], the integration host factor (IHF) [172] and Krebs cycle enzymes [173]; the noisy expressions of the final two result in a bacterial subpopulation with low ATP levels and hence enhanced persistence [172, 173]. In *Mycobacteria*, the stress response pathway involving the σ factor SigE is characterized by a bimodal expression of the stringent response modulator Rel, resulting in a subpopulation with a high Rel translation (H-state) [174, 175], and since SigE has also been implicated in persister formation [176], the H-state might represent persistence, although this speculation has not been validated. Additionally, it was recently reported that stochasticity associated with acetate kinase essential for acetate to enter the Krebs cycle mediates persistence in *M. tuberculosis* [177]. In *Bacillus subtillis*, the noise-driven ComKS system regulates competence and growth arrest, imposing a triggered persistence [178]. Interestingly, the epigenetic computational model mentioned in the section above shows that epigenetic inheritance, in conjunction with cellular noise, expounds on many aspects of persistence (including the triggered vs. spontaneous classification), underlining the possibility that persistence is not solely a genetically coded tactic [164]. However, the precise role of biological noise is still under debate [170], and future studies probing noise-mediated persistence should validate computational findings with persister assays.

In an attempt to unify the different persister mechanisms, a ppGpp ribosome dimerization persister (PRDP) model that involves cAMP, (p)ppGpp, RaiA and other factors has been proposed, where the activation and inactivation of ribosomes play a major role in persister formation and resuscitation [179]. Shortly after the PRDP model was published, it was proposed that indole signalling activates RaiA to mediate persistence in *V. cholerae* [147]. Therefore, exploring whether indole signalling fits in the PRDP model deserves future investigation. Notably, this model also argues that persistence is a deterministic stress response rather than a stochastic event, in line with the findings that external stress transforms practically the entire population into persister cells, albeit this model's applicability requires further investigation [179].

Although Bigger recognized the difference between triggered and spontaneous persisters in the 1940s [5], the classification remains somewhat rudimentary since it is still primarily based on phenotypic variance, and no molecular insight can clearly distinguish between the two types. Furthermore, the measurements of spontaneous persisters are often confounded by lingering triggered persisters from the preceding stationary phase [7]. Given the ambiguity in the classification, additional efforts should

be made in the future to standardize the classification and evaluate its applicability to connect it with other related mechanisms and determine to what extent and under what conditions persister formation is stochastic or deterministic.

TECHNICAL LIMITATIONS AND PROSPECT: FROM BULK TO SINGLE-CELL

Since persistence depends highly on medium composition, age of pre-culture, aeration rate, bacterial stage of growth, and many other persister assay parameters [180], it is critical to utilize a standardized and optimized method for persister measurements to improve the reproducibility of results. It is crucial to highlight the most robust protocol of persister assay published by Goormaghtigh and Van Melderen in 2016 [180], which may merit further use in the future. Furthermore, in an effort to further unify the field, a consensus statement issued in 2019 gave clear-cut guidelines for persister measurements, highlighting approaches to distinguish persister cells from tolerant cells or VBNCs [7].

While the traditional persister assay is a bulk measurement that offers information primarily at the population level, novel microfluidic approaches might provide a plethora of information regarding populational heterogeneity at single-cell resolution [181]. Notably, microfluidic approaches have been used to investigate the physiology of persisters and VBNCs previously undiscovered using bulk measurements, suggesting microfluidics as a promising approach for future persistence research [141, 182, 183].

BRIDGING PERSISTENCE RESEARCH AND CLINICAL PRACTICE

To translate persistence research into more therapeutically relevant insights, future research should study persister mechanisms in a more clinically relevant context. Therefore, it is critical to utilize commonly prescribed antibiotics in persister assays, an issue that has been less considered in previous studies. For example, despite being one of the most commonly used drugs for urinary tract infections [184], trimethoprim-sulfamethoxazole (TMP-SMX) is rarely used in persister assays, emphasizing the importance of deliberately selecting antibiotics in persister assays to understand persister mechanisms in a clinical context. Table 2 lists examples of diseases and antibiotic treatments against pathogenic bacterial species discussed in this review. These antibiotics might be utilized in persister assays to design more therapeutically relevant assays. Notably, *Streptococcus mutans* is not included in the table because, while it is considered a major aetiological agent of dental caries [185], the most recent expert consensus on dental caries recommends chlorhexidine gluconate as the antibacterial agent rather than antibiotics [186], implying that *Streptococcus mutans* antibiotic persisters may not be very relevant in the context of dental caries. Similarly, other species mentioned above, including *C. crescentus*, *Edwardsiella tarda*, *B. thailandensis*, *M. smegmatis*, *P. fluorescens*, and *Bacillus subtilis*, generally do not cause disease in humans [187–192], and hence they are not included in the table.

CONCLUDING REMARKS

In this review, we have discussed our current understanding of persister mechanisms. Although TA modules have long been thought to be major regulators of persistence [14], their primary roles in persistence have lately been called into doubt [9]. Furthermore, there is mounting evidence that QS molecules and indole play critical roles in mediating persistence [10, 11], which has sparked interest in developing anti-persister drugs that regulate the activity of QS molecules or indole [150, 193]. Among the mechanisms discussed in this review, epigenetics is the least researched. Recent advances in genomic profiling, such as single-molecule real-time (SMRT) and nanopore-based sequencing, which enable high-resolution epigenetic detection in bacteria on a single-cell level, could perhaps assist future researchers in deciphering how epigenetics correlate with the cell-to-cell heterogeneity in a persistent population [12].

Chronic infection is a serious challenge in global healthcare, and recalcitrant infections induced by lingering persisters are a major obstacle to achieving successful treatments. Decoding the underlying mechanism of persister formation in a clinical setting is critical to developing specialized anti-persister therapies. Besides deliberately choosing clinically relevant antibiotics in persister assays, it is critical to examine persister development in a physiological setting. Therefore, toxin overexpression experiments or QS/indole signals given exogenously at high concentrations might not be very relevant in clinical settings. Nonetheless, because the physiological concentration of QS molecules such as PCN is controversial due to limited sample sizes utilized in past measurements [194], larger sample sizes are required to determine the clinically relevant concentration range in the future. Finally, because synergistic interactions between bacterial species may be prevalent at infection sites, it is critical to examine persister generation in bacterial communities to find previously unknown community-level persister mechanisms. In summary, future research should employ consistent approaches while keeping clinical contexts in mind in order to unravel important persister mechanisms that have yet to be uncovered.

Funding information
A.Z. is a recipient of a Transition To Independence (TTI) fellowship from the School of Biological Sciences at the University of Cambridge and thus supported by funding from the Rosetrees Trust (grant number JS16/TTI2021\1) and the Isaac Newton Trust (grant number 21.22(a)iii) and the School of Biological Sciences at the University of Cambridge.

Table 2. Selected bacterial species, examples of associated infectious diseases and antibiotics that could be utilized in persister assays

Species	Example diseases	Example antibiotic treatment	Ref.
Escherichia coli	Diarrhoea in children	TMP-SMX, NOR, CIP	[216]
	Urinary tract infection	TMP-SMX, quinolone, NFN	[184, 217]
Pseudomonas aeruginosa	Infections in CF patients	CST, TOB, GEN, CIP	[218–220]
Staphylococcus aureus	Skin infections	MSSA strain: cephalosporin, OXA, NAF	[221]
		MRSA strain: VAN, TEC	
Staphylococcus epidermidis	Endocarditis	VAN, NAF, OXA	[222]
Klebsiella pneumoniae	Pneumonia	Cephalosporin, quinolone, aminoglycoside	[223]
	Bloodstream infection	Third-generation cephalosporin, carbapenem, aminoglycoside	[224]
Mycobacterium tuberculosis	Pneumonia	RIF, STR, INH, KAN, CIP, ETO	[225]
Salmonella enterica serovar Typhimurium	Gastroenteritis	CRO, AZM, fluoroquinolone	[226]
Burkholderia pseudomallei	Melioidosis	CAZ, TMP-SMX, AMC, IPM, CFP-SUL	[227]
Burkholderia cenocepacia	Cepacia syndrome	ATM, DOR, TOB	[228]
Streptococcus pyogenes	Pharyngitis	PEN, benzathine penicillin	[229]
Streptococcus pneumoniae	Pneumonia	AMX, CLR, ERY	[230]
Acinetobacter baumannii	Pneumonia, urinary tract infections	IPM, MEM, DOR	[231]
Clostridium difficile	Diarrhoea	VAN, FDX, MTZ	[232]
Vibrio cholerae	Cholera	DOX, AZM, CIP	[233]
Legionella pneumophila	Legionnaires' disease	Macrolide, fluoroquinolone, TET	[234]

AMC, amoxicillin–clavulanic acid; AMX, amoxicillin; ATM, aztreonam; AZM, azithromycin; CAZ, ceftazidime; CF, cystic fibrosis; CFP-SUL, cefoperazone-sulbactam; CIP, ciprofloxacin; CLR, clarithromycin; CRO, ceftriaxone; CST, colistin; DOR, doripenem; DOX, doxycycline; ERY, erythromycin; ETO, ethionamide; FDX, fidaxomicin; GEN, gentamicin; INH, isoniazid; IPM, imipenem; KAN, kanamycin; MEM, meropenem; MRSA, methicillin-resistant S. aureus; MSSA, methicillin-susceptible S. aureus; MTZ, metronidazole; NAF, nafcillin; NFN, nitrofurantoin; NOR, norfloxacin; OXA, oxacillin; PEN, penicillin; RIF, rifampicin; STR, streptomycin; TEC, teicoplanin; TET, tetracycline; TMP-SMX, trimethoprim-sulfamethoxazole; TOB, tobramycin; VAN, vancomycin.

Acknowledgements
We thank Dr Jehangir Cama, Dr Stephen Trigg and Dr Wendy Figueroa-Chavez for providing constructive comments on the initial draft of the manuscript. All figures were created with BioRender.com.

Author contributions
X.S. wrote the review and conducted the literature research. A.Z. reviewed the writing, assisted in the literature search and provided supervision.

Conflicts of interest
The authors have no conflicts of interest to disclose.

References

1. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022;399:629–655.
2. Blanchard SC. A much-needed boost for the dwindling antibiotic pipeline. *Mol Cell* 2018;70:3–5.
3. Resistance R on A. *Antimicrobial resistance: tackling a crisis for the health and wealth of nations*. Review on Antimicrobial Resistance, 2014.
4. Wilmaerts D, Windels EM, Verstraeten N, Michiels J. General mechanisms leading to persister formation and awakening. *Trends Genet* 2019;35:401–411.
5. Bigger JosephW. Treatment of staphylococcal infections with penicillin by intermittent sterilisation. *The Lancet* 1944;244:497–500.
6. Harms A, Maisonneuve E, Gerdes K. Mechanisms of bacterial persistence during stress and antibiotic exposure. *Science* 2016;354:aaf4268.
7. Balaban NQ, Helaine S, Lewis K, Ackermann M, Aldridge B, *et al*. Definitions and guidelines for research on antibiotic persistence. *Nat Rev Microbiol* 2019;17:441–448.
8. Levin-Reisman I, Ronin I, Gefen O, Braniss I, Shoresh N, *et al*. Antibiotic tolerance facilitates the evolution of resistance. *Science* 2017;355:826–830.
9. Fraikin N, Goormaghtigh F, Van Melderen L. Toxin-Antitoxin Systems and Persistence. In: Lewis K (eds). *Persister Cells and*

Infectious Disease. Cham: Springer International Publishing; 2019. pp. 181–202.

10. Fernandez-García L, Blasco L, Trastoy R, García R, Wood TK, *et al*. *Quorum Sensing Systems and Persistence 11*.
11. Zarkan A, Liu J, Matuszewska M, Gaimster H, Summers DK. Local and universal action: the paradoxes of indole signalling in bacteria. *Trends Microbiol* 2020;28:566–577.
12. Riber L, Hansen LH. Epigenetic memories: the hidden drivers of bacterial persistence? *Trends Microbiol* 2021;29:190–194.
13. Shultis MW, Mulholland CV, Berney M. Are all antibiotic persisters created equal? *Front Cell Infect Microbiol* 2022;12:933458.
14. Singh G, Yadav M, Ghosh C, Rathore JS. Bacterial toxin-antitoxin modules: classification, functions, and association with persistence. *Curr Res Microb Sci* 2021;2:100047.
15. Ramage HR, Connolly LE, Cox JS. Comprehensive functional analysis of *Mycobacterium tuberculosis* toxin-antitoxin systems: implications for pathogenesis, stress responses, and evolution. *PLOS Genet* 2009;5:e1000767.
16. Yu X, Gao X, Zhu K, Yin H, Mao X, *et al*. Characterization of a toxin-antitoxin system in *Mycobacterium tuberculosis* suggests neutralization by phosphorylation as the antitoxicity mechanism. *Commun Biol* 2020;3:216.
17. Berghoff BA, Wagner EGH. Persister Formation Driven by TisB-Dependent Membrane Depolarization. In: Lewis K (eds). *Persister Cells and Infectious Disease*. Cham: Springer International Publishing; 2019. pp. 77–97.
18. Podlesek Z, Žgur Bertok D. The DNA damage inducible SOS response is a key player in the generation of bacterial persister cells and population wide tolerance. *Front Microbiol* 2020;11:1785.
19. LeRoux M, Laub MT. Toxin-antitoxin systems as phage defense elements. *Annu Rev Microbiol* 2022;76:21–43.
20. Lou C, Li Z, Ouyang Q. A molecular model for persister in *E. coli*. *J Theor Biol* 2008;255:205–209.
21. Moyed HS, Bertrand KP. hipA, a newly recognized gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis. *J Bacteriol* 1983;155:768–775.
22. Schumacher MA, Balani P, Min J, Chinnam NB, Hansen S, *et al*. HipBA-promoter structures reveal the basis of heritable multidrug tolerance. *Nature* 2015;524:59–64.
23. Narimisa N, Kalani BS, Amraei F, Mohammadzadeh R, Mirkalantari S, *et al*. Type II toxin/antitoxin system genes expression in persister cells of *Klebsiella pneumoniae*. *Reviews in Medical Microbiology* 2020;31:215–220.
24. Narimisa N, Amraei F, Kalani BS, Azarnezhad A, Jazi FM. Biofilm establishment, biofilm persister cell formation, and relative gene expression analysis of type II toxin-antitoxin system in *Klebsiella pneumoniae*. *Gene Reports* 2020;21:100846.
25. Falla TJ, Chopra I. Joint tolerance to β-lactam and fluoroquinolone antibiotics in *Escherichia coli* results from overexpression of *hipA* . *Antimicrob Agents Chemother* 1998;42:3282–3284.
26. Korch SB, Hill TM. Ectopic overexpression of wild-type and mutant hipA genes in *Escherichia coli*: effects on macromolecular synthesis and persister formation. *J Bacteriol* 2006;188:3826–3836.
27. Vázquez-Laslop N, Lee H, Neyfakh AA. Increased persistence in *Escherichia coli* caused by controlled expression of toxins or other unrelated proteins. *J Bacteriol* 2006;188:3494–3497.
28. Keren I, Shah D, Spoering A, Kaldalu N, Lewis K. Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J Bacteriol* 2004;186:8172–8180.
29. Hansen S, Lewis K, Vulić M. Role of global regulators and nucleotide metabolism in antibiotic tolerance in *Escherichia coli*. *Antimicrob Agents Chemother* 2008;52:2718–2726.
30. Luidalepp H, Jõers A, Kaldalu N, Tenson T. Age of inoculum strongly influences persister frequency and can mask effects of mutations implicated in altered persistence. *J Bacteriol* 2011;193:3598–3605.
31. Korch SB, Henderson TA, Hill TM. Characterization of the hipA7 allele of Escherichia coli and evidence that high persistence is governed by (p)ppGpp synthesis. *Mol Microbiol* 2003;50:1199–1213.
32. Germain E, Roghanian M, Gerdes K, Maisonneuve E. Stochastic induction of persister cells by HipA through (p)ppGpp-mediated activation of mRNA endonucleases. *Proc Natl Acad Sci U S A* 2015;112:5171–5176.
33. Huang CY, Gonzalez-Lopez C, Henry C, Mijakovic I, Ryan KR. hipBA toxin-antitoxin systems mediate persistence in *Caulobacter crescentus*. *Sci Rep* 2020;10:2865.
34. Hosseini M, Nowroozi J, Amirmozafari N. The effect of type II toxin-antitoxin systems on methicillinresistant *Staphylococcus aureus* persister cell formation and antibiotic tolerance. *Acta Biol Szeged* 2021;65:113–117.
35. Karimaei S, Kazem Aghamir SM, Foroushani AR, Pourmand MR. Antibiotic tolerance in biofilm persister cells of *Staphylococcus aureus* and expression of toxin-antitoxin system genes. *Microb Pathog* 2021;159:105126.
36. Conlon BP, Rowe SE, Gandt AB, Nuxoll AS, Donegan NP, *et al*. Persister formation in *Staphylococcus aureus* is associated with ATP depletion. *Nat Microbiol* 2016;1:16051.
37. Kamruzzaman M, Iredell J. A ParDE-family toxin antitoxin system in major resistance plasmids of *Enterobacteriaceae* confers antibiotic and heat tolerance. *Sci Rep* 2019;9:9872.
38. Kim Y, Wood TK. Toxins Hha and CspD and small RNA regulator Hfq are involved in persister cell formation through MqsR in *Escherichia coli*. *Biochem Biophys Res Commun* 2010;391:209–213.
39. Shah D, Zhang Z, Khodursky AB, Kaldalu N, Kurg K, *et al*. Persisters: a distinct physiological state of *E. coli*. *BMC Microbiol* 2006;6:53.
40. Fraikin N, Rousseau CJ, Goeders N, Van Melderen L. Reassessing the role of the type II MqsRA toxin-antitoxin system in stress response and biofilm formation: *mqsA* is transcriptionally uncoupled from *mqsR mBio* 2019;10:e02678-19.
41. Qi X, Brothers KM, Ma D, Mandell JB, Donegan NP, *et al*. The *Staphylococcus aureus* toxin-antitoxin system YefM-YoeB is associated with antibiotic tolerance and extracellular dependent biofilm formation. *J Bone Jt Infect* 2021;6:241–253.
42. Ma D, Gu H, Shi Y, Huang H, Sun D, *et al*. *Edwardsiella piscicida* YefM-YoeB: a type II toxin-antitoxin system that is related to antibiotic resistance, biofilm formation, serum survival, and host infection. *Front Microbiol* 2021;12:646299.
43. Ramisetty BCM, Ghosh D, Roy Chowdhury M, Santhosh RS. What is the link between stringent response, Endoribonuclease encoding type II toxin-antitoxin systems and persistence? *Front Microbiol* 2016;7:1882.
44. Chowdhury N, Kwan BW, Wood TK. Persistence increases in the absence of the alarmone guanosine tetraphosphate by reducing cell growth. *Sci Rep* 2016;6:20519.
45. Harrison JJ, Wade WD, Akierman S, Vacchi-Suzzi C, Stremick CA, *et al*. The chromosomal toxin gene yafQ is a determinant of multidrug tolerance for *Escherichia coli* growing in a biofilm. *Antimicrob Agents Chemother* 2009;53:2253–2258.
46. Masuda Y, Sakamoto E, Honjoh K-I, Miyamoto T. Role of toxin-antitoxin-regulated persister population and indole in bacterial heat tolerance. *Appl Environ Microbiol* 2020;86:e00935-20.
47. Bernier SP, Lebeaux D, DeFrancesco AS, Valomon A, Soubigou G, *et al*. Starvation, together with the SOS response, mediates high biofilm-specific tolerance to the fluoroquinolone ofloxacin. *PLoS Genet* 2013;9:e1003144.
48. Slattery A, Victorsen AH, Brown A, Hillman K, Phillips GJ. Isolation of highly persistent mutants of *Salmonella enterica* serovar typhimurium reveals a new toxin-antitoxin module. *J Bacteriol* 2013;195:647–657.
49. Helaine S, Cheverton AM, Watson KG, Faure LM, Matthews SA, *et al*. Internalization of Salmonella by macrophages induces formation of nonreplicating persisters. *Science* 2014;343:204–208.

50. Claudi B, Spröte P, Chirkova A, Personnic N, Zankl J, *et al*. Phenotypic variation of Salmonella in host tissues delays eradication by antimicrobial chemotherapy. *Cell* 2014;158:722–733.
51. Tripathi A, Dewan PC, Siddique SA, Varadarajan R. MazF-induced growth inhibition and persister generation in *Escherichia coli*. *J Biol Chem* 2014;289:4191–4205.
52. Ma D, Mandell JB, Donegan NP, Cheung AL, Ma W, *et al*. The toxin-antitoxin MazEF drives *Staphylococcus aureus* biofilm formation, Antibiotic Tolerance, and Chronic Infection. *mBio* 2019;10.
53. Bleriot I, Blasco L, Delgado-Valverde M, Gual-de-Torrella A, Ambroa A, *et al*. Mechanisms of tolerance and resistance to chlorhexidine in clinical strains of *Klebsiella pneumoniae* producers of carbapenemase: role of new type II toxin-antitoxin system, PemIK. *Toxins* 2020;12:566.
54. Sharma A, Chattopadhyay G, Chopra P, Bhasin M, Thakur C, *et al*. VapC21 toxin contributes to drug-tolerance and interacts with non-cognate VapB32 Antitoxin in *Mycobacterium tuberculosis*. *Front Microbiol* 2020;11.
55. Xia K, Han C, Xu J, Liang X. Toxin-antitoxin HicAB regulates the formation of persister cells responsible for the acid stress resistance in *Acetobacter pasteurianus*. *Appl Microbiol Biotechnol* 2021;105:725–739.
56. Cheverton AM, Gollan B, Przydacz M, Wong CT, Mylona A, *et al*. A Salmonella toxin promotes persister formation through acetylation of tRNA. *Mol Cell* 2016;63:86–96.
57. Zhou J, Li S, Li H, Jin Y, Bai F, *et al*. Identification of a toxin-antitoxin system that contributes to persister formation by reducing NAD in *Pseudomonas aeruginosa*. *Microorganisms* 2021;9:753.
58. Dufour D, Mankovskaia A, Chan Y, Motavaze K, Gong S-G, Lévesque CM. 2018. A tripartite toxin-antitoxin module induced by quorum sensing is associated with the persistence phenotype in *Streptococcus mutans*. Mol Oral Microbiol 33:420–429.
59. Norton JP, Mulvey MA, Tran Van Nhieu G. Toxin-antitoxin systems are important for niche-specific colonization and stress resistance of uropathogenic *Escherichia coli*. *PLoS Pathog* 2012;8:e1002954.
60. Fino C, Vestergaard M, Ingmer H, Pierrel F, Gerdes K, *et al*. PasT of *Escherichia coli* sustains antibiotic tolerance and aerobic respiration as a bacterial homolog of mitochondrial Coq10. *Microbiologyopen* 2020;9:e1064.
61. Van Acker H, Crabbé A, Jurėnas D, Ostyn L, Sass A, *et al*. The role of small proteins in *Burkholderia cenocepacia* J2315 biofilm formation, persistence and intracellular growth. *Biofilm* 2019;1:100001.
62. Ross BN, Thiriot JD, Wilson SM, Torres AG. Predicting toxins found in toxin-antitoxin systems with a role in host-induced *Burkholderia pseudomallei* persistence. *Sci Rep* 2020;10:16923.
63. Verstraeten N, Knapen WJ, Kint CI, Liebens V, Van den Bergh B, *et al*. Obg and membrane depolarization are part of a microbial bet-hedging strategy that leads to antibiotic tolerance. *Mol Cell* 2015;59:9–21.
64. Edelmann D, Oberpaul M, Schäberle TF, Berghoff BA. Post-transcriptional deregulation of the tisB/istR-1 toxin-antitoxin system promotes SOS-independent persister formation in *Escherichia coli*. *Environ Microbiol Rep* 2021;13:159–168.
65. Berghoff BA, Hoekzema M, Aulbach L, Wagner EGH. Two regulatory RNA elements affect TisB-dependent depolarization and persister formation. *Molecular Microbiology* 2017;103:1020–1033.
66. Edelmann D, Leinberger FH, Schmid NE, Oberpaul M, Schäberle TF, *et al*. Elevated expression of toxin TisB protects persister cells against ciprofloxacin but enhances susceptibility to Mitomycin C. *Microorganisms* 2021;9:943.
67. Edelmann D, Berghoff BA. A shift in perspective: a role for the type I toxin TisB as persistence-stabilizing factor. *Front Microbiol* 2022;13:871699.
68. Coray DS, Wheeler NE, Heinemann JA, Gardner PP. Why so narrow: distribution of anti-sense regulated, type I toxin-antitoxin systems compared with type II and type III systems. *RNA Biol* 2017;14:275–280.
69. Tsilibaris V, Maenhaut-Michel G, Mine N, Van Melderen L. What is the benefit to *Escherichia coli* of having multiple toxin-antitoxin systems in its genome? . *J Bacteriol* 2007;189:6101–6108.
70. LeRoux M, Culviner PH, Liu YJ, Littlehale ML, Laub MT. Stress can induce transcription of toxin-antitoxin systems without activating toxin. *Mol Cell* 2020;79:280–292.
71. Pontes MH, Groisman EA. Slow growth dictates non-heritable antibiotic resistance in *Salmonella enterica*. *Sci Signal* 2019;12:eaax3938.
72. Gray MJ. Inorganic polyphosphate accumulation in *Escherichia coli* is regulated by DksA but not by (p)ppGpp. *J Bacteriol* 2019;201:e00664-18.
73. Boutte CC, Henry JT, Crosson S. ppGpp and polyphosphate modulate cell cycle progression in *Caulobacter crescentus*. *J Bacteriol* 2012;194:28–35.
74. Racki LR, Tocheva EI, Dieterle MG, Sullivan MC, Jensen GJ, *et al*. Polyphosphate granule biogenesis is temporally and functionally tied to cell cycle exit during starvation in *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 2017;114:E2440–E2449.
75. Bhaskar A, De Piano C, Gelman E, McKinney JD, Dhar N. Elucidating the role of (p)ppGpp in mycobacterial persistence against antibiotics. *IUBMB Life* 2018;70:836–844.
76. Harms A, Fino C, Sørensen MA, Semsey S, Gerdes K, *et al*. Prophages and growth dynamics confound experimental results with antibiotic-tolerant persister cells. *mBio* 2017;8.
77. Osbourne DO, Soo VW, Konieczny I, Wood TK. Polyphosphate, cyclic AMP, guanosine tetraphosphate, and c-di-GMP reduce in vitro Lon activity. *Bioengineered* 2014;5:264–268.
78. Theodore A, Lewis K, Vulić M. Tolerance of *Escherichia coli* to fluoroquinolone antibiotics depends on specific components of the SOS response pathway . *Genetics* 2013;195:1265–1276.
79. Song S, Wood TK. Toxin/antitoxin system paradigms: toxins bound to antitoxins are not likely activated by preferential antitoxin degradation. *Adv Biosys* 2020;4:1900290.
80. Wang X, Lord DM, Hong SH, Peti W, Benedik MJ, *et al*. Type II toxin/antitoxin MqsR/MqsA controls type V toxin/antitoxin GhoT/GhoS. *Environ Microbiol* 2013;15:1734–1744.
81. Chandra S, Gupta K, Khare S, Kohli P, Asok A, *et al*. The high mutational sensitivity of ccdA antitoxin is linked to codon optimality. *Mol Biol Evol* 2022;39:msac187.
82. Van Melderen L. Toxin-antitoxin systems: why so many, what for? *Curr Opin Microbiol* 2010;13:781–785.
83. Pecota DC, Wood TK. Exclusion of T4 phage by the hok/sok killer locus from plasmid R1. *J Bacteriol* 1996;178:2044–2050.
84. Song S, Wood TK. A primary physiological role of toxin/antitoxin systems is phage inhibition. *Front Microbiol* 2020;11:1895.
85. Jurėnas D, Fraikin N, Goormaghtigh F, Van Melderen L. Biology and evolution of bacterial toxin-antitoxin systems. *Nat Rev Microbiol* 2022;20:335–350.
86. Li M, Gong L, Cheng F, Yu H, Zhao D, *et al*. Toxin-antitoxin RNA pairs safeguard CRISPR-Cas systems. *Science* 2021;372:eabe5601.
87. Fineran PC, Blower TR, Foulds IJ, Humphreys DP, Lilley KS, *et al*. The phage abortive infection system, ToxIN, functions as a protein-RNA toxin-antitoxin pair. *Proc Natl Acad Sci U S A* 2009;106:894–899.
88. Bobonis J, Mitosch K, Mateus A, Kritikos G, Elfenbein JR, *et al*. Phage proteins block and trigger retron toxin/antitoxin systems. *Microbiology*. DOI: 10.1101/2020.06.22.160242
89. Vassallo CN, Doering CR, Littlehale ML, Teodoro GIC, Laub MT. A functional selection reveals previously undetected anti-phage defence systems in the *E. coli* pangenome. *Nat Microbiol* 2022;7:1568–1579.

90. Makarova KS, Wolf YI, Iranzo J, Shmakov SA, Alkhnbashi OS, *et al*. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. *Nat Rev Microbiol* 2020;18:67–83.
91. LeRoux M, Srikant S, Teodoro GIC, Zhang T, Littlehale ML, *et al*. The DarTG toxin-antitoxin system provides phage defence by ADP-ribosylating viral DNA. *Nat Microbiol* 2022;7:1028–1040.
92. Otsuka Y, Yonesaki T. Dmd of bacteriophage T4 functions as an antitoxin against *Escherichia coli* LsoA and RnlA toxins. *Mol Microbiol* 2012;83:669–681.
93. Freire DM, Gutierrez C, Garza-Garcia A, Grabowska AD, Sala AJ, *et al*. An NAD^+ phosphorylase toxin triggers Mycobacterium tuberculosis cell death. *Mol Cell* 2019;73:1282–1291..
94. Sberro H, Leavitt A, Kiro R, Koh E, Peleg Y, *et al*. Discovery of functional toxin/antitoxin systems in bacteria by shotgun cloning. *Mol Cell* 2013;50:136–148.
95. Li Y, Liu X, Tang K, Wang W, Guo Y, *et al*. Prophage encoding toxin/antitoxin system PfiT/PfiA inhibits Pf4 production in *Pseudomonas aeruginosa*. *Microb Biotechnol* 2020;13:1132–1144.
96. Guegler CK, Laub MT. Shutoff of host transcription triggers a toxin-antitoxin system to cleave phage RNA and abort infection. *Molecular Cell* 2021;81:2361–2373..
97. Srikant S, Guegler CK, Laub MT. The evolution of a counter-defense mechanism in a virus constrains its host range. *eLife* 2022;11.
98. Fillol-Salom A, Rostøl JT, Ojiogu AD, Chen J, Douce G, *et al*. Bacteriophages benefit from mobilizing pathogenicity islands encoding immune systems against competitors. *Cell* 2022;185:3248–3262..
99. Tuller T, Kupiec M, Ruppin E, Gerstein MB. Determinants of protein abundance and translation efficiency in *S. cerevisiae*. *PLOS Comput Biol* 2007;3:e248.
100. Nie L, Wu G, Zhang W. Correlation between mRNA and protein abundance in *Desulfovibrio vulgaris*: A multiple regression to identify sources of variations. *Biochem Biophys Res Commun* 2006;339:603–610.
101. Freiberg JA, Le Breton Y, Tran BQ, Scott AJ, Harro JM, *et al*. Global analysis and comparison of the transcriptomes and proteomes of group A *Streptococcus* Biofilms. *mSystems* 2016;1:e00149-16.
102. Taniguchi Y, Choi PJ, Li G-W, Chen H, Babu M, *et al*. Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* 2010;329:533–538.
103. Urbanczyk H, Ast JC, Higgins MJ, Carson J, Dunlap PVY. Reclassification of *Vibrio fischeri*, Vibrio logei, *Vibrio salmonicida* and Vibrio wodanis as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov. *Int J Syst Evol Microbiol* 2007;57:2823–2829.
104. Turovskiy Y, Kashtanov D, Paskhover B, Chikindas ML. Quorum sensing: fact, fiction, and everything in between. *Adv Appl Microbiol* 2007;62:191–234.
105. Henke JM, Bassler BL. Bacterial social engagements. *Trends Cell Biol* 2004;14:648–656.
106. Venturi V. Regulation of quorum sensing in *Pseudomonas*. *FEMS Microbiol Rev* 2006;30:274–291.
107. Lin J, Cheng J, Wang Y, Shen X. The *Pseudomonas* quinolone signal (PQS): not just for quorum sensing anymore. *Front Cell Infect Microbiol* 2018;8:230.
108. Gardan R, Besset C, Gitton C, Guillot A, Fontaine L, *et al*. Extracellular life cycle of ComS, the competence-stimulating peptide of *Streptococcus thermophilus*. *J Bacteriol* 2013;195:1845–1855.
109. Griffith F. The significance of *Pneumococcal* types. *J Hyg (Lond)* 1928;27:113–159.
110. Håvarstein LS, Coomaraswamy G, Morrison DA. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*. *Proc Natl Acad Sci U S A* 1995;92:11140–11144.
111. Yang Y, Tal-Gan Y. Exploring the competence stimulating peptide (CSP) N-terminal requirements for effective ComD receptor activation in group1 *Streptococcus pneumoniae*. *Bioorg Chem* 2019;89:102987.
112. Winzer K, Hardie KR, Williams P. Bacterial cell-to-cell communication: sorry, can't talk now - gone to lunch! *Curr Opin Microbiol* 2002;5:216–222.
113. Kim J, Park W. Indole: a signaling molecule or a mere metabolic byproduct that alters bacterial physiology at a high concentration? *J Microbiol* 2015;53:421–428.
114. Leung V, Dufour D, Lévesque CM. Death and survival in Streptococcus mutans: differing outcomes of a quorum-sensing signaling peptide. *Front Microbiol* 2015;6:1176.
115. Dietrich LEP, Price-Whelan A, Petersen A, Whiteley M, Newman DK. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Mol Microbiol* 2006;61:1308–1321.
116. Möker N, Dean CR, Tao J. *Pseudomonas aeruginosa* increases formation of multidrug-tolerant persister cells in response to quorum-sensing signaling molecules. *J Bacteriol* 2010;192:1946–1955.
117. Hall S, McDermott C, Anoopkumar-Dukie S, McFarland AJ, Forbes A, *et al*. Cellular effects of pyocyanin, a secreted virulence factor of *Pseudomonas aeruginosa*. *Toxins (Basel)* 2016;8:236.
118. Meirelles LA, Newman DK. Both toxic and beneficial effects of pyocyanin contribute to the lifecycle of *Pseudomonas aeruginosa*. *Mol Microbiol* 2018;110:995–1010.
119. Pu Y, Zhao Z, Li Y, Zou J, Ma Q, *et al*. Enhanced efflux activity facilitates drug tolerance in dormant bacterial cells. *Molecular Cell* 2016;62:284–294.
120. Que Y-A, Hazan R, Strobel B, Maura D, He J, *et al*. A quorum sensing small volatile molecule promotes antibiotic tolerance in bacteria. *PLoS ONE* 2013;8:e80140.
121. Allegretta G, Maurer CK, Eberhard J, Maura D, Hartmann RW, *et al*. In-depth profiling of MvfR-regulated small molecules in *Pseudomonas aeruginosa* after quorum sensing inhibitor treatment. *Front Microbiol* 2017;8:924.
122. Pan J, Xie X, Tian W, Bahar AA, Lin N, *et al*. (Z)-4-Bromo-5-(bromomethylene)-3-methylfuran-2(5H)-one sensitizes *Escherichia coli* persister cells to antibiotics. *Appl Microbiol Biotechnol* 2013;97:9145–9154.
123. Pan J, Song F, Ren D. Controlling persister cells of *Pseudomonas aeruginosa* PDO300 by (Z)-4-bromo-5-(bromomethylene)-3-methylfuran-2(5H)-one. *Bioorg Med Chem Lett* 2013;23:4648–4651.
124. Pan J, Bahar AA, Syed H, Ren D. Reverting antibiotic tolerance of *Pseudomonas aeruginosa* PAO1 persister cells by (Z)-4-bromo-5-(bromomethylene)-3-methylfuran-2(5H)-one. *PLoS One* 2012;7:e45778.
125. Schafhauser J, Lepine F, McKay G, Ahlgren HG, Khakimova M, *et al*. The stringent response modulates 4-hydroxy-2-alkylquinoline biosynthesis and quorum-sensing hierarchy in *Pseudomonas aeruginosa*. *J Bacteriol* 2014;196:1641–1650.
126. Pacios O, Blasco L, Bleriot I, Fernandez-Garcia L, Ambroa A, *et al*. (p)ppGpp and Its Role in Bacterial Persistence: New Challenges. *Antimicrob Agents Chemother* 2020;64:e01283-20.
127. Leung V, Ajdic D, Koyanagi S, Lévesque CM. The formation of *Streptococcus mutans* persisters induced by the quorum-sensing peptide pheromone is affected by the LexA regulator. *J Bacteriol* 2015;197:1083–1094.
128. Jarosz LM, Deng DM, van der Mei HC, Crielaard W, Krom BP. Streptococcus mutans competence-stimulating peptide inhibits *Candida albicans* hypha formation. *Eukaryot Cell* 2009;8:1658–1664.
129. Leung V, Lévesque CM. A stress-inducible quorum-sensing peptide mediates the formation of persister cells with noninherited multidrug tolerance. *J Bacteriol* 2012;194:2265–2274.
130. Dufour D, Zhao H, Gong S-G, Lévesque CM. A DNA-damage inducible gene promotes the formation of antibiotic persisters

in response to the quorum sensing signaling peptide in *Streptococcus mutans Genes (Basel)* 2022;13:1434.

131. Shanker E, Federle MJ. Quorum sensing regulation of competence and bacteriocins in *Streptococcus pneumoniae* and mutans. *Genes* 2017;8:15.
132. Zu Y, Li W, Wang Q, Chen J, Guo Q. ComDE two-component signal transduction systems in oral *Streptococci*: structure and function. *Curr Issues Mol Biol* 2019;32:201–258.
133. Lee J-H, Lee J. Indole as an intercellular signal in microbial communities. *FEMS Microbiol Rev* 2010;34:426–444.
134. Gaimster H, Cama J, Hernández-Ainsa S, Keyser UF, Summers DK. The indole pulse: a new perspective on indole signalling in *Escherichia coli*. *PLoS One* 2014;9:e93168.
135. Vega NM, Allison KR, Khalil AS, Collins JJ. Signaling-mediated bacterial persister formation. *Nat Chem Biol* 2012;8:431–433.
136. Lee J-H, Kim Y-G, Gwon G, Wood TK, Lee J. Halogenated indoles eradicate bacterial persister cells and biofilms. *AMB Express* 2016;6:123.
137. Zarkan A, Matuszewska M, Trigg SB, Zhang M, Belgami D, *et al*. Inhibition of indole production increases the activity of quinolone antibiotics against *E. coli* persisters. *Sci Rep* 2020;10:11742.
138. Han J-T, Li D-Y, Zhang M-Y, Yu X-Q, Jia X-X, *et al*. EmhR is an indole-sensing transcriptional regulator responsible for the indole-induced antibiotic tolerance in *Pseudomonas fluorescens*. *Environ Microbiol* 2021;23:2054–2069.
139. Zarkan A, Caño-Muñiz S, Zhu J, Al Nahas K, Cama J, *et al*. Indole pulse signalling regulates the cytoplasmic pH of *E. coli* in a memory-like manner. *Sci Rep* 2019;9:3868.
140. Chimerel C, Field CM, Piñero-Fernandez S, Keyser UF, Summers DK. Indole prevents *Escherichia coli* cell division by modulating membrane potential. *Biochim Biophys Acta* 2012;1818:1590–1594.
141. Goode O, Smith A, Zarkan A, Cama J, Invergo BM, *et al*. Persister *Escherichia coli* cells have a lower intracellular pH than susceptible cells but maintain their pH in response to antibiotic treatment. *mBio* 2021;12:e0090921.
142. Hong SH, Wang X, O'Connor HF, Benedik MJ, Wood TK. Bacterial persistence increases as environmental fitness decreases. *Microb Biotechnol* 2012;5:509–522.
143. Wu N, He L, Cui P, Wang W, Yuan Y, *et al*. Ranking of persister genes in the same *Escherichia coli* genetic background demonstrates varying importance of individual persister genes in tolerance to different antibiotics. *Front Microbiol* 2015;6:1003.
144. Hu Y, Kwan BW, Osbourne DO, Benedik MJ, Wood TK. Toxin YafQ increases persister cell formation by reducing indole signalling. *Environ Microbiol* 2015;17:1275–1285.
145. Sommerfeldt N, Possling A, Becker G, Pesavento C, Tschowri N, *et al*. Gene expression patterns and differential input into curli fimbriae regulation of all GGDEF/EAL domain proteins in E*scherichia coli*. *Microbiology (Reading)* 2009;155:1318–1331.
146. Kwan BW, Osbourne DO, Hu Y, Benedik MJ, Wood TK. Phosphodiesterase DosP increases persistence by reducing cAMP which reduces the signal indole. *Biotechnol Bioeng* 2015;112:588–600.
147. Lang M, Krin E, Korlowski C, Sismeiro O, Varet H, *et al*. Sleeping ribosomes: bacterial signaling triggers RaiA mediated persistence to aminoglycosides. *iScience* 2021;24:103128.
148. Xu T, Wang X-Y, Cui P, Zhang Y-M, Zhang W-H, *et al*. The Agr quorum sensing system represses persister formation through regulation of phenol soluble modulins in *Staphylococcus aureus Front Microbiol* 2017;8:2189.
149. Personnic N, Striednig B, Hilbi H. Quorum sensing controls persistence, resuscitation, and virulence of Legionella subpopulations in biofilms. *ISME J* 2021;15:196–210.
150. Song S, Wood TK. Combatting persister cells with substituted indoles. *Front Microbiol* 2020;11:1565.
151. Tomberlin JK, Crippen TL, Wu G, Griffin AS, Wood TK, *et al*. Indole: an evolutionarily conserved influencer of behavior across kingdoms. *Bioessays* 2017;39:1600203.
152. Darkoh C, Plants-Paris K, Bishoff D, DuPont HL. Clostridium difficile modulates the gut microbiota by inducing the production of indole, an interkingdom signaling and antimicrobial molecule. *mSystems* 2019;4:e00346-18.
153. Vega NM, Allison KR, Samuels AN, Klempner MS, Collins JJ. *Salmonella typhimurium* intercepts *Escherichia coli* signaling to enhance antibiotic tolerance. *Proc Natl Acad Sci U S A* 2013;110:14420–14425.
154. Chu W, Zere TR, Weber MM, Wood TK, Whiteley M, *et al*. Indole production promotes *Escherichia coli* mixed-culture growth with Pseudomonas aeruginosa by inhibiting quorum signaling. *Appl Environ Microbiol* 2012;78:411–419.
155. Wang Y, Li H, Cui X, Zhang X-H. A novel stress response mechanism, triggered by indole, involved in quorum quenching enzyme MomL and iron-sulfur cluster in *Muricauda olearia* Th120. *Sci Rep* 2017;7:4252.
156. Kim J, Park W. Indole inhibits bacterial quorum sensing signal transmission by interfering with quorum sensing regulator folding. *Microbiology (Reading)* 2013;159:2616–2625.
157. Rahman P, Pasirayi G, Auger V, Ali Z. Development of a simple and low cost microbioreactor for high-throughput bioprocessing. *Biotechnol Lett* 2009;31:209–214.
158. Bhargava N, Sharma P, Capalash N. Pyocyanin stimulates quorum sensing-mediated tolerance to oxidative stress and increases persister cell populations in *Acinetobacter baumannii*. *Infect Immun* 2014;82:3417–3425.
159. McCready AR, Paczkowski JE, Henke BR, Bassler BL. Structural determinants driving homoserine lactone ligand selection in the *Pseudomonas aeruginosa* LasR quorum-sensing receptor . *Proc Natl Acad Sci USA* 2019;116:245–254.
160. Cabuhat KSP, Moron-Espiritu LS. Quorum sensing orchestrates antibiotic drug resistance, biofilm formation, and motility in *Escherichia coli* and quorum quenching activities of plant-derived natural products: a review. *J Pure Appl Microbiol* 2022;16:1538–1549.
161. Lee J, Maeda T, Hong SH, Wood TK. Reconfiguring the quorum-sensing regulator SdiA of *Escherichia coli* to control biofilm formation via indole and N-acylhomoserine lactones. *Appl Environ Microbiol* 2009;75:1703–1716.
162. Culler HF, Couto SCF, Higa JS, Ruiz RM, Yang MJ, *et al*. Role of SdiA on biofilm formation by atypical enteropathogenic *Escherichia coli*. *Genes (Basel)* 2018;9:253.
163. Yan J, Bassler BL. Surviving as a community: antibiotic tolerance and persistence in bacterial biofilms. *Cell Host Microbe* 2019;26:15–21.
164. Day T. Interpreting phenotypic antibiotic tolerance and persister cells as evolution via epigenetic inheritance. *Mol Ecol* 2016;25:1869–1882.
165. Hingley-Wilson SM, Ma N, Hu Y, Casey R, Bramming A, *et al*. Loss of phenotypic inheritance associated with *ydcI* mutation leads to increased frequency of small, slow persisters in *Escherichia coli*. *Proc Natl Acad Sci U S A* 2020;117:4152–4157.
166. Xu Y, Liu S, Zhang Y, Zhang W. Role of DNA methylation in persister formation in uropathogenic *E. coli*. *Microbiology* 2020. DOI: 10.1101/2020.01.07.897686.
167. Ghosh D, Veeraraghavan B, Elangovan R, Vivekanandan P. Antibiotic resistance and epigenetics: more to it than meets the eye. *Antimicrob Agents Chemother* 2020;64.
168. Muhammad JS, Khan NA, Maciver SK, Alharbi AM, Alfahemi H, *et al*. Epigenetic-mediated antimicrobial resistance: host versus pathogen epigenetic alterations. *Antibiotics (Basel)* 2022;11:809.
169. Levin-Reisman I, Balaban NQ. Quantitative measurements of type I and type II persisters using scanlag. *Methods Mol Biol* 2016;1333:75–81.

170. Eling N, Morgan MD, Marioni JC. Challenges in measuring and understanding biological noise. *Nat Rev Genet* 2019;20:536–548.
171. Feng J, Kessler DA, Ben-Jacob E, Levine H. Growth feedback as a basis for persister bistability. *Proc Natl Acad Sci U S A* 2014;111:544–549.
172. Nicolau SE, Lewis K. The role of integration host factor in *Escherichia coli* persister formation. *mBio* 2022;13:e0342021.
173. Zalis EA, Nuxoll AS, Manuse S, Clair G, Radlinski LC, *et al*. stochastic variation in expression of the tricarboxylic acid cycle produces persister cells. *mBio* 2019;10:e01930-19.
174. Sureka K, Ghosh B, Dasgupta A, Basu J, Kundu M, *et al*. Positive feedback and noise activate the stringent response regulator rel in mycobacteria. *PLoS One* 2008;3:e1771.
175. Zorzan I, Del Favero S, Giaretta A, Manganelli R, Di Camillo B, *et al*. Mathematical modelling of SigE regulatory network reveals new insights into bistability of mycobacterial stress response. *BMC Bioinformatics* 2021;22:558.
176. Boldrin F, Provvedi R, Cioetto Mazzabò L, Segafreddo G, Manganelli R. Tolerance and persistence to drugs: a main challenge in the fight against *Mycobacterium tuberculosis Front Microbiol* 2020;11:1924.
177. Quigley J, Lewis K. Noise in a metabolic pathway leads to persister formation in *Mycobacterium tuberculosis*. *Microbiol Spectr* 2022;10:e0294822.
178. Hahn J, Tanner AW, Carabetta VJ, Cristea IM, Dubnau D. ComGA-RelA interaction and persistence in the *Bacillus subtilis* K-state. *Mol Microbiol* 2015;97:454–471.
179. Wood TK, Song S. Forming and waking dormant cells: The ppGpp ribosome dimerization persister model. *Biofilm* 2020;2:100018.
180. Goormaghtigh F, Van Melderen L. 2016. Optimized Method for Measuring Persistence in Escherichia coli with Improved Reproducibility, p. 43–52. In Michiels, J, Fauvart, M (eds.), Bacterial Persistence. Springer New York, New York, NY.
181. Cama J, Pagliara S. Microfluidic single-cell phenotyping of the activity of peptide-based antimicrobials. *Methods Mol Biol* 2021;2208:237–253.
182. Bamford RA, Smith A, Metz J, Glover G, Titball RW, *et al*. Investigating the physiology of viable but non-culturable bacteria by microfluidics and time-lapse microscopy. *BMC Biol* 2017;15:121.
183. Goode O, Smith A, Łapińska U, Bamford R, Kahveci Z, *et al*. Heterologous protein expression favors the formation of protein aggregates in persister and viable but nonculturable bacteria. *ACS Infect Dis* 2021;7:1848–1858.
184. Stuck AK, Täuber MG, Schabel M, Lehmann T, Suter H, *et al*. Determinants of quinolone versus trimethoprim-sulfamethoxazole use for outpatient urinary tract infection. *Antimicrob Agents Chemother* 2012;56:1359–1363.
185. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, *et al*. Bacteria of dental caries in primary and permanent teeth in children and young adults. *J Clin Microbiol* 2008;46:1407–1417.
186. Cheng L, Zhang L, Yue L, Ling J, Fan M, *et al*. Expert consensus on dental caries management. *Int J Oral Sci* 2022;14:17.
187. Gu H-J, Sun Q-L, Luo J-C, Zhang J, Sun L. A first study of the virulence potential of A *Bacillus subtilis* isolate from deep-sea hydrothermal vent. *Front Cell Infect Microbiol* 2019;9:183.
188. T JAS, J R, Rajan A, Shankar V. Features of the biochemistry of *Mycobacterium smegmatis*, as a possible model for *Mycobacterium tuberculosis*. *J Infect Public Health* 2020;13:1255–1264.
189. West TE, Frevert CW, Liggitt HD, Skerrett SJ. Inhalation of *Burkholderia thailandensis* results in lethal necrotizing pneumonia in mice: a surrogate model for pneumonic melioidosis. *Trans R Soc Trop Med Hyg* 2008;102 Suppl 1:S119–26.
190. Scales BS, Dickson RP, LiPuma JJ, Huffnagle GB. Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. *Clin Microbiol Rev* 2014;27:927–948.
191. Park SB, Aoki T, Jung TS. Pathogenesis of and strategies for preventing *Edwardsiella tarda* infection in fish. *Vet Res* 2012;43:67.
192. Zeng Y, Liu B. Self-propelling and rolling of a sessile-motile aggregate of the bacterium *Caulobacter crescentus*. *Commun Biol* 2020;3:1–8.
193. Singh S, Bhatia S. Quorum sensing inhibitors: curbing pathogenic infections through inhibition of bacterial communication. *Iran J Pharm Res* 2021;20:486–514.
194. Rada B, Leto TL. Pyocyanin effects on respiratory epithelium: relevance in *Pseudomonas aeruginosa* airway infections. *Trends Microbiol* 2013;21:73–81.
195. Uruén C, Chopo-Escuin G, Tommassen J, Mainar-Jaime RC, Arenas J. Biofilms as promoters of bacterial antibiotic resistance and tolerance. *Antibiotics (Basel)* 2020;10:3.
196. Hauryliuk V, Atkinson GC, Murakami KS, Tenson T, Gerdes K. Recent functional insights into the role of (p)ppGpp in bacterial physiology. *Nat Rev Microbiol* 2015;13:298–309.
197. Zhang S-P, Wang Q, Quan S-W, Yu X-Q, Wang Y, *et al*. Type II toxin–antitoxin system in bacteria: activation, function, and mode of action. *Biophys Rep* 2020;6:68–79.
198. Rycroft JA, Gollan B, Grabe GJ, Hall A, Cheverton AM, *et al*. Activity of acetyltransferase toxins involved in Salmonella persister formation during macrophage infection. *Nat Commun* 2018;9:1993.
199. Walling LR, Butler JS. Homologous VapC toxins inhibit translation and cell growth by sequence-specific cleavage of $tRNA^{fMet}$ *J Bacteriol* 2018;200:e00582-17.
200. Germain E, Castro-Roa D, Zenkin N, Gerdes K. Molecular mechanism of bacterial persistence by HipA. *Mol Cell* 2013;52:248–254.
201. Han Y, Lee E-J. Substrate specificity of bacterial endoribonuclease toxins. *BMB Rep* 2020;53:611–621.
202. Jiang Y, Pogliano J, Helinski DR, Konieczny I. ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of *Escherichia coli* gyrase. *Mol Microbiol* 2002;44:971–979.
203. Cook GM, Robson JR, Frampton RA, McKenzie J, Przybilski R, *et al*. Ribonucleases in bacterial toxin-antitoxin systems. *Biochim Biophys Acta* 2013;1829:523–531.
204. Butt A, Higman VA, Williams C, Crump MP, Hemsley CM, *et al*. The HicA toxin from *Burkholderia pseudomallei* has a role in persister cell formation. *Biochem J* 2014;459:333–344.
205. Dörr T, Vulić M, Lewis K. 2010. Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. PLOS Biology 8:e1000317.
206. Schütz C, Empting M. Targeting the *Pseudomonas* quinolone signal quorum sensing system for the discovery of novel anti-infective pathoblockers . *Beilstein J Org Chem* 2018;14:2627–2645.
207. Whiteley M, Diggle SP, Greenberg EP. Progress in and promise of bacterial quorum sensing research. *Nature* 2017;551:313–320.
208. Ding F, Oinuma K-I, Smalley NE, Schaefer AL, Hamwy O, *et al*. The *Pseudomonas aeruginosa* Orphan Quorum Sensing Signal Receptor QscR Regulates Global Quorum Sensing Gene Expression by Activating a Single Linked Operon . *mBio* 2018;9:mBio .
209. Groleau M-C, de Oliveira Pereira T, Dekimpe V, Déziel E, Shank EA, *et al*. PqsE Is essential for RhlR-dependent quorum sensing regulation in *Pseudomonas aeruginosa*. *mSystems* 2020;5.
210. Carloni S, Macchi R, Sattin S, Ferrara S, Bertoni G. The small RNA ReaL: a novel regulatory element embedded in the *Pseudomonas aeruginosa* quorum sensing networks . *Environmental Microbiology* 2017;19:4220–4237.
211. Lee J, Zhang L. The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. *Protein Cell* 2015;6:26–41.
212. Chugani SA, Whiteley M, Lee KM, D'Argenio D, Manoil C, Greenberg EP. 2001. QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. Proceedings of the National Academy of Sciences 98:2752–2757.

213. Duplantier M, Lohou E, Sonnet P. Quorum sensing inhibitors to quench *P. aeruginosa* pathogenicity. *Pharmaceuticals (Basel)* 2021;14:1262.

214. Leung V, Ajdic D, Koyanagi S, Lévesque CM. The formation of *Streptococcus mutans* persisters induced by the quorum-sensing peptide pheromone is affected by the LexA regulator. *J Bacteriol* 2015;197:1083–1094.

215. Wang S, Zhao C, Xue B, Li C, Zhang X, *et al*. Nanoalumina triggers the antibiotic persistence of Escherichia coli through quorum sensing regulators lrsF and qseB. *J Hazard Mater* 2022;436:129198.

216. Denham JD, Nanjappa S, Greene JN. Treatment of Enteropathogenic *Escherichia coli* diarrhea in cancer patients: a series of three cases. *Case Rep Infect Dis* 2018;2018:8438701.

217. Overview | Urinary tract infection (lower): antimicrobial prescribing | Guidance | NICE. NICE; (n.d.). https://www.nice.org.uk/guidance/ng109 [accessed 16 August 2022].

218. Wu W, Jin Y, Bai F, Jin S. Chapter 41 - Pseudomonas aeruginosa. In: Tang Y-W, Sussman M, Liu D, Poxton I and Schwartzman J (eds). *Molecular Medical Microbiology*, Second Edition. Boston: Academic Press; 2015. pp. 753–767.

219. Langton Hewer SC, Smyth AR. Antibiotic strategies for eradicating *Pseudomonas aeruginosa* in people with cystic fibrosis. *Cochrane Database Syst Rev* 2017;4:CD004197.

220. Banerjee D, Stableforth D. The treatment of respiratory *Pseudomonas* infection in cystic fibrosis: what drug and which way? *Drugs* 2000;60:1053–1064.

221. David MZ, Daum RS. Treatment of Staphylococcus aureus Infections. In: Bagnoli F, Rappuoli R and Grandi G (eds). *Staphylococcus Aureus: Microbiology, Pathology, Immunology, Therapy and Prophylaxis*. Cham: Springer International Publishing; 2017. pp. 325–383.

222. Lee E, Anjum F. *Staphylococcus Epidermidis*. Treasure Island (FL): StatPearls Publishing; 2022.

223. Ashurst JV, Dawson A. *Klebsiella Pneumonia*. Treasure Island (FL): StatPearls Publishing; 2022.

224. Trecarichi EM, Pagano L, Martino B, Candoni A, Di Blasi R, *et al*. Bloodstream infections caused by *klebsiella pneumoniae* in onco-hematological patients: clinical impact of carbapenem resistance in a multicentre prospective survey. *Am J Hematol* 2016;91:1076–1081.

225. Wei M, Yongjie Z, Zhuoyu Q, Biao Y, Xi J, *et al*. Pneumonia caused by Mycobacterium tuberculosis. *Microbes Infect* 2020;22:278–284.

226. Ajmera A, Shabbir N. *SalmonellaStatPearls*. Treasure Island (FL): StatPearls Publishing; 2022.

227. Ross BN, Myers JN, Muruato LA, Tapia D, Torres AG. Evaluating new compounds to treat *Burkholderia pseudomallei* Infections. *Front Cell Infect Microbiol* 2018;8.

228. Scoffone VC, Chiarelli LR, Trespidi G, Mentasti M, Riccardi G, *et al*. *Burkholderia cenocepacia* infections in cystic fibrosis patients: drug resistance and therapeutic approaches. *Front Microbiol* 2017;8:1592.

229. Kanwal S, Vaitla P. *Streptococcus Pyogenes*. StatPearls Publishing, Treasure Island (FL), 2022.

230. Summary of the evidence | Pneumonia (community-acquired): antimicrobial prescribing | Guidance | NICE. NICE; (n.d.). https://www.nice.org.uk/guidance/ng138/chapter/summary-of-the-evidence [accessed 23 August 2022].

231. Michalopoulos A, Falagas ME. Treatment of Acinetobacter infections. *Expert Opin Pharmacother* 2010;11:779–788.

232. Recommendations | clostridioides difficile infection: antimicrobial prescribing | guidance | NICE. NICE; (n.d.). https://www.nice.org.uk/guidance/ng199/chapter/Recommendations#choice-of-antibiotic [accessed 22 August 2022].

233. Antibiotic Treatment | Treatment | Cholera | CDC; 2022. https://www.cdc.gov/cholera/treatment/antibiotic-treatment.html [accessed 22 August 2022].

234. Arget M, Kosar J, Suen B, Peermohamed S. Successful treatment of Legionnaires' disease with tigecycline in an immunocompromised man with a legion of antibiotic allergies. *Cureus* 2019;11:e4577.