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Quantitative and synthetic biology approaches to combat bacterial pathogens

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Abstract

Antibiotic resistance is one of the biggest threats to public health. The rapid emergence of resistant bacterial pathogens endangers the efficacy of current antibiotics and has led to increasing mortality and economic burden. This crisis calls for more rapid and accurate diagnosis to detect and identify pathogens, as well as to characterize their response to antibiotics. Building on this foundation, treatment options also need to be improved to use current antibiotics more effectively and develop alternative strategies that complement the use of antibiotics. We here review recent developments in diagnosis and treatment of bacterial pathogens with a focus on quantitative biology and synthetic biology approaches.

Introduction

The discovery of penicillin by Alexander Fleming brought forth the golden age of antibiotics. Safe and highly effective prevention and treatment of bacterial infections enabled modern surgeries and immunosuppressive therapies. Due to the success of antibiotics, it was once thought that bacterial infections would be fully curable [1]. Decades later, clinically significant resistance against all current antibiotics has been observed [2,3]. Furthermore, due to the low financial incentives for developing new antibiotics in the pharmaceutical industry, the antibiotic pipeline is drying up, leaving few options to treat infections caused by resistant pathogens [1]. As a result, the mortality and economic burden caused by resistant bacteria have been increasing globally [4].

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Antibiotic resistance is an inevitable and complex issue. Being a natural feature of microbial systems [5], antibiotic resistance has been present on earth for millions of years without human influence [6]. In the past several decades, however, humans have generated considerable selection pressure for resistance, partly through antibiotic misuse and overuse [7]. Notably, human use is only a fraction of the total, with livestock receiving the majority of antibiotics—often for non-therapeutic purposes [8]. Following consumption, an estimated 75% to 90% of antibiotics are excreted unmetabolized into water systems and the environment at large [9,10], that generate prolonged sub-lethal selection gradients that can enrich resistant bacteria [11]. The complex dynamics of bacterial response to antibiotics is a fundamental, but often overlooked aspect of this issue [12,13]. For example, bacterial cells that enter dormant states (persistence), where the cells do not divide or divide slowly, can tolerate higher doses of antibiotic than growing cells [14,15]. Bacterial populations can also collectively survive antibiotic treatments that are lethal to individual cells, leading to collective antibiotic tolerance [13,16]. From an evolutionary perspective, bacteria can gain antibiotic resistance through *de novo* mutations or horizontal gene transfer, which are processes that are confounded by environmental and genetic context [17].

Advances in both diagnostics and treatments are critical for addressing the antibiotic resistance crisis (Figure 1). Rapid and accurate diagnosis is a necessary first step to develop effective, targeted treatment to reduce overuse and misuse of antibiotics. Timely administration of appropriate antibiotics is associated with significant improvement of treatment outcome [18]. In contrast, improper diagnosis can lead to ineffective treatments that promote evolution of antimicrobial resistance [19] or increase the susceptibility of patients to secondary infections [20]. As for treatment, antibiotics will likely remain the most dominant approach to combat bacterial infections in the near future despite increasing resistance. A better understanding of bacterial response to antibiotics is key to optimizing antibiotic treatment design. In the long term, if dependency on antibiotics continues, resistance may continue to rise. To overcome this, alternative treatments are needed to diversify treatment options, which can potentially alleviate our dependence on antibiotics. Here we review recent developments in quantitative biology and synthetic biology that improve diagnosis and treatment of bacterial pathogens in light of the antibiotic resistance crisis.

Diagnosis: identification, detection, and drug response

In general, diagnosis aims to detect and identify specific pathogens and characterize how they respond to different drugs. Detection and identification can be carried out with biochemical assays such as immunoassays [21] or sequence-dependent techniques such as whole-genome sequencing [22,23], ribosomal RNA sequencing [24,25] and polymerase chain reactions [26,27]. Characterization of drug responses is often accomplished by measuring growth of bacterial isolates in liquid culture or on solid agar in the presence of various antibiotics at different concentrations [28,29]. Recent studies have demonstrated the promise of new engineered genetic devices and quantitative techniques in complementing existing technologies for both goals.

Detection and identification

Synthetic biology uses rationally designed biological parts to sense and report biomarkers for the detection and identification of bacterial pathogens and their resistance. Synthetic biology approaches aim to be modular: with proper design and implementation, individual modules, such as sensing, processing, and effector modules can be replaced depending on design goals without drastically affecting functions of other modules [30,31]. This quality could speed up design-implementation cycles [32,33], which is beneficial for accommodating the expanding genotypic and phenotypic diversity of bacterial pathogens. Synthetic gene circuits can also process signals to achieve different computational capabilities, including toggle switches [34], logic gates [35,36] and counters [37], which, if accurately tuned, may integrate sophisticated decision making power to diagnosis tools.

A major challenge in predictable engineering of genetic circuits in living cells is the complex interactions between cellular physiology and the designed circuits [38-40]. To address this challenge, cell-free systems provide a simplified platform for gene circuits whose functions primarily depend on gene expression or simple gene regulation [41]. They are particularly suited for rapid prototyping of certain gene circuits [42] or for engineering circuits as sensors. Recent studies have demonstrated the engineering of RNA toehold in living cells [43] and in cell-free systems [44] to detect diverse RNA molecules (Figure 2A). These cell-free sensors were rapidly prototyped to detect specific viral genes and antibiotic resistance genes, suggesting its potential to serve as a novel diagnostic tool. The versatility of sensing the RNA expression level can perhaps take advantage of known mapping between RNA expression profiles of pathogens and the resistance they carry [45,46]. Aside from detecting RNA molecules, cell-free systems can also sense non-RNA molecules that are associated with certain bacterial pathogens [44].

Cell-free systems are not self-sustainable for *in vivo* long-term monitoring and the sophistication of circuit functions is often limited due to the platform constraints. In comparison, sensors based on living cells can offer greater versatility in circuit functions and in their operation. For instance, a recent study demonstrated the use of engineered *Escherichia coli* carrying a genetic toggle switch to record an environmental signal in living animals [47] (Figure 2B). A model chemical (anhydrotetracycline) is used in this study but the design can be adapted to sense biomarkers of interest, including those associated with bacterial infections [48]. Along the same line, another study demonstrated the engineering of a probiotic strain of *E. coli* (Nissle 1917) to sense thiosulfate and tetrathionate, which are associated with a gut inflammation mouse model infected by *Salmonella typhimurium* [49,50]. Such whole-cell sensors have the potential of being used for continuous monitoring of host environments for biomarkers associated with bacterial infections.

In the long-term, such engineered cell-based sensors can benefit from a number of recent developments in synthetic biology. For instance, the examples listed above focus on sensing small molecules. The sensing capability can be further expanded by incorporating additional sensing and actuating functions, such as those based on engineered protein switches [51,52]. Moreover, instead of storing the sensory information by regulating gene expression, the memory recorded in DNA molecules in single cells through stimuli-responsive genome

editing can provide relatively more stable memory storage [53,54]. In addition, engineered microbial communities can also be used to implement memory capabilities [55].

As viruses that infect bacteria, phages typically have a narrow host range, which can also be exploited for the detection of specific living pathogens [56] (Figure 2C). To facilitate detection and quantification, engineered phages have been equipped with a wide range of reporter genes, which code for fluorescence [57] or enzymatic activities [58-60]. These readouts are often more rapid to quantify and require less steps than plaque assays or immunoassays [61]. In addition to detection, engineered phages can be used to quantify bacterial response to antibiotic treatments with high sensitivity [62-64]. Although most phages have narrow host ranges, which enables detection with high specificity, this property often necessitates the identification and optimization of phages to target one or a group of specific bacterial hosts of interest. However, detection and quantification of multiple different host bacteria can be achieved by using well-defined phage cocktails [65].

Diagnostics based on phenotypic profiling

Sequence-based techniques may not provide sufficient information on drug responses due to the complexity of genotype-phenotype mapping [17,66]. Direct measurements of drug responses may then be preferable. However, current drug susceptibility assays (e.g. disk diffusion assays) have long turnaround time (typically 16~24 hours after isolation) [67] and usually indicate only minimum inhibitory concentration [21], which is the lowest concentration required to completely inhibit bacterial growth.

Technologies that enable faster and more accurate quantifications of antimicrobial susceptibility are required. These technologies are based on phenotypic signatures from either cell populations [68-70] or single cells [71]. Bacterial growth in terms of biomass accumulation can be detected using cantilever fluctuation patterns [68]. With its ultra-sensitivity, this method can return drug susceptibility test results within an hour and can also profile bacterial metabolism [69]. Furthermore, the increase in biomass can also be detected by electrical impedance, which enables another method to quantify bacterial antibiotic response [70]. Besides population-level phenotypes, single cell morphology can also indicate antibacterial susceptibility. Using high-resolution microscopy, single cell morphology analysis can provide test results with high accuracy within 4 hours [71]. However, the cost of high-resolution microscopy can be restrictive for regular clinical use. Note that since many detection methods with high sensitivity start with low number of cells, certain antibiotic responses like collective antibiotic tolerance may not be captured [13].

Besides improving technologies for better phenotypic profiling, it is equally, if not more important to utilize quantitative biology to reveal the rich information content imbedded in these measurements. Because of the complex interplay between bacterial populations and their environment, the growth dynamics of a population can serve as unique phenotypic signatures of the population. To demonstrate feasibility, a recent study used growth curves in a liquid culture with high temporal resolution to identify bacteria at a strain level (including pathogenic strains) [72]. Bacterial growth in the spatial dimension, such as bacterial colony growth can also provide information for diagnosis. High-resolution quantification of bacterial growth on agar plates (quantified by image scanners) has been used to deduce

phenotypic parameters associated with antibiotic susceptibility, antibiotic tolerance and potential to develop resistance [73,74]. Due to the abundant information, temporal and spatial growth dynamics of bacteria can potentially serve as the basis for strain-level identification of pathogens, antibiotic susceptibility test, and evaluation of the likelihood of resistance acquisition.

Treatments

Considering that much of the antibiotic resistance crisis is likely due to overuse or misuse of antibiotics, more effective use of antibiotics is critical for alleviating the crisis. At the global level, this goal has been partially addressed through restricting non-medical use of antibiotics [75] and, in the clinical setting, through antibiotic stewardship programs [76]. For individual patients, proper use of antibiotics requires the optimal design of dosing protocols and drug combinations. Both needs can benefit from a better quantitative understanding of bacterial antibiotic response. In addition, alternative treatment strategies, including those based on synthetic biological systems, can complement the use of antibiotics.

Effective use of existing antibiotics

Improvement in antibiotic usage, especially multi-dose regimens and multi-drug combinations, has shown promise in increasing treatment efficacy [77], delaying emergence of antibiotic resistance [78], and reversing resistance [12]. Such design is often built on a quantitative understanding and characterizations of bacterial responses to certain antibiotics. Using computational modeling, a recent study demonstrates how the temporal response of a bacterial population to a single dose of antibiotic treatment can be used to optimize multi-dosing protocols [79]. The strategy can also be generalized to mixed populations consisting of persister cells and sensitive populations [79].

While combination therapy represents a promising strategy to bacterial pathogens [80], determining optimal combinations can be a daunting task: the number of possible pairs and triplets increase combinatorially with the number of available drugs. To address this challenge, a recent study used quantitative biology tools coupled with appropriate experimental design to demonstrate that the response to drug pairs at several doses can predict responses to three or more drugs at all doses [81]. This allows the design of an optimal multi-drug combination with manageable experiments. Prediction of potent drug combinations can also benefit from integrating other sources of information, such as chemogenomics data which indicate the drug targets of antibiotics [82].

When designing an antibiotic treatment, evolutionary dynamics should also be exploited to reduce selection for or even select against resistant bacteria. Evidence is mounting that mutational pathways toward resistance are constrained depending on the antibiotics used [83,84]. Exploiting these limited mutational pathways can allow design of dosing protocols that eliminate infection while minimizing resistance evolution. It is then important to predict how quickly resistance will develop and whether the mutational pathway is consistent for a given drug. For this, variability in resistance strength and perturbation of growth rates across different antibiotic treatments may serve as predictive quantitative parameters [85]. In the case of multi-drug treatments, negative cross resistance (i.e. the phenomenon that increasing

resistance to one antibiotic leads to collateral sensitivity of another) is predictive of whether cycling two antibiotics will slow down evolution [86]. These strategies may extend beyond *de novo* mutation, with selection potentially governing horizontal gene transfer dynamics as well [87]. Ultimately, treatment strategy may become a balance between applying strong selective pressure (i.e. rapid clearance of infection) and minimizing chance of resistance (i.e. drawn out evolutionary manipulation) [12].

Alternative treatments using synthetic biology

Beyond better use of existing antibiotics, alternative treatment strategies involving synthetic biology may decrease our dependence on antibiotics and reduce overall selection pressure on resistant bacteria in the long term.

In addition to serving as a novel diagnostic tool, the ability of phages to infect and potentially lyse specific hosts have also been evaluated for their utility as therapeutics. Phage therapies are attractive especially for its ability to treat infections caused by multidrug resistant bacterial pathogens [88]. In contrast to antibiotics, phage therapeutics have several distinct properties. One property is the ability of phages to increase in number during treatments, which allows low doses and the potential to use a single dose for successful treatments [89]. Along the same line, phages can also coevolve with their bacterial hosts during treatment, keeping even the most abundant bacteria in check [88,90]. Although bacterial pathogens can also develop resistance to phages, ineffectual phages can be improved via mutagenesis [91] and phage-resistant bacteria may also exhibit reduced virulence [92]. Another property of natural phages is their narrow host ranges which allow the protection of commensal microflora, resulting in fewer side effects than therapies based on broad-spectrum antibiotics [89]. However, when the narrow host range limits the therapeutic generalizability of phages, it may be overcome through the development of carefully-designed phage cocktails [93] or by engineering phages with broadened host range through, for example, modulation of tail fiber compositions [94,95].

To enhance their efficacy, phages have been engineered to encode effectors to target different aspects of the host bacteria. Biofilms are complex structures formed by bacterial cells and a matrix of extracellular polymeric substances, which serve as a physical barrier that can reduce penetration of antibiotics [96]. Furthermore, bacterial cells in biofilms exhibit a high degree of genetic and phenotypic heterogeneity and often contain subpopulations (e.g. persisters) that are highly tolerant against antibiotics. As such, the function to target biofilms makes engineered phages more effective in targeting bacterial pathogens or serve as an adjuvant to antibiotic treatment. For instance, an engineered T7 phage encoding a biofilm-dispersal enzyme exhibited greater efficiency than the wild-type T7 phage in targeting biofilm-forming bacteria [97].

In addition, phages have also been engineered to target antibiotic resistant pathogens by silencing the responsible genes (Figure 3A) [98,99] or by using these genes as a cue to increase specificity [100-102]. These developments have benefited from the recent advancements in genome-editing tools, such as clustered regularly interspaced short palindromic repeats-CRISPR associated genes (CRISPR-Cas). Specifically, RNA-guided Cas9 has been used in engineered phages to sense the presence of specific antibiotic

resistance genes and to kill the corresponding host cells by cleaving their chromosomal DNA (Figure 3B) [100-102]. Due to the specificity of the treatment, the method can preserve commensal bacteria, which if disturbed can lead to adverse effect such as *Clostridium difficile* infection [20]. Alternatively, CRISPR-Cas system carried by a temperate phage has been used to remove antibiotic resistance genes while giving protection against lysis. By providing a fitness benefit in exchange for resistance, such treatments have the potential to enhance the longevity of antibiotics against evolving targets [99].

Due to their small genome size, engineered phages have limited number of exogenous genes that can be introduced. Engineered whole-cell systems therefore offer higher flexibility and programmability. Engineered probiotics and commensal bacterial cells can have a diverse range of capabilities, including delivery of antimicrobial, delivery of antibiotic adjuvants, virulence inhibition, toxin neutralization and adhesion prevention [103]. When coupled with sensory capabilities, engineered bacteria could provide streamlined treatment and prevention of bacterial infections. This capability has been demonstrated in several cases. *Lactococcus lactis* was engineered to detect *Enterococcus faecalis* by sensing enterococcal pheromone and subsequently expressing an antimicrobial peptide to inhibit *E. faecium* [104]. *E. coli* was engineered to sense small molecules secreted by *P. aeruginosa* to induce expression of a bacteriocin that targets *P. aeruginosa* [105]. *E. coli* was programmed to localize to *P. aeruginosa* and deploy an antimicrobial peptide and biofilm dispersing enzyme to kill *P. aeruginosa* [106] (Figure 3C). A recent study demonstrated *in vivo* efficacy of an engineered *E. coli* strain to sense, treat and prevent *P. aeruginosa* gut infection in animal models [107]. Moving beyond traditional model organisms, a recent study demonstrated the capability to engineer a non-model organism *Bacteroides thetaiotaomicron*, an abundant and stable component of the human gut microbiome. The engineered *B. thetaiotaomicron* can colonize in the mouse gut to sense and respond to stimuli [108].

Although engineered bacteria have great potential to become living therapeutics, introduction of engineered bacteria into human host can pose safety concerns as engineered bacteria may evolve and spread to the environment, causing unintended negative impacts to the human host and beyond. To combat this, engineered safeguard mechanisms have been developed to prevent unintended proliferation of engineered bacteria by killing bacteria that escape from spatial confinement [109] or by controlling the death or survival of engineered bacteria through specific chemical cues [110].

Complex population responses to antibiotic treatment

Bacterial pathogens have complex community dynamics [111,112], which play a role in their ability to infect hosts. Quantitative biology approaches, such as computational modeling provide a way to explain experimentally-observed complex community dynamics as well as distilling general rules to guide treatment designs. Cooperation within and between bacterial populations is ubiquitous and represents alternative targets in developing new treatments. One form of this cooperation is collective antibiotic tolerance [13], where a population survives antibiotic treatment only when initial population density is sufficiently high (Figure 4A). This density-dependent survival can emerge from different mechanisms [13]. A common example of collective antibiotic tolerance is the response to β -lactam

antibiotics where cells express β -lactamases to collectively degrade the antibiotic (Figure 4B). As such, treating β -lactam producing strains by blocking the activity of β -lactamases through adjuvant inhibitors like tazobactam and sulbactam has emerged as an effective strategy. However, one study found through computational modeling and experimental results that insufficient blocking can also lead to unintended spread of antibiotic resistance due to the interaction between a β -lactamase producing strain and a cheater strain [113]. This study highlights the importance of population dynamics in treatment design.

Antibiotic treatments can also alter the interplay between cooperators and cheaters which can impact treatment outcomes (Figure 4C). *P. aeruginosa* can cooperate through production of siderophores that chelate iron in the environment as a public good. In a mixture of siderophore-producing cooperators and non-producing cheaters, sub-inhibitory concentrations of gentamicin can increase the fitness advantage of the cheaters, causing a decline in the cooperator strain [114]. Interestingly, this study found that the evolved resistance reaches the highest frequency in a mixture of the producer strain and the non-producer strain compared with monocultures. Using a mathematical model, the authors provided explanations to the experimental observations and established a general principle underlying the interplay between stress resistance and cheater-cooperator interaction [114]. In a different example, *Salmonella typhimurium* is a pathogen that exhibits cooperative virulence. Expression of virulence factors is costly for individual *S. typhimurium*, but is beneficial at the population level. In contrast to the antibiotic treatment response in *P. aeruginosa*, antibiotic treatment of *S. typhimurium* has the potential to increase the virulent cooperator strain and increase disease transmission, which is possibly due to differential selection created by spatial structure [115]. These opposite responses to antibiotic treatment demonstrate the importance of understanding cooperator-cheater dynamics during the response to specific antibiotics.

Quorum sensing (QS) is a mechanism by which bacteria produce and sense small molecule signals to regulate gene expressions based on population size. Given the diverse roles of QS in regulating antibiotic resistance, virulence and biofilm formation, it has been suggested as a potential target for inhibiting bacterial survival or virulence [116,117] (Figure 4D). Indeed, degradation or inactivation of QS signals has been demonstrated to inhibit growth or virulence of several pathogens, including *Vibrio cholerae* [118], *P. aeruginosa* [119] and *Staphylococcus aureus* [120]. However, inhibition of QS signaling can also lead to unintended evolutionary consequences. A clinical study suggests that inhibiting QS signals can lead to the selection for a more virulent variant of a pathogen [121]. Furthermore, QS inhibitors could also interfere with QS among commensal or other beneficial bacterial communities. Due to the different outcomes, better quantitative characterizations can help tease out what conditions dictate efficient treatments that targeting QS activities of bacterial pathogens.

Conclusion

Advances in diagnostics and treatments are critical to improve our ability to combat the antibiotic resistance crisis. Quantitative biology can provide a fundamental understanding of population and evolutionary dynamics in response to antibiotics, which includes cheater-

cooperator interactions [122], antibiotic stress responses [16], resistome diversity [5], and horizontal gene transfer [123]. This understanding can help us associate treatment outcomes with quantitative parameters that can be collected from diagnostic tests which in return opens new strategies to guide diagnosis and treatments. Using this principle, rational design of antibiotic treatment has been demonstrated to be effective in treating resistant pathogens and reversing resistance. In concert with quantitative biology, synthetic biology holds great promise to combat bacterial pathogens by providing platforms that have increased sensory capacities for diagnostics and versatile options for treatments.

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References

1. Power E. Impact of antibiotic restrictions: the pharmaceutical perspective. *Clinical Microbiology and Infection*. 2006; 12:25–34.
2. Coates ARM, Halls G, Hu YM. Novel classes of antibiotics or more of the same? *British Journal of Pharmacology*. 2011; 163:184–194. [PubMed: 21323894]
3. Clatworthy AE, Pierson E, Hung DT. Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chemical Biology*. 2007; 3:541–548. [PubMed: 17710100]
4. Laxminarayan R, Matsoso P, Pant S, Brower C, Rottingen JA, Klugman K, Davies S. Access to effective antimicrobials: a worldwide challenge. *Lancet*. 2016; 387:168–175. [PubMed: 26603918]
5. Crofts TS, Gasparrini AJ, Dantas G. Next-generation approaches to understand and combat the antibiotic resistome. *Nat Rev Microbiol*. 2017
6. Bhullar K, Waglechner N, Pawlowski A, Koteva K, Banks ED, Johnston MD, Barton HA, Wright GD. Antibiotic resistance is prevalent in an isolated cave microbiome. *PLoS One*. 2012; 7:e34953. [PubMed: 22509370]
7. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P T*. 2015; 40:277–283. [PubMed: 25859123]
8. Landers TF, Cohen B, Wittum TE, Larson EL. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. *Public Health Reports*. 2012; 127:4–22. [PubMed: 22298919]
9. Sarmah AK, Meyer MT, Boxall AB. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*. 2006; 65:725–759. [PubMed: 16677683]
10. Kummerer K. Antibiotics in the aquatic environment--a review--part I. *Chemosphere*. 2009; 75:417–434. [PubMed: 19185900]
11. Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. *Nat Rev Microbiol*. 2014; 12:465–478. [PubMed: 24861036]
12. Baym M, Stone LK, Kishony R. Multidrug evolutionary strategies to reverse antibiotic resistance. *Science*. 2016; 351
13. Meredith HR, Srimani JK, Lee AJ, Lopatkin AJ, You L. Collective antibiotic tolerance: mechanisms, dynamics and intervention. *Nat Chem Biol*. 2015; 11:182–188. [PubMed: 25689336]
14. Harms A, Maisonneuve E, Gerdes K. Mechanisms of bacterial persistence during stress and antibiotic exposure. *Science*. 2016; 354
15. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Bacterial persistence as a phenotypic switch. *Science*. 2004; 305:1622–1625. [PubMed: 15308767]
16. Chait R, Palmer AC, Yelin I, Kishony R. Pervasive selection for and against antibiotic resistance in inhomogeneous multistress environments. *Nat Commun*. 2016; 7:10333. Natural resistance is the product of evolution in multi-stress environments. Mixed susceptible and resistant *E. coli*

were exposed to diverse combinations of stresses, resulting in differential population growth. These results reveal situations where the interaction of multiple stresses appear to dictate resistance dynamics, rather than direct selection by the antibiotic itself. [PubMed: 26787239]

17. Hughes D, Andersson DI. Environmental and genetic modulation of the phenotypic expression of antibiotic resistance. *FEMS Microbiol Rev.* 2017; 41:374–391. [PubMed: 28333270]
18. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Peterson LE, Musser JM. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. *J Infect.* 2014; 69:216–225. [PubMed: 24841135]
19. Bruniera FR, Ferreira FM, Saviolli LRM, Bacci MR, Feder D, Pedreira MDG, Peterlini MAS, Azzalis LA, Junqueira VBC, Fonseca FLA. The use of vancomycin with its therapeutic and adverse effects: a review. *European Review for Medical and Pharmacological Sciences.* 2015; 19:694–700. [PubMed: 25753888]
20. Leffler DA, Lamont JT. *Clostridium difficile* Infection. *New England Journal of Medicine.* 2015; 373:287–288.
21. Washington JA. Principles of Diagnosis. In: Baron S, editor *Medical Microbiology.* 4th. 1996.
22. Reuter S, Ellington MJ, Cartwright EJP, Koser CU, Torok ME, Gouliouris T, Harris SR, Brown NM, Holden MTG, Quail M, et al. Rapid Bacterial Whole-Genome Sequencing to Enhance Diagnostic and Public Health Microbiology. *Jama Internal Medicine.* 2013; 173:1397–1404. [PubMed: 23857503]
23. Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, Ip CLC, Golubchik T, Batty EM, Finney JM, et al. Diverse Sources of *C. difficile* Infection Identified on Whole-Genome Sequencing. *New England Journal of Medicine.* 2013; 369:1195–1205. [PubMed: 24066741]
24. Byron SA, Van Keuren-Jensen KR, Engelthaler DM, Carpten JD, Craig DW. Translating RNA sequencing into clinical diagnostics: opportunities and challenges. *Nature Reviews Genetics.* 2016; 17:257–271.
25. Salipante SJ, Sengupta DJ, Rosenthal C, Costa G, Spangler J, Sims EH, Jacobs MA, Miller SI, Hoogestraat DR, Cookson BT, et al. Rapid 16S rRNA Next-Generation Sequencing of Polymicrobial Clinical Samples for Diagnosis of Complex Bacterial Infections. *Plos One.* 2013; 8
26. Chang SS, Hsieh WH, Liu TS, Lee SH, Wang CH, Chou HC, Yeo YH, Tseng CP, Lee CC. Multiplex PCR System for Rapid Detection of Pathogens in Patients with Presumed Sepsis - A Systemic Review and Meta-Analysis. *Plos One.* 2013; 8
27. Brittain-Long R, Nord S, Olofsson S, Westin J, Anderson LM, Lindh M. Multiplex real-time PCR for detection of respiratory tract infections. *Journal of Clinical Virology.* 2008; 41:53–56. [PubMed: 18093871]
28. Jorgensen JH, Turnidge JD. *Manual of Clinical Microbiology.* 9th. 2007. Susceptibility Test Methods: Dilution and Disk Diffusion Methods; 1152–1172.
29. Turnidge JD, Ferraro MJ, Jorgensen JH. *Manual of Clinical Microbiology.* 9th. 2007. Susceptibility Test Methods: General Considerations; 1146–1151.
30. Mishra D, Rivera PM, Lin A, Del Vecchio D, Weiss R. A load driver device for engineering modularity in biological networks. *Nat Biotechnol.* 2014; 32:1268–1275. [PubMed: 25419739]
31. Del Vecchio D. Modularity, context-dependence, and insulation in engineered biological circuits. *Trends Biotechnol.* 2015; 33:111–119. [PubMed: 25544476]
32. Khalil AS, Collins JJ. Synthetic biology: applications come of age. *Nat Rev Genet.* 2010; 11:367–379. [PubMed: 20395970]
- 33•. Nielsen AA, Der BS, Shin J, Vaidyanathan P, Paralanov V, Strychalski EA, Ross D, Densmore D, Voigt CA. Genetic circuit design automation. *Science.* 2016; 352:aac7341. The authors utilize principles from electronic design automation to build a software that enable users to describe the circuit function and implementation constraints. The software then output specific circuit designs. With no additional tuning, the automated design generated 92% of output states correctly. [PubMed: 27034378]
34. Gardner TS, Cantor CR, Collins JJ. Construction of a genetic toggle switch in *Escherichia coli.* *Nature.* 2000; 403:339–342. [PubMed: 10659857]
35. Bonnet J, Yin P, Ortiz ME, Subsoontorn P, Endy D. Amplifying Genetic Logic Gates. *Science.* 2013; 340:599–603. [PubMed: 23539178]

36. Tamsir A, Tabor JJ, Voigt CA. Robust multicellular computing using genetically encoded NOR gates and chemical ‘wires’. *Nature*. 2011; 469:212–215. [PubMed: 21150903]
37. Friedland AE, Lu TK, Wang X, Shi D, Church G, Collins JJ. Synthetic gene networks that count. *Science*. 2009; 324:1199–1202. [PubMed: 19478183]
38. Zhang C, Tsoi R, You LC. Addressing biological uncertainties in engineering gene circuits. *Integrative Biology*. 2016; 8:456–464. [PubMed: 26674800]
39. Venturelli OS, Egbert RG, Arkin AP. Towards Engineering Biological Systems in a Broader Context. *Journal of Molecular Biology*. 2016; 428:928–944. [PubMed: 26546279]
40. Tan C, Marguet P, You L. Emergent bistability by a growth-modulating positive feedback circuit. *Nat Chem Biol*. 2009; 5:842–848. [PubMed: 19801994]
41. Hodgman CE, Jewett MC. Cell-free synthetic biology: thinking outside the cell. *Metab Eng*. 2012; 14:261–269. [PubMed: 21946161]
42. Smith MT, Wilding KM, Hunt JM, Bennett AM, Bundy BC. The emerging age of cell-free synthetic biology. *FEBS Lett*. 2014; 588:2755–2761. [PubMed: 24931378]
43. Green AA, Silver PA, Collins JJ, Yin P. Toehold Switches: De-Novo-Designed Regulators of Gene Expression. *Cell*. 2014; 159:925–939. [PubMed: 25417166]
44. Pardee K, Green AA, Ferrante T, Cameron DE, DaleyKeyser A, Yin P, Collins JJ. Paper-Based Synthetic Gene Networks. *Cell*. 2014; 159:940–954. [PubMed: 25417167]
45. Barczak AK, Gomez JE, Kaufmann BB, Hinson ER, Cosimi L, Borowsky ML, Onderdonk AB, Stanley SA, Kaur D, Bryant KF, et al. RNA signatures allow rapid identification of pathogens and antibiotic susceptibilities. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:6217–6222. [PubMed: 22474362]
46. Suzuki S, Horinouchi T, Furusawa C. Prediction of antibiotic resistance by gene expression profiles. *Nature Communications*. 2014; 5
47. Kotula JW, Kerns SJ, Shaket LA, Siraj L, Collins JJ, Way JC, Silver PA. Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:4838–4843. [PubMed: 24639514]
48. Wallis RS, Kim P, Cole S, Hanna D, Andrade BB, Maeurer M, Schito M, Zumla A. Tuberculosis biomarkers discovery: developments, needs, and challenges. *Lancet Infectious Diseases*. 2013; 13:362–372. [PubMed: 23531389]
49. Daeffler KNM, Galley JD, Sheth RU, Ortiz-Velez LC, Bibb CO, Shroyer NF, Britton RA, Tabor JJ. Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. *Molecular Systems Biology*. 2017; 13 The authors genetically engineered *E. coli* with thiosulfate and tetrathionate sensors, which were identified using computational methods from other bacterial species. Together with flow cytometry, the bacterial whole-cell sensors were shown efficacy in detecting both thiosulfate and tetrathionate.
50. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Russell JM, Bevins CL, Adams LG, Tsolis RM, et al. Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature*. 2010; 467:426–429. [PubMed: 20864996]
51. Stein V, Alexandrov K. Synthetic protein switches: design principles and applications. *Trends Biotechnol*. 2015; 33:101–110. [PubMed: 25535088]
52. Nicholes N, Date A, Beaujean P, Hauk P, Kanwar M, Ostermeier M. Modular protein switches derived from antibody mimetic proteins. *Protein Eng Des Sel*. 2016; 29:77–85. The authors demonstrated the feasibility of building a modular protein switch by the fusion of enzyme TEM-1 β -lactamase with antibody mimetic proteins. The activity of sensors increased up to 14-fold in the presence of target, which offered an opportunity to be used to detect specific proteins in diagnostic tools. [PubMed: 26637825]
53. Farzadfard F, Lu TK. Genomically encoded analog memory with precise in vivo DNA writing in living cell populations. *Science*. 2014; 346:825–+.
54. Siuti P, Yazbek J, Lu TK. Synthetic circuits integrating logic and memory in living cells. *Nature Biotechnology*. 2013; 31:448–+.
55. Urrios A, Macia J, Manzoni R, Conde N, Bonforti A, de Nadal E, Posas F, Sole R. A Synthetic Multicellular Memory Device. *Acs Synthetic Biology*. 2016; 5:862–873. Single cell memory

devices that use positive feedback loops tend to subject to fluctuations and leaky expressions which can potentially lead to false output. The authors implemented a mutual repressor motif with two cell types to create a bistable system to sense and maintain sustained memory response to two chemicals in a more robust manner. [PubMed: 27439436]

56. Anany H, Chou Y, Cucic S, Derda R, Evoy S, Griffiths MW. From Bits and Pieces to Whole Phage to Nanomachines: Pathogen Detection Using Bacteriophages. *Annual Review of Food Science and Technology*. Vol 8. 2017; 8:305–329.
57. Vinay M, Franche N, Gregori G, Fantino JR, Pouillot F, Ansaldi M. Phage-Based Fluorescent Biosensor Prototypes to Specifically Detect Enteric Bacteria Such as *E. coli* and *Salmonella enterica* Typhimurium. *PLoS One*. 2015; 10:e0131466. The phages were engineered with genes encoded green fluorescence protein that would be expressed when the bacterial host *Salmonella enterica* Typhimurium is present. This method detects bacteria with high speed and sensitivity, allowing the detection of 10 bacterial cells per ml. [PubMed: 26186207]
58. Burnham S, Hu J, Anany H, Brovko L, Deiss F, Derda R, Griffiths MW. Towards rapid on-site phage-mediated detection of generic *Escherichia coli* in water using luminescent and visual readout. *Anal Bioanal Chem*. 2014; 406:5685–5693. [PubMed: 24969469]
59. Javed MA, Poshtiban S, Arutyunov D, Evoy S, Szymanski CM. Bacteriophage receptor binding protein based assays for the simultaneous detection of *Campylobacter jejuni* and *Campylobacter coli*. *PLoS One*. 2013; 8:e69770. [PubMed: 23874996]
60. Irwin P, Gehring A, Tu SI, Brewster J, Fanelli J, Ehrenfeld E. Minimum detectable level of *Salmonellae* using a binomial-based bacterial ice nucleation detection assay (BIND). *J AOAC Int*. 2000; 83:1087–1095. [PubMed: 11048849]
61. Pires DP, Cleto S, Sillankorva S, Azeredo J, Lu TK. Genetically Engineered Phages: a Review of Advances over the Last Decade. *Microbiology and Molecular Biology Reviews*. 2016; 80:523–543. [PubMed: 27250768]
62. Chen JH, Alcaine SD, Jackson AA, Rotello VM, Nugen SR. Development of Engineered Bacteriophages for *Escherichia coli* Detection and High-Throughput Antibiotic Resistance Determination. *Acs Sensors*. 2017; 2:484–489. T7 phages were engineered to overexpress β -galactosidase during phage infection, which is detected by a colorimetric substrate. The engineered phage enabled the detection of *E. coli* cells at 10 CFU·mL⁻¹ within 7 h, greatly increasing the speed and sensitivity of *E. coli* detection. [PubMed: 28723178]
63. Rondon L, Piuri M, Jacobs WR, de Waard J, Hatfull GF, Takiff HE. Evaluation of Fluoromycobacteriophages for Detecting Drug Resistance in *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology*. 2011; 49:1838–1842. [PubMed: 21346042]
64. O'Donnell MR, Pym A, Jain P, Munsamy V, Wolf A, Karim F, Jacobs WR, Larsene MH. A Novel Reporter Phage To Detect Tuberculosis and Rifampin Resistance in a High-HIV-Burden Population. *Journal of Clinical Microbiology*. 2015; 53:2188–2194. [PubMed: 25926493]
65. Schmelcher M, Loessner MJ. Application of bacteriophages for detection of foodborne pathogens. *Bacteriophage*. 2014; 4:e28137. [PubMed: 24533229]
66. Piddock LJ. Assess drug-resistance phenotypes, not just genotypes. *Nat Microbiol*. 2016; 1:16120. [PubMed: 27573119]
67. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis*. 2009; 49:1749–1755. [PubMed: 19857164]
68. Gfeller KY, Nugaeva N, Hegner M. Micromechanical oscillators as rapid biosensor for the detection of active growth of *Escherichia coli*. *Biosensors & Bioelectronics*. 2005; 21:528–533. [PubMed: 16076445]
69. Longo G, Alonso-Sarduy L, Rio LM, Bizzini A, Trampuz A, Notz J, Dietler G, Kasas S. Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors. *Nature Nanotechnology*. 2013; 8:522–526.
70. Safavi M, Pandya HJ, Venkataraman M, Thirumalaraju P, Kanakasabapathy MK, Singh A, Prabhakar D, Chug MK, Shafiee H. Rapid Real-Time Antimicrobial Susceptibility Testing with Electrical Sensing on Plastic Microchips with Printed Electrodes. *Acs Applied Materials & Interfaces*. 2017; 9:12832–12840. The authors designed and produced a microfluidic device to isolate bacterial pathogens from whole blood samples using specific antibodies and treat the

- bacteria either with or without antibiotics. The drug response is captured with a high sensitivity by an impedance analyzer in real-time during 1 hour of incubation. [PubMed: 28291334]
71. Choi J, Yoo J, Lee M, Kim EG, Lee JS, Lee S, Joo S, Song SH, Kim EC, Lee JC, et al. A rapid antimicrobial susceptibility test based on single-cell morphological analysis. *Sci Transl Med*. 2014; 6:267ra174.
 72. Tan C, Smith RP, Tsai MC, Schwartz R, You LC. Phenotypic Signatures Arising from Unbalanced Bacterial Growth. *Plos Computational Biology*. 2014; 10
 73. Levin-Reisman I, Gefen O, Fridman O, Ronin I, Shwa D, Sheftel H, Balaban NQ. Automated imaging with ScanLag reveals previously undetectable bacterial growth phenotypes. *Nature Methods*. 2010; 7:737–U100. [PubMed: 20676109]
 - 74•. Levin-Reisman I, Ronin I, Gefen O, Braniss I, Shores N, Balaban NQ. Antibiotic tolerance facilitates the evolution of resistance. *Science*. 2017; 355:826–830. Tolerance is the ability of bacteria to survive under antibiotic treatments without developing resistance. The study found that prolonged tolerance indicated by long lag phase of colony growth dynamic is predictive of evolution of resistance to antibiotic treatments. [PubMed: 28183996]
 75. Meek RW, Vyas H, Piddock LJ. Nonmedical Uses of Antibiotics: Time to Restrict Their Use? *PLoS Biol*. 2015; 13:e1002266. [PubMed: 26444324]
 76. Ventola CL. The antibiotic resistance crisis: part 2: management strategies and new agents. *P T*. 2015; 40:344–352. [PubMed: 25987823]
 77. Monogue ML, Kuti JL, Nicolau DP. Optimizing Antibiotic Dosing Strategies for the Treatment of Gram-negative Infections in the Era of Resistance. *Expert Review of Clinical Pharmacology*. 2016; 9:459–476. [PubMed: 26678036]
 78. Soothill G, Hu Y, Coates A. Can we prevent antimicrobial resistance by using antimicrobials better? *Pathogens*. 2013; 2:422–435. [PubMed: 25437042]
 - 79•. Meredith HR, Lopatkin AJ, Anderson DJ, You L. Bacterial temporal dynamics enable optimal design of antibiotic treatment. *PLoS Comput Biol*. 2015; 11:e1004201. Through computational modeling, the study found a general metric: recovery time, which is the time for a population to return to its initial density after a single dose of antibiotics. This metric can guide the design and optimization of β -lactam antibiotic dosing regimen to treat pathogens that produce low or moderate level of β -lactamases. [PubMed: 25905796]
 80. Daikos GL, Tsousi S, Tzouveleki LS, Anyfantis I, Psychogiou M, Argyropoulou A, Stefanou I, Sypsa V, Miriagou V, Nepka M, et al. Carbapenemase-Producing *Klebsiella pneumoniae* Bloodstream Infections: Lowering Mortality by Antibiotic Combination Schemes and the Role of Carbapenems. *Antimicrobial Agents and Chemotherapy*. 2014; 58:2322–2328. [PubMed: 24514083]
 - 81•. Zimmer A, Katzir I, Dekel E, Mayo AE, Alon U. Prediction of multidimensional drug dose responses based on measurements of drug pairs. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113:10442–10447. The authors built a simple mechanism-independent model to predict drug responses to combinations of three or four drugs with any dose combinations by utilizing drug responses to single drug and drug pairs at a small number of doses. The model predictions fit well with experimental data and potentially provide a computational tool to optimize drug combinations. [PubMed: 27562164]
 - 82•. Chandrasekaran S, Cokol-Cakmak M, Sahin N, Yilancioglu K, Kazan H, Collins JJ, Cokol M. Chemogenomics and orthology-based design of antibiotic combination therapies. *Molecular Systems Biology*. 2016; 12 Leveraging chemogenomics data, which indicates drug targets of antibiotics, the authors trained a model using a machine learning algorithm based on *E. coli* data to predict response of any drug pairs with known chemogenomics data. The model is also predictive of drug responses of *Mycobacterium tuberculosis* and *Staphylococcus aureus* based on ortholog of genes found in the *E. coli* model.
 83. Weinreich DM, Delaney NF, DePristo MA, Hartl DL. Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science*. 2006; 312:111–114. [PubMed: 16601193]
 84. Toprak E, Veres A, Michel JB, Chait R, Hartl DL, Kishony R. Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. *Nat Genet*. 2011; 44:101–105. [PubMed: 22179135]

- 85•. Chevereau G, Dravecka M, Batur T, Guvenek A, Ayhan DH, Toprak E, Bollenbach T. Quantifying the Determinants of Evolutionary Dynamics Leading to Drug Resistance. *PLoS Biol.* 2015; 13:e1002299. Resistance does not develop in the same way for different antibiotics, but why these differences manifest is unclear. Variability in mutants' resistance strengths and perturbation of growth rates under antibiotic stress may be predictive of how resistance develops. Predictability and overall understanding of resistance evolution enables the design of effective counter strategies. [PubMed: 26581035]
86. Kim S, Lieberman TD, Kishony R. Alternating antibiotic treatments constrain evolutionary paths to multidrug resistance. *Proceedings of the National Academy of Sciences of the United States of America.* 2014; 111:14494–14499. [PubMed: 25246554]
- 87•. Lopatkin AJ, Sysoeva TA, You LC. Dissecting the effects of antibiotics on horizontal gene transfer: Analysis suggests a critical role of selection dynamics. *Bioessays.* 2016; 38:1283–1292. Though horizontal gene transfer plays an important role in the acquisition of resistance, assay design issues confound our understanding of horizontal gene transfer dynamics. To this end, it is critical to test how bacterial populations respond to selection pressures for proper horizontal gene transfer quantification and ultimately reducing the spread of resistance. [PubMed: 27699821]
88. Samson JE, Magadan AH, Sabri M, Moineau S. Revenge of the phages: defeating bacterial defences. *Nature Reviews Microbiology.* 2013; 11:675–687. [PubMed: 23979432]
89. Loc-Carrillo C, Abedon ST. Pros and cons of phage therapy. *Bacteriophage.* 2011; 1:111–114. [PubMed: 22334867]
90. Koskella B, Brockhurst MA. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *Fems Microbiology Reviews.* 2014; 38:916–931. [PubMed: 24617569]
91. Weigle JJ. Induction of Mutations in a Bacterial Virus. *Proc Natl Acad Sci U S A.* 1953; 39:628–636. [PubMed: 16589315]
92. Leon M, Bastias R. Virulence reduction in bacteriophage resistant bacteria. *Front Microbiol.* 2015; 6:343. [PubMed: 25954266]
93. Gu J, Liu X, Li Y, Han W, Lei L, Yang Y, Zhao H, Gao Y, Song J, Lu R, et al. A method for generation phage cocktail with great therapeutic potential. *PLoS One.* 2012; 7:e31698. [PubMed: 22396736]
94. Yoichi M, Abe M, Miyanaga K, Unno H, Tanji Y. Alteration of tail fiber protein gp38 enables T2 phage to infect *Escherichia coli* O157 : H7. *Journal of Biotechnology.* 2005; 115:101–107. [PubMed: 15607229]
95. Lin TY, Lo YH, Tseng PW, Chang SF, Lin YT, Chen TS. A T3 and T7 Recombinant Phage Acquires Efficient Adsorption and a Broader Host Range. *Plos One.* 2012; 7
96. de la Fuente-Nunez C, Reffuveille F, Fernandez L, Hancock REW. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Current Opinion in Microbiology.* 2013; 16:580–589. [PubMed: 23880136]
97. Lu TK, Collins JJ. Dispersing biofilms with engineered enzymatic bacteriophage. *Proc Natl Acad Sci U S A.* 2007; 104:11197–11202. [PubMed: 17592147]
98. Libis VK, Bernheim AG, Basier C, Jaramillo-Riveri S, Deyell M, Aghoghogbe I, Atanaskovic I, Bencherif AC, Benony M, Koutsoubelis N, et al. Silencing of Antibiotic Resistance in *E. coli* with Engineered Phage Bearing Small Regulatory RNAs. *Acs Synthetic Biology.* 2014; 3:1003–1006. [PubMed: 25524110]
- 99•. Yosef I, Manor M, Kiro R, Qimron U. Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. *Proc Natl Acad Sci U S A.* 2015; 112:7267–7272. This study merges phage and CRISPR-Cas for resistance reversal with ecological incentivization for susceptibility. Their results not only highlight the efficacy of CRISPR-Cas and phage therapies, but also the importance of manipulating selection pressure to maintain susceptibility. [PubMed: 26060300]
100. Citorik RJ, Mimee M, Lu TK. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nature Biotechnology.* 2014; 32:1141–1145.

101. Bikard D, Euler CW, Jiang WY, Nussenzweig PM, Goldberg GW, Duportet X, Fischetti VA, Marraffini LA. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nature Biotechnology*. 2014; 32:1146–1150.
102. Goma AA, Klumpe HE, Luo ML, Selle K, Barrangou R, Beisel CL. Programmable Removal of Bacterial Strains by Use of Genome-Targeting CRISPR-Cas Systems. *Mbio*. 2014; 5
103. Goh YL, He H, March JC. Engineering commensal bacteria for prophylaxis against infection. *Curr Opin Biotechnol*. 2012; 23:924–930. [PubMed: 22459613]
104. Borrero J, Chen YQ, Dunny GM, Kaznessis YN. Modified Lactic Acid Bacteria Detect and Inhibit Multiresistant Enterococci. *Acs Synthetic Biology*. 2015; 4:299–306. *Lactococcus lactis* was engineered to sense pheromone secreted by *Enterococci* which triggers expression of two antimicrobial peptides to target *Enterococci faecalis*, including a multidrug-resistant strain. The authors showed efficacy of the sensing and killing in both liquid and solid culturing conditions. [PubMed: 24896372]
105. Gupta S, Bram EE, Weiss R. Genetically Programmable Pathogen Sense and Destroy. *Acs Synthetic Biology*. 2013; 2:715–723. [PubMed: 23763381]
106. Hwang IY, Tan MH, Koh E, Ho CL, Poh CL, Chang MW. Reprogramming Microbes to Be Pathogen-Seeking Killers. *Acs Synthetic Biology*. 2014; 3:228–237. [PubMed: 24020906]
107. Hwang IY, Koh E, Wong A, March JC, Bentley WE, Lee YS, Chang MW. Engineered probiotic *Escherichia coli* can eliminate and prevent *Pseudomonas aeruginosa* gut infection in animal models. *Nat Commun*. 2017; 8:15028. The authors engineered *E. coli* Nissle 1917 to sense and kill *P. aeruginosa* and destabilize biofilm structure. The engineered bacteria were tested in *Caenorhabditis elegans* and mouse infection models, in which the system efficiently reduced the infection of *P. aeruginosa*. [PubMed: 28398304]
108. Mimeo M, Tucker AC, Voigt CA, Lu TK. Programming a Human Commensal Bacterium, *Bacteroides thetaiotaomicron*, to Sense and Respond to Stimuli in the Murine Gut Microbiota. *Cell Syst*. 2015; 1:62–71. [PubMed: 26918244]
109. Huang S, Lee AJ, Tsoi R, Wu F, Zhang Y, Leong KW, You L. Coupling spatial segregation with synthetic circuits to control bacterial survival. *Mol Syst Biol*. 2016; 12:859. Interaction between biomaterials and cell growth dynamics can serve as a safeguard strategy to contain bacteria in microcapsules. The bacteria only survive within the capsules due to high local cell density and will be killed if they escape from the capsules. [PubMed: 26925805]
110. Chan CTY, Lee JW, Cameron DE, Bashor CJ, Collins JJ. ‘Deadman’ and ‘Passcode’ microbial kill switches for bacterial containment. *Nature Chemical Biology*. 2016; 12:82–86. Containment of engineered bacteria can be accomplished by environment-dependent killing programmed in genetic circuits. Deadman kill switches are circuits that express a toxin when the survival cue in the environment is not present. Passcode kill switches use hybrid transcription factor design to use distinct combinations of environmental inputs as survival cues. [PubMed: 26641934]
111. Ghoul M, Griffin AS, West SA. Toward an evolutionary definition of cheating. *Evolution*. 2014; 68:318–331. [PubMed: 24131102]
112. Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. *Nat Rev Microbiol*. 2016; 14:576–588. [PubMed: 27510864]
113. Yurtsev EA, Chao HX, Datta MS, Artemova T, Gore J. Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids. *Molecular Systems Biology*. 2013; 9
114. Vasse M, Noble RJ, Akhmetzhanov AR, Torres-Barcelo C, Gurney J, Benateau S, Gougat-Barbera C, Kaltz O, Hochberg ME. Antibiotic stress selects against cooperation in the pathogenic bacterium *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America*. 2017; 114:546–551. Antibiotics as stresses can alter social dynamics between public good producers and non-producers of *Pseudomonas aeruginosa*. In mixtures of non-producers are enriched with antibiotic treatment and exhibit high antibiotic resistance than either monoculture. [PubMed: 28049833]
115. Diard M, Sellin ME, Dolowschiak T, Arnoldini M, Ackermann M, Hardt WD. Antibiotic Treatment Selects for Cooperative Virulence of *Salmonella Typhimurium*. *Current Biology*. 2014; 24:2000–2005. [PubMed: 25131673]
116. Miller MB, Bassler BL. Quorum sensing in bacteria. *Annual Review of Microbiology*. 2001; 55:165–199.

117. Antunes LCM, Ferreira RBR, Buckner MMC, Finlay BB. Quorum sensing in bacterial virulence. *Microbiology-Sgm*. 2010; 156:2271–2282.
118. Duan FP, March JC. Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:11260–11264. [PubMed: 20534565]
119. O'Loughlin CT, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, Bassler BL. A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. *Proc Natl Acad Sci U S A*. 2013; 110:17981–17986. [PubMed: 24143808]
120. Wright JS, Traber KE, Corrigan R, Benson SA, Musser JM, Novick RP. The agr radiation: an early event in the evolution of staphylococci. *Journal of Bacteriology*. 2005; 187:5585–5594. [PubMed: 16077103]
121. Kohler T, Perron GG, Buckling A, van Delden C. Quorum sensing inhibition selects for virulence and cooperation in *Pseudomonas aeruginosa*. *PLoS Pathog*. 2010; 6:e1000883. [PubMed: 20463812]
122. Damore JA, Gore J. Understanding microbial cooperation. *J Theor Biol*. 2012; 299:31–41. [PubMed: 21419783]
123. Soucy SM, Huang J, Gogarten JP. Horizontal gene transfer: building the web of life. *Nat Rev Genet*. 2015; 16:472–482. [PubMed: 26184597]

- Antibiotic resistance in bacterial pathogens is becoming a global crisis
- Addressing this crisis requires development of innovative tools for diagnosis and treatments
- These developments will benefit from advances in quantitative biology and synthetic biology.

Figure 1. The antibiotic resistance cycle

Antimicrobial use selects for resistance (red), which necessitates new antibiotics (blue). This cycle has become imbalanced in the midst of an antibiotic discovery void, with few remaining antibiotics and prevalent resistance. Through rapid diagnostics, dosing strategies that minimize resistance, and therapies targeting resistance itself, we can begin to reduce the prevalence of resistance. In the meantime, new antimicrobials that replace or supplement existing antibiotics are needed to treat multidrug resistant infections.

Figure 2. Synthetic biology based diagnostic tools

A. A toehold switch can detect a RNA molecule with a pre-defined sequence. This sequence can be designed to detect the expression of resistant genes or other genes that can identify a certain pathogen. When the target RNA molecule is not present, a hairpin structure forms and represses the expression of reporter proteins. Once the target RNA molecule is present and bound, the hairpin opens and the expression of the reporter is activated.

B. A whole cell sensor that records environmental signal (red) through a trigger module that activates the memory module to generate a sustained output signal (purple).

C. Phages provide a sensitive detection method for the presence of a certain bacterial host through rapid propagation of phages and expression of an easy-to-detect readout.

Figure 3. Synthetic biology based treatment tools

A. Engineered M13 phage deliver antisense antimicrobial RNA (blue) to silence the mRNA of resistance gene (red) through enhancing degradation and inhibit translation through base pairing.

B. Engineered bacteriophages deliver CRISPR-Cas systems that kill the host cell by cleaving the host chromosomal DNA in response to the sensing of target genes (e.g., those encoding resistance and virulence).

C. Engineered probiotics can recognize specific molecules which then trigger the secretion of antibiobiofilm and antimicrobials that disrupt bacterial pathogens with different mode of activities.

Figure 4. Population-level mechanisms that enable bacterial survival under antibiotic treatments (adapted from [12])

A. Collective antibiotic tolerance describes the survival of a population during antibiotic treatment only when its density is sufficiently high. Blue rounded rectangles represent bacterial cells. Solid outlines denote live cells; dashed outlines denote dead cells.

B. Cells producing β -lactamase can collectively degrade β -lactam antibiotics. Antibiotic can diffuse into cells and be degraded intracellularly. Antibiotic-mediated cell lysis can trigger the release of β -lactamase to the environment to degrade antibiotics extracellularly.

C. Cooperator produces public good, which imposes a cost to itself. Cheaters exploit public good without producing it. In a mixture of cooperators and cheaters, depending on the conditions, antibiotic selection can lead to selection of either cooperators or cheaters.

D. Quorum sensing (QS) is a mechanism by which a signal molecule is produced and sensed by a population to regulate the expression of effector proteins. When the population density is high, QS signaling molecules accumulate to a high concentration and trigger the expression of proteins involved in virulence, biofilm formation and antibiotic resistance. When population density is low, the expression of these proteins is not triggered (denoted by $\emptyset$).