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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.

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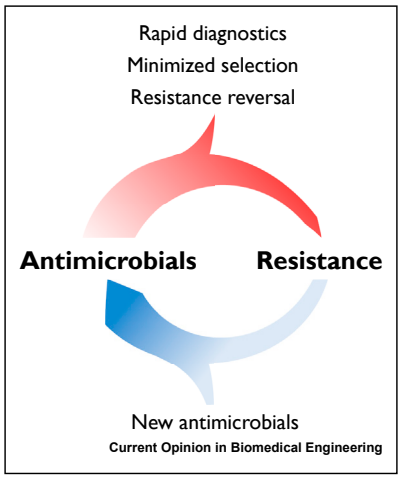
Introduction

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].

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%–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 (Fig. 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

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.

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 (Fig. 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] (Fig. 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.