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Review

New approaches to antimicrobial discovery

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ABSTRACT

The spread of resistant organisms is producing a human health crisis, as we are witnessing the emergence of pathogens resistant to all available antibiotics. An increase in chronic infections presents an additional challenge – these diseases are difficult to treat due to antibiotic-tolerant persister cells. Overmining of soil Actinomycetes ended the golden era of antibiotic discovery in the 60s, and efforts to replace this source by screening synthetic compound libraries was not successful. Bacteria have an efficient permeability barrier, preventing penetration of most synthetic compounds. Empirically establishing rules of penetration for antimicrobials will form the knowledge base to produce libraries tailored to antibiotic discovery, and will revive rational drug design. Two untapped sources of natural products hold the promise of reviving natural product discovery. Most bacterial species, over 99%, are uncultured, and methods to grow these organisms have been developed, and the first promising compounds are in development. Genome sequencing shows that known producers harbor many more operons coding for secondary metabolites than we can account for, providing an additional rich source of antibiotics. Revival of natural product discovery will require high-throughput identification of novel compounds within a large background of known substances. This could be achieved by rapid acquisition of transcription profiles from active extracts that will point to potentially novel compounds.

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1. The dual problem: resistance and tolerance

The rise and spread of antibiotic resistance presents a unique challenge to both science and medicine. Infection is the only disease that threatens not only individuals but societies. Resistance to chemotherapy evolves in only two types of diseases – cancer and infection, and for this reason, both are very difficult to treat. However, cancer has no memory, resistance evolves in somatic cells and is not passed through the germline. By contrast, our pathogens not only develop their own resistance mechanisms, but constantly borrow those that had evolved over billions of years in environmental bacteria. We lack an adequate knowledge base to support rational development of novel antimicrobials to keep up with the threat of resistant bacteria. Today, the result of the standoff between us and our pathogens is epitomized by the spread of multidrug resistant ESKAPE organisms (*Enterococcus*, *Staphylococcus aureus*, *Klebsiella species*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter*) [1]. In the case of some Gram-negative bacteria such as *A. baumannii*, there are strains resistant to all currently available antibiotics [2].

Antibiotics shut down or subvert essential cellular functions, and resistance mechanisms appear to exploit every possible way of preventing a drug from hitting its target. The major types of clinically-relevant resistance mechanisms are destruction of the antibiotic; target modification; and restricted penetration/efflux of the drug [3,4]. Resistance has been studied for a long time and is fairly well understood. The same cannot be said about tolerance.

It is given that new antibiotics are needed to combat drug-resistant pathogens [5]. However, we also need antibiotics capable of effectively eradicating an infection. Currently used antibiotics have been developed against rapidly-growing bacteria, and most of them have no activity against stationary state organisms, and even the best have limited activity against dormant persister cells [6]. The relative effectiveness of antibiotics in treating disease is largely a result of cooperation with the immune system, which eliminates non-growing cells and persisters after antibiotics block growth or kill the bulk of a susceptible population. The deficiency of existing antibiotics against ostensibly drug-susceptible pathogens is becoming increasingly apparent with the rise of immunocompromised patients (HIV infected, undergoing chemotherapy, diabetics) and the wide use of indwelling devices (catheters, prostheses, heart valves), where the pathogen forms biofilms protecting cells from the immune system. The ineffectiveness of the immune system leads to chronic diseases, which make up approximately half of all infectious disease cases in the developed world.

The main culprit responsible for tolerance of pathogens to antibiotics is a specialized survivor, a persister cell [6,7]. What we learned about persisters so far can be summarized as follows: persisters are not mutants but phenotypic variants of regular cells produced stochastically in the population, and their relative abundance rises at late-exponential state, reaching 1% [8]; all pathogens form persisters [6]; toxin-antitoxin modules (TA) are the principal mechanism of persister formation in the model organism *E. coli* [9–12]; and a drop in ATP drives persister formation in *S. aureus* [13]; pathways of persister formation are highly redundant [14]; persisters are non-growing [15], dormant [11,16] cells, which explains their tolerance to bactericidal antibiotics that depend on the presence of active targets for killing the cell [9,17].

We find that mutants producing elevated levels of persisters are selected for in the course of antimicrobial therapy in infections caused by all pathogens tested – *C. albicans* [18], *P. aeruginosa* [19], *E. coli* [20] and *M. tuberculosis* [21]. In the case of *E. coli* from patients with urinary tract infections, we determined the mechanism by which high persister (hip) mutants form. Mutations in the *hipA* toxin loosen the interaction of the two subunits in the inactive dimer [20], activating this kinase which causes dormancy by phosphorylating glu-tRNA [22]. This heritable drug tolerance is parallel to the more familiar heritable drug resistance.

Finding of hip mutants underscores the significance of persisters and drug tolerance in the clinical manifestation of disease. An important aspect of tolerance is its link to resistance. Persisters are killed only slowly, if at all, and resume growth when the antibiotic concentration falls. The result is a relapsing infection with a large effective population size which favors development of resistance [23]. The importance of persisters in recalcitrance of infectious diseases raises the bar for drug discovery, we need to develop therapies that effectively kill both regular and dormant cells.

2. Antibiotic discovery – a brief history of a challenging problem

The genesis of the antibiotic crisis is evident, though not widely appreciated – it is the breakdown of the once successful discovery platform introduced by Selman Waksman in the 40s. The platform was simple, screening soil streptomycetes for antimicrobial activity against a susceptible test organism by detecting zones of growth inhibition on an overlay plate [24]. The method is similar to what Fleming discovered by accident when he saw a *Penicillium notatum* colony clearing a plate seeded with *Staphylococcus aureus* [25]. Streptomycetes are the best producers of antimicrobials, and application of a systematic screen is what made the difference between luck and a discovery platform. Ironically, the *Penicillium* fungus apparently acquired the penicillin biosynthetic operon from streptomycetes by horizontal transmission [26]. The screening of streptomycetes led to the discovery of streptomycin, the first effective compound to act against tuberculosis. This "Waksman platform" was widely adopted by the Pharma industry and produced the major classes of antibiotics over the next 20 years (Fig. 1). The fortunes of the companies, Merck, and Pfizer, and Eli Lilly, among others, rose with the new cures for infectious diseases they were developing. But after 20 years of success, overmining of the soil streptomycetes (and other actinomycetes) resulted in diminishing returns – rediscovery of known compounds, and collapse of the platform.

Resistance to existing compounds was emerging, but modifying antibiotics produced active analogs. This approach was fairly successful for a while, resulting in effective cures [27]. An excellent class of synthetics, the fluoroquinolones, was also developed by the Pharma industry in the 60s, and the tempo of drug discovery seemed to be outpacing the spread of resistance. National resources were shifted indeed, and both the NIH and the Pharma refocused their attention on other problems. However, by the 1990s, it became clear that our victory over the pathogens was an illusion, with resistance spreading faster than discovery of new antibiotics, and we find ourselves in an alarming position of being on the losing side of the war. The story of the most successful class of antibiotics – the β-lactams – is particularly illuminating. Resistance to penicillin was recorded shortly after its introduction in 1947, and traced to hydrolysis of the antibiotic by β-lactamase [28]. Naturally-produced β-lactamase inhibitors were discovered, and combined with β-lactams. One of the most successful antibiotics currently on the market is augmentin, amoxicillin + clavulanic acid. Pathogens however continuously develop resistance by modifying/replacing the target, penicillin-binding proteins, and acquiring new β-lactamases, probably from soil microorganisms. As a result, we are witnessing the fourth generation of semi-synthetic β-lactams. Novexell together with AstraZeneca developed a new β-lactamase inhibitor, avibactam, which is active against a majority of β-lactamases [29]. The latest response from