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Confronting the challenges of discovery of novel antibacterial agents

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ABSTRACT

Bacterial resistance is inevitable and is a growing concern. It can be addressed only by discovery and development of new agents. However the discovery and development of new antibacterial agents are at an all time low. This article broadly examines the historical as well as current status of antibacterial discovery and provides some perspective as how to address some of the challenges.

Bacterial infections and the fight against them have been a focus of mankind since the dawn of time with application of interventions including mercury salts and herbs. The earliest known chemotherapeutic antibacterial discovery began in the 20th century by screening of compounds from the dye industry leading to discovery of salvarsan (arsenic derivative of hydroxy aniline, 1910) and the sulfa drugs (1930's).[1] With understanding of the mechanism of action of sulfa drugs as inhibitors of folate pathway, targeted screening and lead optimization of the pyrimidine class of compounds led to discovery and development of trimethoprim (early 1960's). However, the true revolution of the antibacterial discovery did not begin until the discovery of penicillin in 1928 from *Penicillium notatum* by Sir Alexander Fleming followed by purification, production and clinical treatment in 1940s. This discovery led to a revolution of not only antibacterial discovery but also the field of microbial natural products. Empirical screening of microbial natural product fermentation broths led to the discovery of the antibacterial natural products in next 20 years (1940–1962) designated as 'Golden-Age' of antibacterial discovery.[1,2]

Microbial derived antibiotics (β-lactams, aminoglycosides, tetracyclines, macrolides, glycopeptides, streptogramins) and synthetic quinolones discovered during Golden Age served as drugs or chemical platforms for drug leads for medicinal chemists. For the next five to seven decades, optimization of these leads produced new antibiotics with incrementally improved potency and properties.[3] Improved chemistry, target identification and availability of ligand-bound 3D structure, and increased understanding of resistance mechanisms led to the discovery and development of as many as six generations of antibiotics of most important classes.[3] Convergent total synthesis of tetracyclines by Myers and coworkers is the most notable development of new chemistry published in a long time.[4,5] The new chemistry allowed for the efficient design of tetracycline analogs not possible before, leading to new classes of structures including pentacyclines.[4,5] While iterative modifications to old classes of chemical leads produced new antibiotics with improved potency, drug properties, and resistance profiles this process is not limitless and may have run its course. Despite these challenges, a limited number of compounds from the established classes of antibiotics are in various stages of development.[6]

While the antibacterial field is grappling with these challenges, bacterial resistance continues to grow to all antibiotics regardless of class and mechanism. Some classes and mechanisms are more prone to resistance selection than other classes. Bacterial resistance is inevitable. It is not if but when it will occur. Therefore, to combat resistance, new antibiotics with new mechanisms or new classes of compounds that bind to new binding sites of the established targets are needed.

After a significant innovation gap[3] post 'Golden Age' linezolid, a new synthetic oxazolidinone class of Gram-positive antibiotic was approved by the FDA in 2000.[1] Since then a few other new classes of Gram-positive antibiotics (daptomycin-2003, retapamutilin-2007, fidaxomicin-2011) were approved for clinical use.[1] Empirical screening was used to discover these classes of compounds in 1980's or before.[1]

So what happened during the last five decades since the 'Golden Age' of antibiotic discovery that led to discovery void[1] of *novel*

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classes of antibiotics? Is it truly an innovation gap due to complacency, application of wrong discovery strategies (apparently prudent at the time), de-emphasis of natural products, not enough emphasis or resources for antibiotic research and development?

Arguably, it is a result of the combination of all the factors described above. First, after a great success during the Golden Age of antibiotic discovery, it was assumed that the antibiotic classes already discovered would be sufficient for the treatment of bacterial infections and it was not sufficiently appreciated that bacterial resistance was inevitable under the selective pressure of antibiotics. Penicillin resistance was known at the time and therefore it was not a surprise. This oversight clearly led to complacency, likely poor funding and lack of innovation, until it was recognized otherwise.

All antibiotics during the Golden Age were discovered by empirical screening using inhibition-of-growth assays. Mechanism of action was determined much later, sometimes many decades later.[1] The advent of molecular biology, expression and production of enzymes and receptors made large-scale in vitro enzyme and receptor based screening routine. This turned out to be highly successful approach for chemical lead finding against a variety of mammalian targets followed by optimization leading to drugs. Unfortunately this approach of in vitro cell free screening was utterly unsuccessful for bacterial targets as reported by GSK in 2007.[7] At the time, in comparison to traditional empiric screening, the in vitro MOA based cell-free approach was very attractive due to its 'obvious' rationality. With that in mind, it is likely that most of the companies applied similar cell-free screening approaches for the discovery of antibacterial agents without much success at the end. Historically, natural products have been a most prolific source for providing novel antibiotics. However, in the intervening period most of the companies terminated or reduced their efforts on screening of natural products due to various reasons not least due to repeated rediscovery of known compounds. After spending huge resources for screening without finding *bona fide* tractable leads for chemical optimization to new drugs resulted in tremendous frustration. This led to de-prioritization of the antibacterial programs in many Pharmaceutical Companies.

The lack of success of the discovery of a good chemical lead for building a medicinal chemistry program for discovery and development of novel antibacterial agents with a novel mechanism of action could be attributed to two main factors. First and perhaps most critical is the lack of novel chemical diversity of antibacterial screening libraries and de-emphasis of natural products, and the second, the screening approach.

Chemical diversity, and lack thereof, for drug discovery space is a topic for much debate. Within the confines of chemical diversity the antibacterial agents appear to occupy unique property space compared to other drugs as demonstrated by O'Shea and Moser[8] from the analysis of the CMC database. These findings show that antibacterial agents: are significantly more polar (Gram-negative agents are much more polar, $\log D_{7.4}$ negative 2.8, than Gram-positive agents, $\log D_{7.4}$ negative 0.2 vs average CMC data set $\log D_{7.4}$ positive 1.6), do not obey Lipinski rule of five for oral bioavailability (some orally active drugs do obey these rules), display wide molecular weight (102–1449) ranges (MW: average CMC data set 338 Da, average Gram-positive antibacterial 813 Da and average Gram-negative antibacterial 414 Da).[1] Most of the corporate chemical collections and screening libraries were designed and built up with oral bioavailability and human targets in mind, leading to compounds that are much more lipophilic and unlikely to fill the property and diversity space suitable for antibacterials.[9]

Failure of the cell free screening approach (wet lab and virtual) of the bacterial targets is generally due to lack of cellular activity and lack of understanding of the characteristics required to endow molecules with cellular entry properties. Unlike mammalian cells, bacterial cells are protected by cell wall (Gram-positive) and by an outer-membrane in addition to cell wall (Gram-negative). Most of the bacterial targets with the exception of cell wall/outer membrane targets are intracellular. Therefore, in order to engage with their biological targets, compounds have to cross these strong protective barriers often with opposite physical properties.[1] Bacteria do express and use active transports to transport nutrients. Fortunately, sometimes these active transporters also help transport some of the drugs to periplasm and cytosol. Unfortunately transporters have not been exploited for drug entry with the exception of iron transporters (vide infra) perhaps due to lack of clear understanding of the structure and function of these transporters and also due to the rapid loss of the transporters, leading to resistance. Transporters often do play a role in transporting natural product antibiotics and show exclusive selectivity for some compounds and not for others even within the same class of antibiotics. Unfortunately, even if the drugs pass the membrane entry barrier efflux pumps expressed in bacteria, pump them out from periplasm/cytosol.

Improvement of potency of a chemical lead against an enzyme target by standard medicinal chemistry approaches, with or without structural information, can be achieved rapidly (e.g., LpxC, vide infra). However they may fail to kill bacteria because of their failure to reach the intracellular target (e.g., certain LpxC inhibitors, vide infra). While some structure-function knowledge exists on efflux pumps, not much is known on cell permeability other than highly acidic compounds are not as permeable as neutral, zwitterionic, and basic compounds. Balancing of permeability and efflux, the two *yin–yang* phenomenon, is critical for designing successful antibiotics. If balancing the target activity, cell penetration and efflux were not challenging enough, their diversity and differential expression in different bacterial species make the discovery and development of broad-spectrum antibiotics even more challenging. Therefore lead optimization of a broad-spectrum antibacterial program is akin to running a dozen single-target mammalian programs simultaneously. This is likely one of the reasons for the failure of the programs originating from cell-free screening efforts even after good tractable enzyme inhibitors were discovered. Despite these challenges empirical screening has allowed for the discovery of many great broad-spectrum antibiotics in the past. Detailed understanding of cell penetration and efflux can make this field wide open for new inventions.

Until resolution of the entry barrier, phenotypic assays represent the best approach for screening for antibacterial leads. A good target-based phenotypic screen can help eliminate/reduce the unwanted detergent-like or poisonous hits. This approach could focus the lead optimization resources to cell active leads. A few examples of target based whole cell phenotypic assays that have been applied for the discovery of new leads are: antisense assays[10] (e.g., platensimycin/platencin[11,12] and kibdelomycin[13]), and Wall Teichoic Acid (WTA) pathway assay.[14] Various other target based whole cell based assays have been reviewed.[1,15,16] However, paramount to the success of the antibacterial discovery is a structurally diverse screening library covering antibacterial property space. Without diverse antibacterial chemical libraries with requisite antibacterial drug properties no screening method would produce the desired outcome.

There are approximately 265–350 genetically validated antibacterial targets. Of these about 60% are broad-spectrum targets.[17] Astonishingly, no more than 20 of these are targeted by currently marketed drugs[2] and thus provide tremendous opportunity for the discovery and development of new agents with novel mode of action without cross-resistance, particularly multi-target mechanisms (chemical leads that interact with more than one biological target). With this kind of analysis and information in hand, the time is ripe for focusing resources and applying scientific acumen