Antibiotics: past, present and future

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Abstract. The first antibiotic, salvarsan, was deployed in 1910. In just over 100 years antibiotics have drastically changed modern medicine and extended the average human lifespan by 23 years. The discovery of penicillin in 1928 started the golden age of natural product antibiotic discovery that peaked in the mid-1950s. Since then, a gradual decline in antibiotic discovery and development and the evolution of drug resistance in many human pathogens has led to the current antimicrobial resistance crisis. Here we give an overview of the history of antibiotic discovery, the major classes of antibiotics and where they come from. We argue that the future of antibiotic discovery looks bright as new technologies such as genome mining and editing are deployed to discover new natural products with diverse bioactivities. We also report on the current state of antibiotic development, with 45 drugs currently going through the clinical trials pipeline, including several new classes with novel modes of action that are in phase 3 clinical trials. Overall, there are promising signs for antibiotic discovery, but changes in financial models are required to translate scientific advances into clinically approved antibiotics.

The development of antibiotics

The introduction of antibiotics into clinical use was arguably the greatest medical breakthrough of the 20th century (Figure 1) [1]. As well as treating infectious diseases, antibiotics made many modern medical procedures possible, including cancer treatment, organ transplants and open-heart surgery. However, misuse of these valuable compounds has resulted in the rapid rise of antimicrobial resistance (AMR) with some infections now effectively untreatable [2]. The dangers of a post-antibiotic era has prompted policymakers to acknowledge this threat to human health and promise additional grant funding, which is gradually driving a resurgence of interest in antibiotic discovery and development [3]. The UK Government-commissioned O'Neill report predicted that without urgent action 10 million people a year will die from drug resistant infections by 2050 [4]. One of the key recommendations is to stimulate early stage drug discovery [4]. Given the relative lack of success in bringing effective synthetic antibiotics to the clinic [5], the best hope for developing a new generation of anti-infective drugs is to discover new microbial natural products (NPs) because these compounds are unrivalled in their chemical diversity and effectiveness as antibiotics [1]. Filamentous actinomycetes make 64% of the known NP antibiotic classes with the remainder made by other bacteria and fungi (Figure 2 and Table 1). Here we give a brief overview of the history of NP antibiotics and our prospects for discovering, developing and safeguarding a new generation of antibiotics.

Table 1. All classes of clinically used antibiotics and their source. [a]Classes are defined by origin, structure and/or mechanism of action, which distinguishes between bacitracin, colistin and daptomycin, for example. [b]Year reported refers

to first report in literature. [c]The European Medicines Agency recommended the withdrawal of fusafungine from the market in February 2016. [d]Salvarsan is no longer in clinical use. [e]Salicylic acids are found in nature, but this was not the source of this class of antibiotic. [f]Compound synthesis was inspired by natural antibiotic classes.

A brief history of antibiotics

The use of antibiotic-producing microbes to prevent disease stretches back millennia, with traditional poultices of mouldy bread being used to treat open wounds in Serbia, China, Greece and Egypt more than 2000 years ago. The Eber's papyrus from 1550 BC is the oldest preserved medical document and includes mouldy bread and medicinal soil amongst its list of remedies [6]. An Anglo-Saxon recipe from 1000 years ago was also recently shown to kill MRSA (methicillin-resistant *Staphylococcus aureus*) [7]. However, the development of anti-infective drugs and the underlying concept of chemotherapy is widely accredited to Paul Ehrlich, who developed the synthetic arsenic-based pro-drugs salvarsan (salvation arsenic) and neo-salvarsan circa 100 years ago to treat *Treponema pallidum*, the causative agent of syphilis [8] (Figure 1). This represented one of the first systematic screens for drug discovery using a library of synthetic compounds and was inspired by Ehrlich's work on dyes that specifically stained bacterial cells. Salvarsan was superseded by the sulfonamide prodrug Prontosil, discovered by Gerhard Domagk [9], a bacteriologist at Bayer who used the drug to save his daughter's arm from amputation. Domagk and colleagues were effectively continuing the work of Paul Ehrlich because the sulfa drugs were inspired by azo dyes. Sulfonamides were the first truly effective,

broad spectrum antimicrobials in clinical use and are still in use today, but they were largely superseded by the discovery of penicillin, observed on a contaminated Petri dish by Alexander Fleming in 1928 [10]. Penicillin was later purified by Norman Heatley, Howard Florey, Ernst Chain and colleagues at Oxford, who were instrumental in the development of penicillin as a drug [11] (Figure 1). Dorothy Hodgkin solved the beta-lactam structure of penicillin in 1945 [12]. resolving the famous debate between Robert Robinson, who favoured a thiazolidine-oxazolone structure, and several other notable chemists including Chain, Abrahams and Woodward, who believed it to be a beta-lactam [13]. This was an important breakthrough because it enabled the development of semi-synthetic derivatives to bypass penicillin resistance.

Antibiosis between microbes was described well before the discovery of penicillin, including by Louis Pasteur, who proposed that microbes could secrete material to kill other bacteria [14]. The production of diffusible and heat-stable compounds by bacteria was being reported by the turn of the 20th century [15], and their utility in combatting infectious diseases had been explored. Arguably the first clinical use of an antibiotic was reported in the 1890s, where Emmerich and Löw used an extract of *Pseudomonas aeruginosa* (then known as *Bacillus pycyaneus*) to treat hundreds of patients and this extract, called pyocyanase, was used until the 1910s [16]. Pyocyanase was active towards multiple pathogens and incorrectly believed to be an enzyme. Instead, the active components of pyocyanase was likely to be a mixture of pyocyanin, a quorum sensing phenazine, and 2-alkyl-4-hydroxy-quinolones [17].

The discoveries of penicillin, tyrocidine and numerous reports of the production of antimicrobial compounds by microorganisms, led Selman

Waksman to start a systematic study of microbes as producers of antimicrobial compounds in the late 1930s. Waksman defined an antibiotic as '*a compound made by a microbe to destroy other microbes'* and was instrumental in identifying soil-dwelling filamentous Actinomycetales ("actinomycetes") as prolific producers of antimicrobial compounds [18]. Waksman discovered numerous antibiotics made by soil-dwelling actinomycetes, including neomycin and streptomycin, the first agent active against tuberculosis [18]. Waksman's pioneering work identified the genus *Streptomyces* as prolific producers of NPs, or secondary metabolites, which are compounds not required for the normal growth, development, or reproduction of an organism in the laboratory. Many streptomycete NPs are active against bacteria, fungi, viruses, nematodes and insects and they have also been developed as anti-cancer and immunosuppressant drugs [19].

Waksman's work initiated the Golden Age of antibiotic discovery from the 1940s to the 1960s. Most of these antibiotics are still in clinical use but their effectiveness has been eroded by the rise of AMR (Figure 1) [1]. In fact, the rapid and relatively easy discovery of multiple classes (and variations therein) of NP antibiotics during a relatively short period led to the excessive use of these drugs. This, coupled with a faltering antibiotic discovery pipeline from the 1970s onwards, has led to the current situation with few new antibiotics in the clinical trials pipeline [1]. Hence, most antibiotics in clinical trials today are derivatives of known classes of NP or synthetic antibiotics rather than new classes of antibiotic (Table S1). Notably, this hiatus in antibiotic discovery aligns with a decline in the discovery of new NP families and the persistent rediscovery of known compounds in screening campaigns using microbial, and predominantly

actinomycete, fermentation extracts [1]. This, in part, led to a belief that all the 'low-hanging fruit' had been harvested and resulted in most of major pharmaceutical and agrochemical companies shutting down their NP discovery departments.

The divestment in NP research was accompanied by an investment in numerous high-throughput screening (HTS) programmes that aimed to discover new synthetic antibiotics, but these have proved unsuccessful. For example, 70 HTS campaigns were conducted by GlaxoSmithKline (GSK) over 7 years using a collection of approximately 500,000 compounds, but this yielded very few leads, and no candidates for development [20]. Similarly, 65 HTS campaigns by AstraZeneca provided a few leads but none that were active against multi-drug resistant Gram-negative bacteria [21]. In recent years however, the discovery of new antibiotic-producing strains in under-explored environments combined with new tools for genome mining has reinvigorated the NP discovery field, e.g. [22-24].

Why do microorganisms make antibiotics?

Of all the antibiotics discovered between 1945 and 1978, 55% came from the genus *Streptomyces* (Figure 1) [25]. Several theories have been proposed to explain why soil microbes make so many bioactive NPs. The most likely explanation is that they have multiple functions, acting as chemical weapons to kill competitors in the soil either as protection (defensive) or predation (offensive), as signalling molecules to close relatives or to mediate interactions with eukaryotic hosts such as insects and plants [26-28]. This is consistent with evidence that *Streptomyces* species and other filamentous actinomycetes evolved

158 circa 440 million years ago, around the same time that plants first colonized land
159 [25,29]. The filamentous growth of these bacteria would have provided an
160 advantage in colonizing plant roots and we speculate that many of their NPs may
161 have evolved or been co-opted to mediate these interactions [30].

162 One of the more surprising discoveries to arise from microbial genome
163 sequencing is that many bacteria and fungi encode many more NP pathways than
164 they actually make in the laboratory [31]. In general, at least three quarters of
165 their potential NP capability is not switched on *in vitro* and this discovery has
166 triggered huge efforts to develop tools and techniques to activate their "cryptic"
167 BGCs in the hope of discovering novel chemical scaffolds with useful
168 bioactivities [32-36]. Many studies have demonstrated that when activated or
169 expressed heterologously, silent BGCs encode functional NP biosynthetic
170 pathways [34]. This suggests that production of these compounds is triggered by
171 environmental cues or by host organisms. Many invertebrates, including insects
172 and marine sponges, form defensive and mutually beneficial symbioses
173 (defensive mutualisms) with antibiotic-producing bacteria and it seems likely that
174 most if not all land plants do the same [26,37-39]. Studying these bacteria in the
175 context of their host using advanced techniques, such as stable isotope probing
176 (SIP) and imaging mass spectrometry (IMS), may be one way to identify the
177 thousands of novel compounds encoded by silent BGCs and to identify the NPs
178 that are most important to their hosts [40].

179

180 **Prospects for natural product antibiotic discovery**

181 In the Golden Age of antibiotic discovery, new antibiotic classes were being
182 discovered on an almost yearly basis by isolation of likely antibiotic-producing

183 organisms from soil samples. However, a finite number of NP classes from easy-
184 to-cultivate bacteria meant that compound rediscovery soon became a problem
185 (Figure 1). More recently, the NP discovery field has been reinvigorated by the
186 discovery of new antibiotic-producing strains in under-explored environments,
187 combined with new tools for genome mining and heterologous pathway
188 expression.
189
190 ***Under-explored environments and ecological niches***
191 It is now clear that only a tiny fraction of the soils on earth have been sampled for
192 antibiotic producers. Sampling more widely is likely to yield numerous new
193 strains and BGCs, even from this traditional sampling environment**.** In addition,
194 sampling under-explored environments that were inaccessible or unknown during
195 the Golden Age is yielding new chemical structures [20,37,38]. These include the
196 marine environment, where the marine actinomycete genus *Salinospora* has
197 proven to be a source of multiple structurally novel NPs [41] such as
198 salinosporamide A (Marizomib) , which has anticancer activity and is currently in
199 Phase III clinical trials for the treatment of glioblastoma [42].
200 Mutualistic co-evolved bacteria might also be an excellent source of new
201 NPs and studying these niches has the added advantage of uncovering interesting
202 underlying biology and the opportunity to understand what these molecules
203 actually do in nature [43,44]. Bacterial symbionts of marine invertebrates such as
204 sponges are a rich source of novel NPs. For example, *Candidatus* Entotheonella
205 species are uncultivated symbionts of the marine sponge *Theonella swinhoei* [45]
206 and were shown to produce almost all the bioactive polyketides and modified
207 peptides isolated from a chemotype of *T. swinhoei*.

Sequencing of the human microbiome has also revealed many NP BGCs across Actinobacteria and other bacterial phyla, and the antibiotic lactocillin was identified from a human vaginal isolate [46]. Another antibacterial compound, lugdunin, was isolated from the commensal nasal bacterium *Staphylococcus lugdunensis* which prohibits colonization by *Staphylococcus aureus* and is active in animal models, with a high barrier to the development of resistance [47].

Difficult to cultivate bacteria

Genomic data suggesting the presence of novel BGCs in *Clostridium* bacteria prompted Hertweck and colleagues to investigate the antibiotic-producing potential of this genus, as no NPs had been characterised from clostridia. *Clostridium cellulolyticum* grown under standard laboratory conditions yielded no NPs, so fermentation was repeated with added aqueous soil extracts, as the bacterium had been isolated from decayed grass compost. This triggered the production of closthioamide, a new class of polythioamide antibiotic [48]. In another elegant example, the antibiotic humimycin was discovered by synthesising a putative peptide NP that was bioinformatically predicted from the genome of the actinomycete *Rhodococcus equi*, an opportunist human pathogen [23].

Other novel approaches have included the isolation of hard to culture bacteria from soil using diffusion chambers that allow for the growth of the pure bacterium in a complex natural environment [49]. This was miniaturised into an isolation chip (iChip) and used to culture 10,000 soil isolates that were otherwise intractable to laboratory fermentation. Extracts generated from these were then screened for antimicrobial activity and one resulted in the identification of the

antibacterial peptide teixobactin that is produced by *Eleftheria terrae* (Figure 4) [50]. These discoveries and recent metagenomics studies [51] highlight the continued relevance of traditional soil environments for antibiotic discovery .

The development of improved sampling methodologies for under-explored environments and difficult to cultivate bacteria, combined with new genetic tools and technologies to activate interesting BGCs, is likely to lead to the discovery of thousands of new bioactive compounds over the next 20 years. It is highly probable that some fraction of these will form the basis of new anti-infectives for clinical medicine, although this will require improved financial models to incentivise the development of new antibiotics.

Prospects for clinical development. As of December 2018, there are 45 new antibiotic candidates in clinical trials for the US market (Table S1) [52]. Of these, 28 belong to known NP classes while 17 are synthetic and comprise 12 classes, of which seven are new. The NP classes include 13 based on beta-lactams, which was the first class of NP antibiotic to be discovered back in 1928 (Fig. 1). Five of these are variant beta-lactams, two are hybrids (to a glycopeptide and a siderophore) and seven are combinations with beta-lactamase inhibitors (Table S1). There are five new tetracyclines, a class which was first described in 1945 and introduced into the clinic in 1948, an aminoglycoside (1943), a distamycin (1962), a fusidane (1945), a macrolide (1952), a pleuromutilin (1950) and two polymyxins (1947). The fusidane (fusidic acid) is a fungal NP which is in Phase III trials in the United States, but it has already been used clinically elsewhere in the world. There are two new synthetic classes in Phase III clinical trials: ridinilazole, which specifically blocks cell division in *C. difficile* through a

mechanism that has not been revealed; and murepavedin, which has a novel mechanism of action, inhibiting LptD to block lipopolysaccharide transport to the outer membrane [53]. Murepavidin is effective against drug resistant *Pseudomonas aeruginosa*, one of the hardest pathogens to treat, particularly in patients with cystic fibrosis. It is also encouraging that four of the nine compounds in Phase II clinical trials represent novel classes, but this is still a modest number for the therapeutic area and is insufficient to combat multidrug-resistant Gram-negative pathogens given the historically high attrition rate for compounds making it through clinical trials to clinical utility. AntibioticDB is an open access database that records candidate antibiotics, including antibiotics under pre-clinical development, those in clinical trials and discontinued drugs [54].

Unfortunately, most of the large pharmaceutical companies have left the field of NP discovery, and this work is now chiefly undertaken by academic labs and small to medium-sized companies. Only two of the 45 drugs currently in development belong to big pharmaceutical companies: the synthetic gepotacidin inhibits topoisomerase II through a mechanism distinct from that of quinolones and is being developed by GSK to treat gonorrhoea (phase 2) while Merck have a beta-lactam/lactamase combination in phase 3 clinical trials. The most notable NP antibiotic success in recent years was the introduction of Cubicin (daptomycin) onto the market by Cubist in 2003, and sales of this drug are now more than $1 billion a year. Cubicin is used by injection to treat vancomycin-resistant *Staphylococcus aureus* (VRSA) and was discovered from *Streptomyces roseosporus* in 1987. In 2011, Cubist also purchased Optimer Pharmaceuticals, who secured clinical approval for Dificid (fidaxomicin, produced by the rare

283 actinomycete *Dactylosporangium auranticus* subsp. *hamdenesis*). This is the
284 newest NP class to be introduced into the clinic, despite being discovered in 1975,
285 before daptomycin [55]. Merck purchased Cubist in 2015 for $9.5B but have
286 since closed the discovery arm of Cubist, which was heavily involved in NP
287 discovery.

288 In 2014 Sanofi and Fraunhofer announced the creation of a NP Centre of
289 Excellence with the goal of identifying novel compounds to accelerate the
290 discovery and development of new antibiotics. In 2016 Sanofi further announced
291 a partnership with Warp Drive Bio to collaborate on the development of novel
292 oncology therapies and antibiotics by using next generation sequencing and
293 genome mining (on a massive scale) to identify new NPs but this ended in 2017.
294 In 2018 Warp Drive Bio was effectively merged with Revolution Medicines,
295 which is now focussed on oncology rather than anti-infectives, although Warp
296 Drive Bio's genome mining platform has recently been acquired by Ginkgo
297 Bioworks. Roche have several strategic alliances, such as with Spero, which
298 currently has two antibiotics in phase 1 and another in phase 3 clinical trials
299 (Table S1). Several companies, including Genetech, are working on antibody-
300 antibiotic conjugates (AACs) [56]. Of the larger to mid-sized companies, Basilea
301 is a major active player and focuses on the development of innovative antibiotics,
302 antifungals and oncology drugs. In addition, there are innovative small to
303 midsized companies in the antibacterial and antifungal discovery space including
304 Tetraphase Pharma, which currently has two antibiotics in phase 1 clinical trials
305 and recently had two more approved for use (Table S1). There is a heavy NP
306 influence on all these companies, which appear to be using semisynthetic or total
307 synthesis approaches within very specific areas of chemical space around known

drugs such as polyenes, macrolides and tetracyclines.

Beyond the scientific difficulties associated with antibiotic discovery and development, there are a plethora of regulatory, economic, business and societal issues that must be addressed in order to protect and maximise the potential of our existing and future arsenal of clinical agents, while at the same time promoting the investment and culture changes required to invigorate antibiotics R&D to meet the challenges raised by AMR [57,58]. These have been analysed and recommendations made in several key reports including those by O'Neill and the Pew Trust [4,52]. Mossialos and colleagues comprehensively reviewed 47 incentive strategies for the development of new antibiotics and concluded that a framework of multiple incentives and policies is required [59].

Summary and outlook. The rise in bacterial infections that are resistant to almost all known antibiotics is alarming, yet it is only in the last few years that governments have begun to tackle this problem seriously. This global wake-up call has stimulated a debate about how best to combat AMR and prompted the UK government to appoint an economist, Lord Jim O'Neill, to lead a strategic review [4]. The appointment of an economist highlighted the complexities of bringing to market a drug that, if functionally successful, will be dosed for only a short time. Combined with historically low prices, and the likelihood that any new antibiotic with a unique mode of action will most probably be restricted as a treatment of last resort, the economics of antibiotic R&D is a major disincentive to investment. To address these problems innovative solutions are required that provide a reimbursement model that delinks revenue from drug sales.

Scientifically, the identification of new chemical matter with the unique

physicochemical characteristics required for antibiotic discovery and development is a key challenge. NPs still represent the most likely source of new materials given the advances described in this review. Even the best-studied antibiotic producers, the streptomycetes, have been vastly under-sampled in terms of their capability, and there is confidence from the study of organisms from underexploited environments, ecological considerations, and genome sequencing that thousands of NP antibiotics await discovery across the bacterial kingdom. New tools and techniques such as CRISPR/Cas9-mediated genome editing are available to exploit these observations, and, although there is no universal strategy for the expression of silent BGCs, recent advances have led to the discovery of many new molecular structures with exceptional biological activities [34]. Further advances in this area will undoubtedly accelerate this rate of discovery further.

Thus, governments are starting to act and there is much to be optimistic about, not least the fact that most of the NP antibiotics that have been discovered come from a small fraction of the microbes on Earth. With suitable global action, this should lead to a renewed antibiotic pipeline to combat AMR alongside other emergent technologies, such as vaccines, antibody-antibiotic conjugates, probiotics, phage therapy and rapid diagnostics [60].

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Competing interests statement

The authors declare no competing interests.

Figures.

Figure 1. Timeline showing the decade new classes of antibiotic reached the clinic. The antibiotics are coloured per their source: green = actinomycetes, blue = other bacteria, purple = fungi and orange = synthetic. At the bottom of the timeline are key dates relating to antibiotic discovery and antimicrobial resistance, including the first reports of drug resistant strains methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant *Staphylococcus aureus* (VRSA) and plasmid-borne colistin resistance in Enterobacteriaceae.

Figure 2. Most clinically relevant classes of antibiotic are derived from natural products.

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