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Antibiotic Drug Discovery

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ABSTRACT:
Due to the threat posed by the increase of highly resistant pathogenic bacteria, there is an urgent need for new antibiotics; all the more so since in the last 20 years, the approval for new antibacterial agents had decreased. The field of natural product discovery has undergone a tremendous development over the past few years. This has been the consequence of several new and revolutionizing drug discovery and development techniques, which is initiating a 'New Age of Antibiotic Discovery'. In this review, we concentrate on the most significant discovery approaches during the last and present years and comment on the challenges facing the community in the coming years.

I. INTRODUCTION:

Nature has been a source of medicinal products for millennia, with many useful active substances developed from plant sources. In the 20th century, the discovery of the penicillins was the starting point for drug discovery from microbial sources. The majority of drugs have been developed from lead structures on the basis of natural products synthesized by bacteria. Drugs derived from bacterial secondary metabolites are in manifold use, for example in diagnosis, mitigation, or in the treatment, or prevention of a disease or relief of discomfort.

Antibacterial Discovery Strategies:

Most of the antibiotics used in the clinic today were derived from natural products (NP) identified in screens of environmental material using variations of the Waksman platform strategy [12]. Synthetic antibacterials trace back to Paul Ehrlich who identified chemical compounds like salvarsan that was active in the treatment of Syphilis. Later, Domagk discovered the dye prontosil and its active metabolite sulfanilamide that inhibited folic acid biosynthesis. This work led to development of the sulfa drugs [13]. Medicinal chemistry and the advent of high throughput screens of compound libraries (target based) replaced NP searches in the mid 1990's and reintroduced the idea of synthetic chemistry as a strategy for creating next generation antibacterials. Advances in computational biology (bioinformatics, systems biology, and metagenomics) and structural biological technologies (crystal structures of protein targets, ligand screens via docking software, the so called designer drug strategy) have led to a third discovery strategy—synthetic biology. While all these technologies engender some optimism, the main reason for the antibiotic discovery void in the first place is that creating new chemical classes is really hard. Is repeating history a viable strategy?—probably not.

Are Natural Products the Answer?

One definition of insanity is repeating the same task over and over again while expecting a different outcome. This is especially applicable to antibiotic discovery research where much emphasis has been placed on finding new natural product (NP) antibiotics [12]. After all, the vast majority of antibiotics used in the clinic are derivatives of NPs. The insanity with continuing on this path is that for all of these NP antibiotics, resistance mechanisms predated their therapeutic use and this is unlikely to change [3,6,7]. Moreover, we know from past discovery efforts that NPs tend to be redundant—with the same targets and mechanisms of action appearing over and over again [14].
Casting a wider net will not change this reality, because the number of broad spectrum drug targets is quite small

Hypothesis:

The number of antibiotic classes is limited by the number of broad spectrum drug targets. It follows that the number of resistance mechanisms will generally parallel the number of antibiotic classes. Hence, modifying the genes associated with secondary metabolism, while

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potentially improving an existing scaffold, is unlikely to produce new chemical classes of antibacterials. Another issue with NPs is that since they have evolved in natural environments and not in the human milieu, most NPs are structurally complex and as such are by default poor pharmacophores (PK/PD), requiring much medicinal cheor to reduce intrinsic toxicities. The pharmaceutical industry abandoned NP divisions long ago in favor of high throughput screens of compound libraries against novel drug targets (another failure, see below); or, more productively to create next generation derivatives of existing antibiotics, a strategy that has worked well and continues today as judged by the number of analogues in the current pipeline. Despite the continuum of second, third and fourth generation β-lactams (penicillin, cephalosporin and carbapenem derivatives) [11], by far the largest group of antibiotics used in the clinic today, has not solved the resistance problem originally pointed out by Alexander Fleming in the late 1940's. While logic dictates that we should abandon further development of this class, advances in pairing them with β-lactamase inhibitors (clavulinate, avibactam, and vaborbactam to mention a few) has restored their effectiveness and extended their clinical lifetimes [15]. Since β-lactamases and β-lactam targets (penicillin binding proteins, PBP) share conserved structural attributes that can be altered via mutation, resistance will eventually defeat them, but for now this strategy is both viable and profitable. The current antibacterial pipeline is populated with new derivatives based on the scaffolds for quinolones, tetracyclines, macrolides and aminoglycosides [16]. New chemistries around existing scaffolds has the potential to overcome current resistance mechanisms by improving binding complexity with the drug target, and or improve pharmacological properties. In this regard, the ketolides were supposed to overcome resistance to macrolides, but the tradeoff is increased toxicities. However, given our experiences with NP antibiotics in general, it is unlikely that continued searches for new ones, regardless of where one looks, will be any more successful than past efforts. mistry to improve bioavailability, to reduce drug metabolism

Antibiotic Resistance Theory:

Every healthcare worker can tell you why antibiotic resistance is bad for patients and why antimicrobial stewardship (judicious use) is so important, but few have any deep understanding of the diversity of underlying molecular and genetic mechanisms. This has created a "Chicken Little" effect; where strong opinions based on shallow thinking, perpetuation of myths and intolerance of new ideas have unnecessarily complicated the discovery process. Much of antibiotic theory has evolved over nearly 100 years of history and mostly from experiences with NPs. Antibiotics and their respective resistance mechanisms have co-evolved over millions or even billions of years of internecine microbial warfare that is ongoing in the environment. These resistance mechanisms are encoded in DNA and can be disseminated throughout the microbial world via mobile genetic elements and horizontal transfer [22]. It is not surprising that soil and aquatic microbes transfer these resistance determinants to human and animal pathogens, often in clinical settings where antibiotics are in heavy use. There is no good outcome to the continued use of NPs and as discussed previously it is unlikely that new ones will fare any better. This is best exemplified by the emergence of resistance (mcr-1 family) to colistin, a polymyxin class antibiotic that has been around for decades, but little used, until the emergence of carbapenem resistance in Klebsiella (KPC) and other carbapenem resistant enteric superbugs (CRE) made it the drug of last resort [23]. The rapid and global emergence of mcr-1 just underscores the point. The resistance problem is further exacerbated by globalization, increasing population density and international travel that has brought us mcr-1 as well as the NDM-1 β-lactamase resistance determinant [24]. The COVID-19 pandemic, unfortunately, is another example of the accelerated global spread of biological agents.

Synthetic Antimicrobials and Mutation Theory Antibiotic Resistance Theory:

It follows from the previous paragraph on NPs, that synthetic antibacterials that inhibit targets "Mother Nature" has yet to find would require millions of years of evolution for resistance determinants to emerge. Note that this hypothesis also holds true for new chemistries against established drug targets, provided that they are also not susceptible to existing resistance mechanisms. A good example is linezolid, a synthetic antibacterial discovered nearly 30 years ago and more recent derivatives (tedizolid), that inhibit protein synthesis in GP bacteria [25]. One might think that synthetic antibacterials would enjoy a long clinical life. Unfortunately, synthetic classes of antibacterials, including linezolid, are often defeated by mutation-based drug resistance.

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Mutations occur naturally (probability of ~1 in 108) in DNA and since microbial infections involve hundreds of billions of bacteria, microbes tend to win the probability game. Survival is the prime directive for any lifeform and in particular, the stress of antibiotics on bacteria, especially at sub-inhibitory levels, provides strong selection for these resistant variants to emerge and flourish. It should be pointed out that mutation-based drug resistance also underlies resistance for nearly all NPs. This reality led Eric Lander and John P. Holdren to conclude in a summary of their report by the committee on antimicrobial resistance to the President's Council on Science and Technology that: "In the fight against microbes, no permanent victory is possible: as new treatments are developed, organisms will evolve new ways to become resistant" [26].

This grim prediction is certainly supported by a recent clinical trial of promising synthetic boron containing heterocycle leucyl tRNA synthetase inhibitor which was halted due to rapid emergence of drug resistance [27]. This novel drug inhibited a non-essential proof-reading region of the essential enzyme in which mutations could accumulate and thereby defeat the inhibitor without total loss of function [27]. The lessons learned here include: (1) focus on the essential catalytic center of a prospective drug target, (2) ensure amino acid conservation within this region (resists mutation), and (3) confirm by co-crystallization that leads indeed are binding within the catalytic pocket of the target. It is also helpful early on to explore possible off target activities against human drug targets related to a particular enzyme class. Attention to these details might lead to medicines that slow the inevitable pace to drug resistance. It raises another critical question "can we find drug targets that by catalytic mechanism are capable of escaping mutation-based drug resistance?

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Three different approaches have been applied for effective drug discovery programmes:

(1) Historically, substances, crude extracts or purified chemicals were screened for biological activity mostly in whole cell-assays without knowing the drug target. Only after an active substance has been identified, serious efforts have been made to analyse the target and the mode of action of the compound. This strategy is known as bioactive-guided screening, classical pharmacology, forward pharmacology (Takenaka, 2001) or phenotypic drug discovery (Lee et al., 2012).

(2) Another approach to identify new drug substances is denominated as chemical screening, which aims to identify novel, chemically diverse molecules without taking into account their biological activity. The substances used in this approach can originate from biological sources (such as metabolites from microorganisms) or from chemical libraries. For this, sophisticated analytical methods are applied, such as high-performance liquid chromatography, mass spectrometry (MS) or nuclear magnetic resonance spectroscopy. Hereby, the structure elucidation is a pivotal/crucial step to avoid the re-discovery of an already known substance. Nowadays, large databases of mass spectra for known compounds are

(3) In contrast to the chemical screening, the targetoriented screening aims to identify compounds that hit a known and validated molecular target. Thereby, the target represents a cellular or molecular structure involved in the pathology of interest that the drug-indevelopment is meant to act on. Different attributes of the target have to be taken into account. For example, a good antibacterial target has no human homologue and is present in a wide range of bacteria where it is essential. Further important attributes are the

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location of the target in bacteria and a low frequency of resistance to new compounds. Hence, multiple targets or targets encoded by multiple genes should be selected because high-level, target-based resistance to these compounds does not occur by single-step mutations

The main disadvantage of these screening programmes was that numerous metabolites have been repeatedly rediscovered (Genilloud et al., 2011). This has led to estimates that the rate of discovery of different classes of microbial metabolites can vary significantly in 'random' screening campaigns (Baltz, 2007). Due to this empirical knowledge, it is assumed that most of the low-hanging fruits have already been picked and only a few new metabolites are left to be harvested unless massive screening programmes are implemented (Zengler et al., 2002; Baltz, 2007, Monciardini et al., 2014).

To overcome this problem, combinatorial chemistry has been developed as a key technology enabling the generation of large screening libraries for the needs of high-throughput screenings. However, now, after more than two decades of combinatorial chemistry, it became apparent that despite the increased number of new chemical substances, no increase in lead structures or drug candidates has been reached (Newman and Cragg, 2007). Instead, the synthetic, combinatorial library compounds seem to cover only a limited and quite uniform chemical space (Rosen et al., 2009), whereas existing drugs and particularly natural products exhibit much greater chemical diversity (Feher and Schmidt, 2003).

General principles of antibiotic biosynthesis:

The actinobacterial secondary metabolome is a source for many compounds. Secondary metabolites are specialized compounds that are distinguished from primary metabolites because they are not directly involved in cell growth or reproduction. They usually are taxonomically restricted and have specialized functions, including signalling, nutrient acquisition or defence (Demain and Fang, 2000; Davies, 2013). The diversity of secondary metabolites is enormous, although their biosynthesis in principle is based on variations of only a few predominant pathways, viz. synthesis of polyketides (PKS), nonribosomal peptides (NRPS) or polypeptides, terpenes or sterols, etc., or combinations thereof. The specificity of individual biosynthetic enzymes determines which building blocks will be incorporated into the compound and how it will be modified. The knowledge on enzyme specificity can be used to predict the resultant compounds (Weber et al., 2015). The enzymes catalysing secondary metabolic biosynthesis are encoded by genes, which generally are organized in so-called biosynthetic gene clusters. Such a biosynthetic gene cluster is defined as the genome region, which harbours all genes required for the synthesis of the natural product as well as genes for the resistance, export and pathway-specific regulation.

Novel approaches for drug discovery :

Drug discovery using unexplored strains:

Although Actinobacteria are a treasure chest for novel natural compounds, presumably only less than 1% of actinobacterial species have been cultivated so far meaning that around 99% of the population is unexplored (Davies, 1999). One main obstacle in the identification of novel species is that many bacteria cannot be cultured under conventional laboratory conditions. However, in recent years, several new and revolutionary cultivation techniques have been developed, which now allow to grow a broad range of so far uncultivable bacteria. One of these new methods is represented by the microfluidic bioreactor cultivation, which provides a highthroughput cultivation system where up to 600 000 pure soil-derived Actinobacteria can be grown in microfluidic droplets per hour (Zang et al., 2013). This technique allows to cultivate slow growers that under normal conditions would be outcompeted by fast-growing strains but potentially may be promising natural compound producers. Actually, the idea of focusing on slow-growing and/ or hard-to-isolate strains has been one basis to set up the company Naicons in 2006, which aims to establish a large library of so far uncultured or unclassified actinomycetes and filamentous fungi and to isolate new metabolites thereof. This screening approach already led to the discovery of several new effective natural compounds, such as the protein synthesis inhibitor orthoformimycin, which is a structurally novel substance that follows a completely new mechanism of action or the class III lanthipeptide NAI-112, which is a potent antiinflammatory agent (Monciardini et al., 2014). Another recent successful example for the identification of a novel anti-infective substance from a previously uncultured bacterium is represented by teixobactin, which is a substance from the novel species of b-proteobacteria named Eleftheria terrae (Ling et al., 2015). E. terrae was isolated with the help of a new cultivation technique, the so-called iChip, which is a multichannel device composed of several hundred

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miniature diffusion chambers, each inoculated with a single environmental cell. The iChip then is placed back in the natural environment, which leads to a significantly increased colony count that subsequently can efficiently be cultivated under laboratory conditions (Nichols et al., 2010).

Besides sophisticated cultivation methods, also cuttingedge methodologies, such as Next-Generation Sequencing techniques, can be applied as efficient tool for the identification of novel natural compounds. An intriguing example is represented by the single-cell and metagenomics-based approach leading to the identification of the novel bacterial taxon 'Entotheonella' that co-inhabits the Red sea marine sponge Theonella swinhoei. Metagenomic analysis showed that more than 40 bioactive polyketides and modified peptides belonging to seven different structural classes are produced by the endosymbiont in association with the sponge (Wilson et al., 2014).

Even, rather 'classic' approaches to search for novel antibiotics can still be very successful if samples are taken from unexplored habitats (e.g. desert, deep sea, endosymbiotic environment) or bacteria are investigated, which do not belong to the classes of well-known antibiotic producers.

A successful example is the relatively novel genus Salinispora, of which several species have been isolated from marine sediments of Guam, Palau and the Red Sea producing salinosporamides, inhibitors of the 20S proteasome (Jensen et al., 2015). A promising underexplored habitat is also the human body: Recently, it has been shown that the nasal colonizing organism Staphylococcus lugduensis produces a novel peptide antibiotic (Peschel pers commun; Nature in press). This shows that also non-actinomycetal bacteria can be promising sources for novel antimicrobials. This is also exemplified by the production of several new antibiotics by anaerobiers, such as Clostridium beijerinckii, which recently has been shown to produce the pentacyclic polyphenol clostrubin, which is a highly active substance against diverse pathogenic bacteria (Letzel et al., 2013; Pidot et al., 2014; Shabuer et al., 2015).

Genome mining :

In contrast to screening for chemical compounds, an alternative strategy has become increasingly popular during the last two decades (Ziemert et al., 2016). The so-called genome mining approach detects and analyses the biosynthetic gene clusters of the chemical compounds and subsequently connects those genes to molecules. Genome mining has various advantages. The vast amount of DNA data available these days provides a large pool of potential compounds encoded in these genomes that, given the bioinformatics tools .

that exist, are relatively fast and easy to screen for almost no costs. Moreover, sophisticated web-based tools, such as anti-SMASH (Medema et al., 2011; Blin et al., 2013, 2014; Weber et al., 2015), PRISM (Skinnider et al., 2015) or NaPDoS (Ziemert et al., 2012), are easily accessible and do not require extended expertise in natural product biosynthesis or bioinformatics. Mining bacteria for their genetic potential also revealed that many more bacteria have the ability to produce natural products than previously thought and that more chemical diversity is waiting for discovery.

Based on structure and content of the biosynthetic gene clusters, chemical classes and structures of the encoded compound can be predicted. This information can be used to either guide a more targeted drug discovery technique (such as reactivity-guided isolation; Castro-Falcon et al., 2016) or peptide and glycogenomic approaches (Kersten et al., 2011, 2013) or allow the heterologous expression in an optimized expression host (activation of silent gene cluster). Furthermore, comprehensive biosynthetic gene cluster databases such as MiBIG (Minimum Information about a Biosynthetic Gene cluster) provide the possibility to easily estimate novelty in the encoded compounds (Medema et al., 2015).

However, it still remains challenging to connect gene clusters to bioactivity. Purely by predicting structures, it is often impossible to say if the encoded compounds have antibiotic or medically relevant activities and are worth further investigations. A recently developed method, therefore, combines the search for biosynthesis genes with detecting potential resistance genes. This socalled target-directed genome mining (Tang et al., 2015) is based on the observation that antibiotic-producing bacteria need to be resistant against the producing compound themselves to avoid suicide. In some cases, a resistant second copy of the target gene is encoded within the biosynthetic gene cluster of the antibiotic allowing a more targeted search for antibiotic compounds with interesting targets.

A lot of the genome mining methods are based on the detection of domains and protein families well known to be involved in secondary metabolism such as PKS and NRPS. To overcome possible limitations and provide the opportunities

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to find biosynthetically unique chemistry and enzymes, two methods have been recently developed. The ClusterFinder algorithm by Cimermancic et al. (2014) is based on a probabilistic approach that compares the presence of Pfam domains of cluster involved in secondary metabolism compared with random areas in the genome. As proof of principles, the authors screened more than 1000 bacterial genomes and detected about 30 000 putative biosynthetic gene clusters. Another method that addresses the problem of finding novel pathways is based on evolutionary distances and was called EvoMining (Cruz-Morales et al., 2016). EvoMining is based on the assumption that most enzymes used in secondary metabolism have been duplicated and evolved from primary metabolism (CruzMorales et al., 2016). By recapitulating the evolutionary history of 23 enzyme families previously uninvestigated in the context of natural product biosynthesis, biosynthetic gene clusters coding for hidden chemical diversity were discovered. This phylogenomic analysis resulted in the discovery of arseno-organic metabolites in Streptomycetes coelicolor and Streptomyces lividans (Cruz-Morales et al., 2016).

A lot of efforts are focusing now on the implementation of the variety of genome mining methods and computational predictions into analytical chemistry and molecular biology approaches to allow a more streamlined drug discovery process. Methods such as peptide and glycogenomics (Kersten et al., 2011, 2013) connect computational predictions with state of the art mass spectrometric methods (alternative methods for drug discovery), whereas pattern-based genome mining, for example, uses the huge amount of DNA sequence data available in a more comparative genomic approach combined with MS analysis (Duncan et al., 2015).

Activation of silent gene cluster:

Genome mining tools uncovered a plethora of so-called silent gene clusters in diverse actinobacterial genomes. These clusters encode the synthesis of potential secondary metabolites that are not produced under standard laboratory culture conditions. In the past few years, a lot of inventive effort has been put into strategies to activate such silent gene clusters in order to find new secondary metabolites. For this, different expression strains have been developed to facilitate gene cluster expression and metabolite detection. For instance, the model organism S. coelicolor has been genetically engineered in a manner that positively affects antibiotic production in general (by altered RNA polymerase and ribosome) (Hosaka et al., 2009) and in addition alleviates the detection of antibiotic production (by inactivated active secondary metabolite gene clusters (Δact Δred Δcpk Δcda)) (Gomez-Escribano and Bibb, 2012). Heterologous expression of antibiotic gene clusters has been a challenge for a long time because cluster sizes can easily reach more than 100 kb, which makes them difficult to clone. However, nowadays more elaborated methods are available, as, for example, the transformation-associated recombination cloning system, which is a method recently adopted from Saccharomyces cerevisiae that promotes cloning and expressing large DNA fragments in actinomycetes (Yamanaka et al., 2014). Besides that, the information available from gene cluster analyses can purposefully be used to manipulate pathway-specific regulators or implement 'refactory bricks', for example, replacing the natural promoter with a strong artificial one, in order to directly activate the transcription of the biosynthesis gene cluster (Rutledge and Challis, 2015).

An example for the activation of a silent glycopeptide cluster was described for Amycolatopsis japonicum. The introduction of a glycopeptide-specific transcriptional activator resulted in the production of ristomycin, a glycopeptide (Spohn et al., 2014) used for the diagnosis of von Willebrand disease and Bernard–Soulier syndrome. In addition to these targeted approaches, a whole array of more pleiotropic strategies can be applied in order to activate silent gene clusters: for instance microbial co-cultivation; addition of rare earth elements or nutrition signals, such as the cell wall metabolite N-acetylglucosamine; or application of stress conditions (e.g. ethanol or heat stress treatment) have successfully been show to elicit silent gene cluster expression (Ochi and Hosaka, 2013; Abdelmohsen et al., 2015; Rutledge and Challis, 2015). Altogether, this allows to exploit the natural product producers more intensively as it has been possible in the years before and will lead to the identification of quite a number of new secondary metabolites in the near future.

Alternative methods for drug discovery :

The major disadvantage of most computational genome mining tools is that they are only able to identify biosynthetic gene clusters, which code for already known biosynthetic principles and thus the associated products may be structurally familiar compounds. However,

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compounds encoded by novel and unique biosynthesis mechanisms cannot be uncovered with these tools. An alternative way for the identification of yet unknown biosynthetic pathway classes is based on the knowledge of gene regulation principles instead of occurrence and structural composition of biosynthetic enzymes. It follows the idea that global, environmental signal-sensing regulators control the production of certain secondary metabolites. Such regulators promote or repress gene transcription by binding to specific DNA motifs upstream of their target genes in response to an environmental signal, such as nutrient starvation, oxidative stress or the presence of competitive organisms. The identification of the regulator, the determination of the specific DNA-binding motifs and the computational screening of genome sequences for known DNA-binding motifs of such regulators are a progression, which is defined as Identification of Natural compound Biosynthesis pathways by Exploiting Knowledge of Transcriptional regulation (INBEKT) approach (Spohn et al., 2016).

The application of INBEKT allowed for instance the identification of the biosynthetic gene cluster of the zincophore ethylene diamine disuccinic acid ([S,S]-EDDS). [S,S]-EDDS synthesis in the producer strain A. japonicum is known to be strictly regulated by the zinc regulator Zur. The identification of the Zur-binding motifs in the A. japonicum genome finally led to the successful discovery of the [S,S]-EDDS biosynthetic genes (Spohn et al., 2016). The [S,S]-EDDS structure indicated a novel and unique biosynthesis mechanism different from the typical NRPS or NIS (NRPS-independent siderophore synthetase) pathways known for the synthesis of chelating agents.

One of the emerging areas in microbiology is detecting specialized metabolites produced by microbial colonies and communities with MS. The newly developed imaging MS and real-time MS allow two- and three-dimensional visualization of the distribution of metabolites from microbial colonies and enable the identification of metabolites directly from microbial colonies (Yang et al., 2009; Fang and Dorrestein, 2014).

Dereplication in natural product discovery:

The different approaches listed in the previous chapter are leading to the synthesis of bacterial metabolites, which then can be analysed for their biological activity. However, before employing them in extensive tests, it has to be examined whether they are really new. For this dereplication, effective novel methods have been developed..

Recently, various bioinformatics approaches have been developed to organize or interpret large sets of MS/MS fragmentation data. For example, solutions such as MAGMa (MS annotation based on in silico generated metabolites) allow matching of multistage fragmentation data against candidate molecules substructures and were successfully applied on complex extracts (van der Hooft et al., 2012; Ridder et al., 2014; Allard et al., 2016). Among these new approaches, molecular networking (MN) is a particularly effective one to organize MS/MS fragmentation spectra. MN compares all MS/MS spectra in a given extract and groups them according to their similarity (Watrous et al., 2012; Bandeira, 2011; Liu et al., 2013; Fang and Dorrestein, 2014). The application of these tools leads to the identification of novel compounds by avoiding re-isolation of known compounds.

Further aspects of antibiotic drug development:

In addition to the progress in finding new secondary metabolites, also new ideas came up and old strategies revived, which may help in the search for novel compounds or in their more effective application.

• In order to overcome the resistance problem, compounds that do not kill the pathogen but only prevent its pathogenic action may be used. This will reduce the pressure on the pathogen to acquire mutations, which will allow it to survive in the presence of the drug. Such ‘antivirulents’ may be used in combination with ‘classical’ antibiotics.

• Another strategy to reduce the occurrence of resistance is the development of new drug combinations. This has been proven to be successful in the treatment of tuberculosis and HIV, but is not a routine procedure in other medicating infections.

• Apparently, many of the most effective drugs used in human therapy have more than one target in the bacterial cell. A criterion for the future selection of drug candidates may be, therefore, the interaction with more than one target. The newly emerging research field of ‘cell biology of antibiotic action’ will deliver a deeper understanding on the mode of action of many antibiotics. This should enable the development of new approaches for the search for novel antibiotics.

• According to recent predictions Gram-negative pathogens will constitute a major threat in future. Although Gram-positives presently cause the majority of deaths in clinics, a greater arsenal is required to combat Gram-negatives. A critical step

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which prevents the application of many compounds in Gram-negatives is the passage across the outer membrane. Here, the combination of known antibiotics with substances, which permeabilize the outer membranes, may help. Alternatively, antibiotics can be combined with siderophore structures that promote the uptake. This 'trojan horse' strategy has successfully been applied in model systems.

Challenges:

There is no doubt that we will need novel antibiotics in future and that natural products are most likely the best source. After a long period of steady decline in identifying novel compounds, we are now in a lucky situation: Genome mining revealed a much bigger potential to synthesize natural products than have been isolated with conventional approaches; new approaches in microbiology, in particular microbial cultivation techniques, made much more producers accessible as antibiotic producers; genetic technologies (combinatorial biosynthesis, synthetic biology, precursor-directed biosynthesis), as well as biochemical knowledge, enable large-scale derivatization of new and old natural compounds to optimize them in a way that they can be applied in medical applications. Presently, we have a significantly enlarged arsenal for the discovery of new antibiotics (Fig. 1). Worldwide, scientists will apply and further develop these technologies. This will undoubtedly result in the identification of novel secondary metabolites in the next years. Whereas the new tools have mainly been developed by academia, we will need cooperation between academia and industry to generate the large numbers of compounds, which are required to end up with an antibiotic that makes it to the hospital.

The described advances in the field significantly increased the probability to find something new, but many drawbacks in drug discovery have still to be overcome. This includes scientific drawbacks, but even more important economic and regulatory aspects. The majority of antibiotics did not end up in the hospitals since severe side-effects prevented their application.

Unfortunately, no rationale is available which reliably predicts such effects from the chemical structure of new compounds.

Whenever a new antibiotic has been found, resistance developed in the pathogens, even if first evidence existed, that this would not happen. We, therefore, need better strategies to prevent resistance development such as much more sensible prescription and application of antibiotics in human medicine and an absolute strict regulation of antibiotic therapies in veterinary medicine. Since decades, big pharma concentrated on the search for broad-spectrum antibiotics due to economic reasons ('block busters'). In order to overcome the 'resistance crisis', it will be important that newly discovered antibiotics will also be further developed if they combat only a narrowspectrum of pathogens.

Fortunately, the regulatory processes to approve new antibiotics have been alleviated in recent years. They can be approved if they are 'non-inferior' to already approved drugs; they do not need to be 'superior'. In addition, special procedures are available for a moresimple and quick approval in the case of limited use. This will increase the number of antibiotics which can be applied and thereby facilitate the treatment of resistant pathogens. Taken together, the recent scientific advances in finding and characterizing new compounds which can serve as antibiotics and the progress in understanding pathogenic processes, there is hope that the pessimistic prediction on the defeat against infections does not come true

Conflict of interest:
The authors declare no conflict of interest.

II. CONCLUSIONS:

Spurring global innovation of new antibiotics, alternative therapies and diagnostics tools is integral to effectively combating AMR. However, demand for new antibiotic products far outweighs supply. Only five novel classes of antibiotics have reached the market since 2000. None of these target gram-negative bacteria. This is not surprising given that there are numerous scientific, regulatory and economic barriers that prevent adequate investment in antibiotic R&D.

We have a partial picture of the global antibiotics market. EU and US antibiotic pipeline data shows that are at least 52 antibiotic products in clinical development, the vast majority of which are in Phases I and II. However, this pipeline may only translate into 15 antibiotic products with varying value; less than half would be systemic antibiotics that could target gram-negative bacteria. Only one antibiotic in the development pipeline uses a novel mechanism of action and it is for a limited purpose.

US investment in antibiotic R&D is expected to grow to $413 million (€380 million) in 2016 after having been constant for five years. However, it is unclear how this difference in

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EU/US funding has affected outcomes in the antibiotics pipeline. European and US governments appear to have limited means of clawing back these significant contributions and sharing in future profits should their funding result in marketable proOur only real insight into private investment in antibiotic R&D is from global data on venture capital funding. Global venture capital in antimicrobial R&D has declined by 28% between the two five year windows of 2004–08 and 2009–13. Venture capital investment in gram-negative antimicrobials has increased by 51% during these two periods, but it still comprises only 12% of total venture capital investment in antimicrobials. The amount of internal capital invested by developers into their own antibiotic projects is unknown.ducts.

Numerous initiatives have been implemented to reinvigorate the antibiotic R&D pipeline. In total, we identified 58 active initiatives and sub-initiatives at global, EU and national levels (UK, France, Germany, Netherlands, Sweden, US and Canada) that directly incentivize antibiotic R&D. Also, there are nine initiatives that indirectly support antibiotic R&D by coordinating strategic actions on AMR and seven initiatives that are either proposed or in the introductory stages of implementation.

The antibiotic R&D initiative environment is now crowded. There is room for improvement regarding the coherence and coordination between and within initiatives. Various models of partnership often form the basis for many initiatives, which improves the possibilities for stakeholder collaboration, but can further confuse coordination efforts.

While almost all these initiatives can be seen to be improving antibiotic project NPV, our analysis shows that a far greater number of push incentives are used over pull incentives. This imbalance between push and pull incentives has led to an unequal distribution of initiatives across the antibiotic value chain. The most common incentives of direct project funding, research collaboration, research grants and fellowships for scientific personnel, tend to favour the basic research side of the antibiotic value chain. Thus, SMEs often find that they lack support throughout the challenging preclinical and early clinical phases of development. Surprisingly, taxation policies (a push incentive) were not used to specifically support firms that engage in antibiotic R&D. In contrast, there are few incentives that support the commercialization end of the value chain such as end prizes, AMCs, and value-based pricing and reimbursement. Moreover, there remains a need for even greater harmonization between the EMA and FDA, as well as other drug regulatory agencies.

Finally, our analysis suggests that antibiotic conservation and patient access objectives are poorly integrated into the existing innovation schemes. Many initiatives have not explicitly linked their incentives to high-priority medical needs in infectious disease.

III. RECOMMENDATION :

Align existing and new antibiotic R&D initiatives to function within the broader One Health approach to AMR.

AMR must be tackled through a unified approach that integrates efforts across human health, veterinary medicine and environmental factors. Antibiotic R&D initiatives must be integrated into a broader AMR agenda that reinforces other aspects of the One Health approach.

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