

Alternatives to antibiotics: future trends

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The need for new antibiotics is greater than ever due to the emergence of multidrug resistant pathogens. The value and importance of antibiotics cannot be overestimated, and they must not be considered as commodities. Antibiotic have revolutionised the field of medicine and we are reliant on them to treat infectious diseases. Since the introduction of penicillin in 1943, there has been a mismanagement of antibiotics. This has allowed bacterial antibiotic resistance to emerge. This has been illustrated by many of the pathogens associated with epidemics of human disease evolving multidrug-resistant (MDR) forms following subsequent use of antibiotics. The situation with regards to antibiotic resistance is grim. There is a substantial financial and clinical burden being placed on health care systems by the pandemic of resistance mechanisms. Fortunately, many bacterial pathogens respond to empirical treatment with antibiotic agents administered in the community. Despite the negative outlook by big pharma companies, it is vital that there should be no let-up in the search for new novel antimicrobial compounds that target novel resistance mechanisms that could potentially be resistant proof. By academia and industry working together novel therapies could be examined in more detail and could potentially slow down the rate at which antibiotic resistance is presently emerging. It is imperative that a concerted offensive approach is taken and that full advantage of new understandings and technology is fully utilised.

Keywords Antibiotic resistance, Alternative antimicrobial agents, Antimicrobial peptides

The Evolution of Antibiotic Resistance

1. Introduction

The successful use of therapeutic agents is compromised by the potential development of resistance or tolerance to that compound from its initial development. This is true for any agent that is used in the treatment of bacterial, parasitic or fungal infections. With pathogenic microbes becoming more resistant to therapeutic agents, it is causing increasing problems for public health services.

In the last 60 years antibiotics have revolutionised the medical field and have been paramount in the fight against infectious disease caused by bacteria and other microbes. Antibiotics are natural products of microorganisms or an equivalent product produced wholly (synthetic) or partially (semi-synthetic) by chemical synthesis and in low concentrations inhibit the growth of or kills microorganisms. When antibiotics are produced naturally, they are usually small organic molecules; usually less than 1000 daltons in size. The majority are produced by the genus *Streptomyces* which is within the filamentous bacterial group (*Actinomycetes*) and by filamentous fungi. Moreover they are generally produced in the stationary phase of growth, parallel to spore formulation and are not essential for growth or sporulation. The three chemical classes of synthetic chemical antibacterial drugs are Quinolones, Sulphonamides and Trimethoprim & Linezolid, these synthetic compounds only contribute to a small amount of the drugs sold/used.

The average life expectancy within the twentieth century has been greatly increased due to antimicrobial therapy. Unfortunately, the use of these wonder drugs has been hampered by the rapid appearance of resistance. An extensive range of physiological and biochemical mechanisms are responsible for antimicrobial resistance. The complexity of the mechanisms that contribute to the emergence and dissemination of resistance cannot be underestimated. The lack of fundamental knowledge around these topics is one of the main reasons that there has been so little significant achievement within the area of prevention and control of resistance development. Experts are now warning of a return to a pre-antibiotic era; a recent database enlisted up to 20,000 potential resistance genes (R genes) of nearly 400 different types, predicted in the main from available bacterial genome sequences [1]. Thankfully the number existing as functional resistance determinants in pathogens is far lower.

This emergence of resistance genes coupled with the increasing use, and misuse, of existing antibiotics in human and veterinary medicine has led to an antibiotic burden on the environment. The development of generations of antibiotic-resistant microbes throughout the planet is the result of years of selective anthropogenic pressure via overuse, underuse and misuse of antimicrobials. This is not a natural process of events, but a manmade one, which has been forced onto nature. Parallels can be clearly drawn from Darwinian notions of selection and survival.

Moreover most international and local authorities recognise this serious problem. Numerous reports and recommendations have been written but to no avail, the development and spread of antibiotic resistance is unyielding. Some new therapies are being investigated such as: Antimicrobial Peptides, Chemically Enhanced Agents and Natural Products. Natural products provide new frontiers and a challenging intellectual exercise into their chemical complexity

and biochemical mode of action [2]. The complete synthesis of natural chemical compounds within the laboratory can be extremely difficult. These small molecules are complex in both functionality and chirality [3]. The antimicrobial properties of naturally occurring chemical compounds and the versatile nature of antimicrobial peptides look to hold the key to unlocking the antibiotic resistance doorway.

2. The first signs of antibiotic resistance

The sequence of discovery of the major classes of antibiotics is illustrated in Figure 1. Penicillin was discovered by Alexander Fleming in 1928. In 1940, several years before the drug was introduced as a therapeutic agent, a bacterial penicillinase was identified by two members of the penicillin discovery team [4, 5]. The new drug came into clinical use in 1946 and made a significant impact on human health. Penicillin had an affinity to kill staphylococci and streptococci bacterial without harming the host which harboured them. The prevalence of resistance strains capable of cleaving the beta-lactam ring by releasing penicillinase was noted and studies were undertaken in an attempt to synthetically modify penicillin chemically. It can now be appreciated in the light of recent findings that the identification of penicillinase preceded the clinical use of penicillin, this is thought to be due to the natural prevalence of resistance genes (R-genes) in natural microbial populations [6]. So, which came first the antibiotic or the resistance?

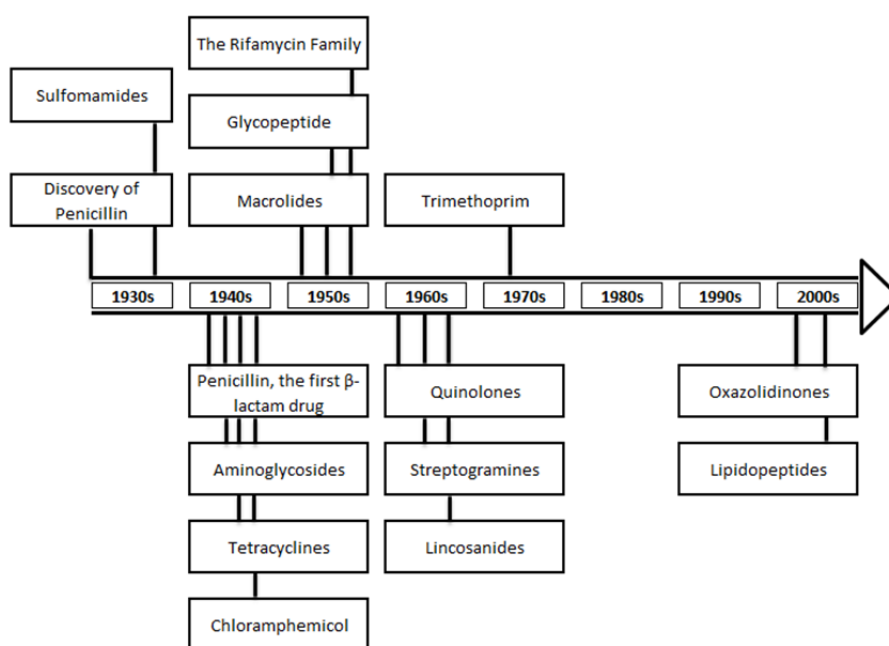


Fig. 1 Timeline of new antibiotic classes. The timeline illustrates when the major antibiotics were developed during the past 70 years. During the 1950's new antibiotics peaked, this is known as the golden era, this is when most of the antibiotics that we used today were discovered. The lean years followed from about 1965, this was a low point in antibiotic discovery and development. Many attempts were made to understand and improve the use of antibiotics by pharmacologic, biochemical and selective target approaches, few were successful.

The late 1940s and early 1950s were the golden age of antibiotic discovery and antibiotic chemotherapy came into full fruition. New antibiotics such as streptomycin was introduced for the treatment of tuberculosis, chloramphenicol and tetracycline were also brought into clinical use [7]. However during patient treatment, mutant strains with resistance to therapeutic concentrations of these drugs soon emerged [8].

Two broad categories of antibiotic resistance are recognised, these are intrinsic and acquired resistance. Intrinsic tolerance refers to the inherent features of the cell, which are primarily responsible for preventing antibiotic action. Intrinsic resistance is usually expressed by chromosomal genes. The second is acquired resistance; this occurs when resistance strains emerge from previously sensitive bacterial strains, this usually happens after exposure to the agent concerned. The identification of genetic transferable antibiotic resistance elements (R genes) in Japan in the mid-1950s introduced the genetic concept that collections of antibiotic R-genes could be disseminated by bacterial conjugation [9]. Acquired resistance may result in mutations in chromosomal agents or the acquisition of extrachromosomal DNA elements such as plasmids or transposons. This transfer of genetic material between bacterial species has occurred throughout eons of microbial evolution and examining the bacterial genomic sequences has illustrated the importance that horizontal gene transfer (HGT) has on genome evolution [3]. Plasmid-mediated transfer of antibiotic resistance has been an area of major focus due to its medical and practical significance [10].

3. Superbugs and super resistance

Many of the bacterial pathogens associated with clinical infection have evolved in multidrug-resistant (MDR) forms following antibiotic use. Some examples of these are MDR *Mycobacterium tuberculosis* which is a major bacterial pathogen found in both developing and industrial nations. Other serious MDR infections include hospital-acquired infections from *Acinetobacter baumannii*, *Burkholderia cepacia*, *Campylobacter jejuni*, *Citrobacter freundii*, *Clostridium difficile*, *Enterobacter* spp., *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli*, *Haemophilus influenza*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Serratia* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia* and *Streptococcus pneumonia*. Microbes with enhanced mortality and morbidity are referred to as “superbugs” this is due to the several mutations which endow high levels of resistance to specific classes of antibiotics recommended for their treatment. Therapeutic options for these microbes are significantly reduced and periods of hospital treatment are considerably lengthened; this results in higher health care costs. It has also been documented that super-resistant strains have also acquired increasing virulence; moreover antibiotic resistance can be considered a virulent factor [3].

4. Multidrug-Resistant Tuberculosis (MDR TB)

Tuberculosis (TB) is a common human pathogen that is transmitted through the air, primarily it affects the lungs, but it can also affect other parts of the body such as the brain, kidneys and spine. Currently as much as one third of the world's population is infected with TB, in the majority of cases TB is treatable and patients will make a full recovery. However, it can easily result in death in patients with underlying immunogenic conditions and those who receive poor treatment. Multidrug-resistance TB is usually classified as an organism that is resistant to at least rifampin and isoniazid; these are two of the most potent drugs used in the treatment of TB [11]. Treatment with a cocktail of anti-TB drugs has become an essential treatment regime, with notable success; however, for a number of reasons multidrug resistance continues to hamper treatment. In the last decade extensively drug resistant tuberculosis (XDR TB) has emerged, these are strains of *M. tuberculosis* that are resistance to rifampin and isoniazid, plus any fluoroquinolone and at least one of the three second-line injectable drugs (i.e. kanamycin, amikacin or capreomycin)[11]. As XDR TB is resistant to four or more of the front-line treatments, patients are left with very few treatment options [12, 13]. Presently there are reports of total drug resistant (TDR) strains [14, 15]. There have been no validated reports suggesting horizontal gene transfer in the development of resistance in *M. tuberculosis*. The resistance is thought to occur due to a spontaneous mutation.

5. Gram negative pathogens

E. coli, *Salmonella enterica* and *Klebsiella pneumoniae* are some of the most prevalent Gram-negative bacterial pathogens; they cause a variety of diseases in both humans and animals. There has been a strong correlation linked between antibiotics used in the treatment of diseases caused by these pathogens and antibiotic resistance that has emerged in the past half century. This has been especially notable in the β -lactam class of antibiotics and their related inactivating enzymes the β -lactamases. There have been several groups and classes of these identified, comprising up to 1,000 resistance-related enzymes. These include new classes of genes and their mutant radiations [16-18]. The transmission and evolution of resistance to the β -lactam antibiotics among enteric bacteria in both hospital and community infection was fundamentally influenced by horizontal gene transfer (HGT).

6. Hospital-acquired diseases: *Pseudomonas aeruginosa*

Pseudomonas aeruginosa has evolved from being a burn wound infection into a leading nosocomial pathogen. It is a notoriously difficult infection to control with the use of antibiotics and disinfectants. Antibiotic resistance mechanisms evolved coincidentally with the introduction of many of the new antibiotic derivatives. This compromised many of the treatment options available, such as aminoglycosides and β -lactams. *P. aeruginosa* is of considerable concern for patients suffering with cystic fibrosis; the pathogen is highly persistent and has evasive strategies to avoid human immune defence mechanisms [19]. The bacteria aggregates in the lungs and becomes surrounded by a layer of alginate polysaccharide. These biofilms are highly resistant as they do not allow for permeability of antibiotics to the bacteria [20]. Resistance development in these patients is associated with lengthy antibiotic treatment.

7. Gram positive pathogen: *Staphylococcus aureus*

At present, the most notorious superbug is the Gram-positive pathogen *Staphylococcus aureus*. This organism has received a negative public reputation due to extensive press coverage. This organism has a close relationship with

mankind. The commensal *S. aureus* is carried in the nasal passage of 30% of the population. It is mainly associated with common skin infections, such as boils. In the past four decades *S. aureus* has evolved from a controllable nuisance into a serious public health concern [21]. After the discovery of penicillin, it was thought that *S. aureus* infections were controllable, however this was to be short lived. In 1959, the discovery and introduction of methicillin (the first designer antiresistance antibiotic) was thought to be a sure defence against the penicillinases, but the appearance of methicillin-resistance *S. aureus* (MRSA) within three years led inevitably to other multidrug resistant variants. Recently MRSA has moved out of the hospital and into a community-acquired (CA) pathogen; it also has enhanced virulence and transmission characteristics. The fundamental differences between CA-MRSA and MRSA is the *mec* gene clusters, it has also acquired new pathogenic genes, such as those that encode the cytotoxic Panton-Valentine leukocidin [22, 23]. These are regulated by defined signalling systems [24].

8. Superbugs in volatile environments

Superbugs are omnipresent in the biosphere; in volatile environments (war zones, civil unrest, famine and natural disasters) and poor or non-existent hospital practises. Superbugs are not the only microbial threats, but are widely recognised as the most menacing when it comes to mortality and morbidity worldwide. With regards the number of infections and consequences *Vibrio cholera* should be at the top of the superbug list [25]. Fortunately it is not common in industrial nations; however, it is endemic in South America and Asia. In regards to the global control of endemic and pandemic infectious diseases, a considerable problem is the availability of reliable systems for tracking outbreaks of serious infections. The World Health Organisation (WHO), despite heroic efforts, identification and reporting of antibiotic resistance outbreaks are still not carried out in many parts of the world. The lack of information with regards to early stages of an epidemic bacterial infection has retarded appropriate remedial action in many cases [25].

9. The true cost of antimicrobial resistance

We know that bacteria are capable of becoming resistant to an antibiotic as soon as they are discovered and placed into use. However this is not necessarily a problem if there are other antibiotics to take their place. This was the predominant situation during the latter half of the 20th century; however this is no longer the case. There has been a rapid decrease in the number of drugs being investigated and approved; many of these have been on the grounds of safety and quality [26]. This has left the antibiotic pipeline dry and it is becoming clear that the existing classes of antibiotics are probably the best we will ever have [27].

In a bid to slow down or stop the progression of resistance to antibiotics there have been efforts to support interventions that should encourage a more conservative and appropriate use of antibiotics. However, too often these efforts are too little and too late [28, 29].

With the danger of antibiotic resistance widely acknowledged, the question is raised as to why more is not being done? The fundamental reason is that antibiotic resistance has fallen victim to evidence based on policy making. The outcome has resulted in prioritising health problems by economic burden and cost effectiveness of intervention [30]. Hence, health economists have been unable to show that antibiotic resistance costs are sufficient to become a health priority.

10. Limitations of health economic research

The current estimated cost of economic burden is modest and varies from less than \$5 to more than \$55,000 per patient episode for hospital costs. Societal costs are thought to be anything up to \$150 billion per year; these figures are far lower than the economic burden for other health problems such as cancers, heart disease and mental disorders [26]. The current estimated cost of resistance is modest and the reason for this is that it is based on the incremental cost and focuses on a specific infectious disease or set of diseases: estimates are based on the cost related to the extra treatment of a resistant infection compared with a susceptible primary infection, such additional cost are: more expensive drugs, side effects from extra treatments, extended hospital stays, costs of additional investigations and greater mortality [31]. The most critical economic burden is masked; this is when resistance leads to the loss of many of the advantages in medical care that antimicrobials have enabled-A world with no effective antibiotics for situations in which they are commonly used, for instance, surgical procedure and cancer chemotherapy. These may become far more dangerous as rates of antibiotic resistance associated with infection arise.

Over the past century the modern healthcare system has been built on the basis that infections can be treated or prevented by the use of antimicrobials. With high levels of resistance it is possible that we will fall back into the pre-antibiotic era. However is this viewpoint optimistic? Our healthcare system is now designed to treat more chronic conditions. It has become more technologically based, and has made a significant improvement in both mortality and morbidity; antimicrobials have become functional unit of this process. From cradle to grave antimicrobial drugs have become fundamental units to safeguarding the overall health of human societies. For example, antimicrobials are given

as standard to women delivering by caesarean section [32], to prevent iatrogenic infection in surgical care [33] and those who are receiving cancer treatment [34].

When the true cost of resistance is viewed in a broader way, the cost is not limited to those associated with the additional treatment of a primary infection, such as “strep throat”. Rather the cost must encompass the loss of the modern healthcare system. Resistance is not just an infectious disease issue; it is also a cancer issue, a surgical issue and a health issue.

To evaluate the true economic burden associated with resistance, we must also consider the burden of not having any effective antimicrobials at our disposal. As witnessed, when there are outbreaks of hospital acquired infections, examining the negative outcomes and observing how very quickly it can come to a standstill. In the future we need to re-evaluate and rethink how the health care system is developed, some facilities may need to be redesigned or perhaps there will be a need for the reintroduction of sanatoriums if effective antimicrobial treatments are no longer available.

11. An uncertain future

The current estimates of the cost of resistance provides false reassurance and this may result in inadequate attention and resources being devoted to resolving this problem [30]. It is difficult to forecast the likely economic burden of resistance and the result that this will have on the health system. A full health system analysis seems to be a more appropriate means of accessing the potential impact of resistance and evaluating the measures to curtail it. Although this is an extremely complex approach, understanding the threat to the public health system overall is of fundamental importance, not just for specific diseases. This could be the single most important step in understanding the economic burden presented by resistance. It is also important to analyse outbreaks of resistance infections e.g. MRSA and estimate the cost of disruption to the healthcare system, this could aid in future estimations [35].

Finally, a change in mind-set and action is needed to plan for a future with more antibiotic resistance bacteria. Considerable inertia remains as regards the radical changes needed in relation to our stewardship of antimicrobials. However a major drive of this inertia is due to the collective uncertainty about the clinical and financial implications that are associated with increased resistance. If we wait for the burden to become a substantial before we take action, then it may be too late. We should not see antimicrobial policies as a cost; we should perhaps look upon them as insurance and a ‘precautionary principle’, albeit one which we hope will never happen.

12. Trends in antimicrobial drug development

The need for new antibiotics is currently greater than ever with the emergence of multidrug resistance in common pathogens and the rapid emergence of new infections. Paradoxically it seems that ‘Big Pharma’ has lost interest in anti-infective R&D at the same time. The reason for the loss of interest is thought to be due to the fact old antibiotics still work in the majority of cases. The enormous cost of new antibiotic discovery coupled with the painstaking clinical trials discouraged new investment and further research. The pharma focus is on the long term profitable illness of aging populations, such as cancer, cardiovascular, psychoneurological, and lifestyle-targeted therapies [36]. Conversely, antimicrobials are usually used for short-course periods that cure disease and thus eliminate their own need to be given to a patient. Pharmaceutical companies know that they will make more money selling a drug that you have to take every day of your life, over a drug that you take for a few weeks [37].

The majority of the drug developers are becoming more selective regarding research and development (R&D) investments. From discovery through to approval the development of an antibiotic drug is estimated to cost between \$0.8 and \$1.7 billion. The figure of \$0.8 was generated through a study conducted in 2003, it was thought to be the average number of US dollar that a pharmaceutical company spent to bringing a new drug to market [38]. Since then, there have been new figures generated and some health economists suggest that the current figure is thought to be around US\$1.3 billion, others estimate US\$1.7 billion [38, 39]. There has been debate regarding the costs, but one thing that is agreed upon is that the cost of R&D within the drug industry and the cost of clinical trials in particular are rising significantly. It is feared that this growing expense of developing drugs has stifled innovation and research.

Further reasons for the reduction in R&D in antibiotics is also due to the following factors; the pressure to reduce drug usage, more difficult regulatory climate, short life span of drugs due to resistance developing, short life-span of patent protection, for example the patents on Augmentin and Ciprofloxacin are out of patent [40, 41]. When a drug is patented, it is protected from being copied for a limited period of time, usually 20 years. Those years are up for some of the industry’s biggest drugs, Glaxo Smith Kline’s (GSK) blockbuster antibiotic Augmentin came off patent in 2002. Finally, the lack of fulfilment in novel high-throughput methodologies to develop screening processes to enable the identification of new antibiotic molecules resulted in significant investment loss [42]. Amoxicillin, a moderate-spectrum, bacteriolytic, -lactam antibiotic is still one of the most prescribed antibiotics today. In 2000, the only antibiotic in the top 20 prescription drugs was amoxicillin-clavulanic acid [43] this highlights the need for new antibiotics.

When examining the regulatory climate it is noted that the Food and Drug Administration (FDA) continues to approve drugs based on disease state, one at a time (urinary tract, pneumonia, etc.) rather than the organism that the antibiotic is designed to kill. Hence companies spend \$100 million to pursue a phase III programme and the result is that they only obtain one small area of treatment. Drug pricing in society is also imbalanced, society will pay \$50,000 for a course of cancer treatment that will prolong life for three months, but are unwilling to pay \$100 for a course of antibiotics that would cure a target infection. This pricing difference is not data-driven or rational; there is no cost-efficacy analysis that supports cancer drug pricing. Rather drug pricing appear to be decided on public perception and fear. The Office of Health Economics illustrated, at discovery that the net present value of antibiotic to a drug company is minus \$50 million. That compared with \$1 billion for a new musculoskeletal drug [44, 45].

However, there does seem to be some signs of change within the industry, the number of FDA approvals has increased and the hope remains that the industry could be about to embark on a new golden era. However, one of the reasons for this upsurge in drug development is that developers have been focussing on diseases which affect smaller numbers of people and where treatments have not always been available.

13. New policies to encourage drug development

With drug development of antimicrobial agents at a historical low point and antimicrobial resistance is one of the greatest threats facing the world, the World Health Organisation (WHO) dedicated last year's World Health Day to the topic. Hospital-acquired superbugs have reached an all-time high, with an estimated 70,000 people a year dying from infections [46]. European public-private partnership research initiative of €224 million has also been launched to speed up the much-needed development of new antimicrobial drugs [48]. In February 2013 The Innovative Medicines Initiative (IMI) funded two new projects through its antimicrobial resistance programme New Drugs for Bad Bugs (ND4BB). The two projects funded are COMBACTE (Combatting Bacterial Resistance in Europe) and TRANSLOCATION (Molecular basis of the bacterial cell wall permeability) will seek to tackle the growing problem of antimicrobial resistance throughout the EU that is currently the cause of over 25000 deaths in the EU every year and cost the European economy over 1.5billion euro a year. There is a lot of promise and hope in these two projects [47]. Europe is not the only region that is taking action. The Generating Antibiotic Incentives Now (GAIN) is currently being debated by Congress in the USA.

14. All pain: No GAIN

The Generating Antibiotic Incentives Now (GAIN) act aims to extend patents granted and give prolonged exclusivity to drug companies that discover and develop new drugs, thus improving their profitability. However there are concerns that this is a short-term fix, rather than a long-term stimulus [48]. It is felt that the current draft of the GAIN Act does not provide any binding requirements to implement surveillance of resistant bacteria, appropriate use and more responsible use of existing antibiotics, conservation and increased incentives to develop new antibiotics. It is feared that this act focuses on bringing the antibiotics to market quickly, without any of the needed change of use in either human use or animal populations.

It has been proposed by the Disease Society of America that a regulatory pathway be included in the GAIN Act. This new regulatory pathways is called the Special Populations Limited Medical Use (SPLMU), these have since been renamed LPAD pathway [49]. This regulatory pathway would allow the FDA to approve drugs to treat life-threatening drug resistant infections with limited available therapies. These therapies would be approved based on smaller, fast and relatively less expensive clinical trials. This is due to the fact that the drugs would have a smaller safety database prior to approval. These drugs would only be approved to patients infected with these highly resistant pathogens within the setting of a studied disease. Thus SPLMU would limit the use of the drug to patients to whom the benefits of the drug outweigh the risk of the shorter faster clinical trial. This would join new development of drugs with stewardships to aid in prolonging the useful lives of these critically needed drugs. It is assumed that the drugs approved by this mechanism would be allowed to charge a premium price rate, given the smaller scope of patient's eligible for the therapy, the dangerous nature of the infection and the lack of availability of other therapies [50]. However enrolling patients into these trails would pose an ethical problem, i.e. that severely infected patients would wait for hours while screening and consenting for clinical trials to take place. Under the FDA's accelerated program Sirturo™ (a bedaquiline developed by Janssen Therapeutics) has been approved. This is the first drug to treat multi-drug resistant TB and to be used in combination with other TB drugs. By Sirturo™ being approved it allows this serious disease to be treated based on clinical data, this illustrated that the drug has an effect on the surrogate endpoint that is reasonably likely to predict a clinical benefit to the patient. This programme will allow patients earlier access to promising new drugs, while the company continues to conduct additional testing to confirm clinical benefit and drug safety [51].

Meanwhile in Europe, the European Medicines Agency (EM) has released a broad guidance document on antibacterial trials conduct, it specifies that: 1) that after a dose of prior antibiotic therapy those patients can be enrolled, making enrolment possible; 2) the possibility of conducting organism-specific rather than disease-specific studies; 3)

the possibility of conducting small studies to support approval of antibiotics that treat resistance, critical infections; 4) and clinical response endpoints at test-of-cure [45].

We must value and revive our antibiotics, they are an invaluable resource. It is imperative that new policies be introduced to encourage research and public interest back into the area of antibiotic resistance. If this problem is to be solved it will require many different regulatory and pharmaceutical bodies to work together to find the solution [52]. By working together novel therapies can be examined and can hopefully slow down the rate at which antibiotic resistance is presently emerging.

15. New novel therapies

With the urgent need for new novel therapies, this has allowed for new opportunities for natural products, old culture collections mining and patent literature to be revisited.

Natural products have provided challenging intellectual exercises with respect to mode of actions, chemical structure and biosynthetic pathways. The synthesis of these natural products in the laboratory is difficult since these molecules are extremely complex. It is for this reason that the demise in natural product research occurred in the 1990's, many novel high-throughput methodologies were developed to aid in screening of known natural products. Some of these novel techniques are combinatorial chemistry which generates vast chemical libraries, high throughput screening technology allows 1000s of chemicals to be tested against potential targets in vitro, genome sequencing of pathogens allows access to all potential protein targets in vitro and finally, structural biology allows drugs to be designed and modified in silico [53, 54]. However most of the efforts came up empty handed. Combinatorial chemistry has not led to antibiotic drug discovery; this is due to insufficient diversity in scaffolds or density in functional groups.

Successful natural products include Daptomycin, (found in the soil saprotroph *Streptomyces roseosporus* Vancomycin (made by the soil bacterium Actinobacteria species) and Macrolides, erythromycin was initially isolated in 1952 from *Streptomyces erythreus*, newer macrolide antibiotics are semi-synthetic derivatives. There are now opportunities for reviewing natural products, as up to 10⁵ bioactive natural products remain undiscovered. These are thought to be derived from environmental sources, for example marine actinomycetes, bacterial symbionts and microorganism that are thought to be unculturable. It is hoped that "metagenome" cloning ~100kb fragments from genomes pooled from the environment without culturing will yield more results [55]. Genomic sequencing has also revealed nascent biosynthetic pathways or "hidden pathways" for non-ribosomal peptides and polyketides. Furthermore, expanding the variations of growth conditions of new natural products could lead to varied expression. Potentially revisiting high throughput (HT) screening and examining growth inhibition instead of enzyme activity could potentiate new drugs.

16. Antimicrobial peptides

A vast number of antimicrobial peptides (AMPs) are considered a major source of potential novel agents (e.g. Magainins, Cathelicidins, Lactoferrins and Defensins) particularly against strains that exhibit multidrug resistances [56]. Particular interest has been shown in cationic AMPs against Gram-negative bacteria since their mechanism of action is biophysical (i.e. it disrupts the cell membrane) and is considered resistance proof, as there is no identified evasion/adaptation method for the target bacteria [57]. This gives AMPs a superior property as they target the Achilles heel of the unique but essential feature of microbial cellular membranes [58]. In 2013 the antimicrobial peptide database (<http://aps.unmc.edu/AP/main.php>) contained: 183 bacteriocins, 291 plant AMPs, and 1648 animal host defense peptides. Despite the large number and variety of AMPs in public and academic databases very few applications of these compounds are being developed. Unfavourable pharmacokinetics requiring high doses and high manufacturing costs are the main drawbacks rather than sub-optimal specificity for bacteria over eukaryotic cells.

17. Chemically novel agents

Another potential source of innovative antimicrobial agents is the chemically-novel antibacterial agents (e.g. Relacin [59]) preferably those which act upon previously unexploited bacterial targets, such as those that inhibit Rel/Spo synthases and aminoacyl-tRNA synthetases (aaRS) enzymes. These new chemical entities inhibit fundamental bacterial pathways. Moreover, chemical entities are being examined to increase potency of current antibiotics within clinical use; these synergistic compounds further potentiate the activity of the antimicrobial compound [60]. Some examples of these interactions with natural products are illustrated in Table 1. This would accelerate the drug development process through use of these compounds with known safety profiles and administration routes.

Table 1 Synergism between natural products and antibiotics against bacterial infections.

Natural Product	Antibiotic	Microorganism	Mechanism of action	Reference
Epigallocatechin-gallate (EGCg)	Ampicillin	MSSA β -lactamase producing <i>S. aureus</i>	Inhibits β -lactamase	[61]
EGCg	Penicillin	Penicillinase producing <i>S. aureus</i>	Inhibits penicillinase	[62]
EGCg	β -Lactam	MSSA, MRSA	EGCg directly binds to the peptidoglycan and inhibits cell wall	[63]
EGCg	Tetracycline	<i>S. aureus</i> with Tet (K) MDR pump	Blocks MDR efflux pumps	[64]
Carnosic acid	Tetracycline	Tet (K) possessing strains	Inhibit the MDR pumps, Tet (K) and Msr (A)	[65]
Isoflavone Bidwillon B from <i>Erythrina variegata</i>	Mupirocin	MRSA	Bidwillon B and mupirocin inhibited the incorporation of thymidine, uridine, glucose and isoleucine	[66]
Baicalin	β -Lactam antibiotics	MRSA	Inhibits β -lactamase	[67]
Diterpenes from <i>Lycopus europaeus</i>	Tetracycline	<i>S. aureus</i> possessing Tet (K), Msr (A) MDR pumps	Blocks MDR pumps	[68]

18. Bacteriophage

The idea of treating bacterial infections using phage therapy is not a new concept, rather it predates the use of antibiotics [69]. Bacteriophages are one of the most diverse and abundant biological agents on the planet and can be found in a variety of environments. They were originally discovered in the 20th Century and have been applied in the control of bacterial diseases. They were first evaluated as potential therapeutic agents in biocontrol in agriculture and veterinary medicine. The use of phage therapy for the treatment of meningitis and septicaemia in calves and chickens, or the use of bacteriophages in the reduction of *salmonella* spp. in poultry has been well documented [70]. It has also been suggested that phages could be used as a control for bacterial infection in high valued crops in place of antibiotic sprays. In 2007 bacteriophages were granted the status of 'GRAS' (generally recognised as safe) for use in all food products [43]. Novel methodologies in molecular biology allow researchers to focus on two key areas of bacteriophage-based therapies, firstly, administration of whole bacteriophages and secondly, administration of phage-lytic enzymes known as enzybiotics.

19. Enzybiotic

The term enzybiotic is a hybridization of "enzyme" and "antibiotic" it refers to those enzymes, independent of their origin, that are able to function as antibacterial or antifungal agents. They are commonly used as preservatives and food additives and are used to control *Listeria monocytogenes* in cheese [71].

Enzybiotics act as bacterial cell wall-degrading enzymes and include lysins, bacteriocins, autolysins, and lysozymes. Certain enzybiotics have a low probability of developing bacterial resistance. Another significant feature is that few undesirable side effects have been reported with the use of enzybiotics. They also have the additional advantage that their immunogenic properties are weak [72].

20. Conclusion

We must consider our antibiotics as a limited precious resource, and like all limited resources conserve, restore and value them. The value and importance of antibiotics cannot be overestimated; we are completely dependent on them for treatment of infectious diseases. The time has come to admit our mismanagement of antibiotics over the past 70 years. The present situation as regards antibiotic resistance is grim, resistance mechanisms are pandemic and this creates a huge clinical and financial burden on the healthcare system worldwide. There is no simple solution for this problem; it requires a multifaceted solution which incorporates the public, governments and industry. Perhaps the best one can expect is that doctors and medical healthcare centres provide their patients with an environment that is not filled with antibiotic resistant pathogens by enforcing stricter measures in infection control together with prudent antibiotic use. The search for new antimicrobial treatments is vital and must be continued, novel mechanisms and innovative bold solutions must be obtained if we are to slow down the rate of resistance. If not, the preantibiotic era awaits our descendants.

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