Antimicrobial resistance is a growing problem across the world, becoming a major threat to global public health. According to the Centre for Disease Control and prevention (CDC), in the USA alone over 2 million patients are infected with drug resistant bacterial infections and 23,000 of these patients die annually of these infections. This not only impacts health care systems but also has a negative impact on the economy as treatments become more expensive due to extended and more complex treatment regimes. It is estimated that the cost of this to the U.S. government is in the region of $20 billion dollars per annum, and this cost is only predicated to increase as drug resistance becomes more prevalent. The antibiotic era began in 1945 with Alexander Fleming’s discovery of penicillin, even then he warned of the potential development of resistance to antibiotics. Now his warning has become a reality with the discovery of numerous drug-resistant (MDR) bacterial strains. There is growing concern that the surge in MDR bacterial strains may result in a ‘post antibiotic era’, where many of our current antibiotics will lose efficacy rendering bacterial infections untreatable. The reality of this is already coming into effect; it has been reported that last resort treatments for MDR gonorrhoea are failing due to drug resistance.

There are numerous classes of antibiotics available in the clinic such as β-lactams, tetracyclines, quinolones, streptogramins, lipopeptides and fluoroquinolones to name a few. However, despite the wide array of antibiotics used in the clinic, resistance has emerged to all classes. Despite our increasing understanding of the drug discovery process and advances in many therapeutic areas, the development of new and novel antibiotics has remained static. This has resulted in the drug discovery void where no new major classes of antibiotic have reached the market for 3 decades. Due to the low financial gain and limited success of antibacterial drug discovery projects less money and research is being invested in this area. In part it is due to this lack of innovation that the incidence of drug resistance is increasing and no new treatments are being developed to combat this problem.

A study published in 2011 evaluated all antibacterial agents in clinical development at that time; these were subjected to certain exclusion criteria resulting in 90 molecules for evaluation. Of these 90 molecules only 73% were new substances and only 17% were identified as possibly affecting a novel target or having a novel mode of action. Shockingly only 13% of these molecules showed in vitro activity in MDR Gram-positive infections and only 4.4% in MDR Gram-negative infections. As it is MDR bacterial infections that are causing this global health crisis this analysis only highlights the drastic need for new and novel therapeutics to combat the growing incidence of MDR bacterial infections.

Why is resistance developing?

The primary cause for antibiotic resistance is widely thought to be due to the improper use of antibiotics as well as the increased use of these agents in veterinary medicine and agriculture. Improper use of antibiotics includes re-use of antibiotics, over prescription of antibiotics and mis-prescription of antibiotics for the appropriate infection. It has been estimated that more than half of prescribed antibiotics in the US are not an appropriate treatment; often patients are prescribed antibiotics for viral infections which are ineffective. Reducing the improper use of antibiotics is a simple yet important tactic in combating antibiotic resistance. Often patients do not take the recommended dose of antibiotics, finishing treatments early or taking them at the wrong intervals. This can lead to sub-therapeutic concentrations of the drug that is known to promote resistance. This is not only due to over-prescription by doctors and social pressure from patients but also due to the often-poor diagnostic tests available to efficiently and effectively diagnose bacterial infections. Current diagnostic test suffer from the need of prior knowledge of drug resistant genes and slow culture based methods. In order to help reduce the spread of resistance several government bodies have established programmes focusing on the rational use of antibiotics and the limited use of drugs for which no resistance has yet been developed.

Resistance mechanisms

There are a number of mechanisms that help facilitate the development of resistance, such as; mutation, horizontal gene transfer, increased efflux of antibiotics and increased drug metabolism.

Some bacteria may have an intrinsic resistance to certain antibiotics. In an environment where the resistant strain coexists with a drug susceptible strain horizontal gene transfer (HGT), also known as horizontal evolution, can occur. This phenomenon results in the transfer of genetic material between organisms via a bacteriophage rather than reproductive means.
between bacteria of the same or different species [14]. The new genetic material is then incorporated into the recipient’s genome, rendering them resistant. Random mutation is another factor attributing to resistance, also known as vertical evolution, and is the transfer of genetic material from the parental generation to the offspring during reproduction [14, 15]. Spontaneous mutations often occur due to exposure to sub-lethal doses of a drug. These may be changes to the drug binding site preventing the drug from binding and having its effect but that does not alter the proteins function.

Another principal mechanism of resistance is the reduced uptake/increased efflux of antibiotics [16]. This is achieved by the over expression of special membrane efflux pumps that remove harmful molecules from the cells cytoplasm and into the extracellular environment. This results in a low cellular concentration of the active drug, often below the therapeutic dose resulting in drug inactivity. These efflux pumps can either be specific to a certain class of molecule or in some cases efflux a wide range of molecules of different classes [17]. An example of resistance due to efflux pump over expression is the fluoroquinolones for which resistance has been identified in a number of bacterial strains [18]. Over expression of drug metabolising enzymes may also be responsible for increased drug resistance. In some bacterial strains enzymes which either degrade or modify/metabolise antibacterial drugs are over expressed, one example is the over expression of beta-lactamase [19]. This enzyme is responsible for the hydrolysis of beta-lactam rings, a key component of many antibiotics such as penicillin and carbapenem, resulting in drug inactivity [20]. New drug targets and therapeutics are desperately needed to address growing resistance to many of our current antibiotics.

Addressing Antibiotic Resistance

Antimicrobial resistance represents a significant challenge to future healthcare provision. The emergence of a number of pathogens which are resistant to three or more of the front-line antibiotic therapies (multi-drug-resistant; MDR), and a number of isolates which are effectively untreatable (pan-drug resistant) has heightened awareness amongst clinicians and regulators alike. An acronym ESKAPEE has been derived from the names of the organisms recognised as the major threats (E. faecalis, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, Enterobacter spp and E. coli) although there are a number of other organisms that may become equally challenging to treat in the clinic [21-26]. Of these organisms, the Gram-negative pathogens are particularly worrying as the pipeline of antibiotics is essentially empty and very few compounds are even in early stage clinical trial [21,22,27-29]. There is an urgent need to develop new antibiotics to develop effective therapy against these superbugs.

New drugs and targets

The identification of new antibiotic drugs and targets is a widely used strategy in trying to develop new therapeutics that can help combat resistance. In 1995 the first complete bacterial genome was sequenced, this led to hope for a new era of antibacterial drug discovery by using the genome to help identify new antimicrobial targets [30]. A number of companies began a new target based antibacterial drug discovery; GSK alone evaluated over 300 genes over a 7 year period. It was shown that more than half of those 300 genes were essential for bacterial survival, which looked promising for identifying new drug targets. A total of 70 high-through-put screens were carried out for single drug targets, however; this yielded only 5 leads. This was a very unsuspected and disappointing outcome, the success rate of this screening campaign was 4-5 fold less successful than for other therapeutic areas. The high cost and low success of this approach lead GSK to drop this strategy, as this multimillion-dollar project had yielded no valuable data for antibiotic development [31]. Despite there being hundreds of conserved essential proteins, the number of targets currently exploited is very small with most current drugs affecting the same three pathways; cell wall biosynthesis, the ribosome and DNA gyrase/topoisomerase. One area that has that recently come to light as a potential antimicrobial target is DNA; this is discussed in more detail in section 1.5.

Over the past decade most new molecules that have reached clinical trials for antibiotic use have been developed by chemical modifications on core scaffolds [21] of well-established antibiotic classes such as oxazolidinone and quinolones [32]. This has helped to increase potency, introduce broader spectrum antibiotic and overcome resistance mechanisms. However, this is not a long-term solution. One very promising new antibiotic discovered in early 2015 is teixobactin, an isolate from previously uncultured soil bacteria, has shown excellent activity against Gram-positive pathogens [33]. Structural analysis revealed that teixobactin contains an entirely unique chemical scaffold, thus introducing a novel chemical class for antibiotic drug discovery. Despite the best efforts of many research groups and companies no novel antibiotic targets or major classes of molecules have been identified in recent years that have led to new drugs in the clinic, this is a bitter disappointment for the antibiotic drug discovery field.

Quorum sensing inhibitors

Interrupting quorum sensing in bacteria is another method under development in order to overcome resistance [34, 35]. Quorum sensing is a system present in bacteria that allows them to regulate their gene expression based on population density [36]. These bacteria produce signalling molecules called autoinducers that lead to the alteration of gene expression. They use this in order to communicate within the bacterial community to coordinate processes like biofilm formation, antibiotic resistance, virulence and symbiosis [37]. There are three main approaches that can be used to supress quorum sensing; destruction of autoinducer, disruption of the autoinducers synthesis or inhibition of auto inducer/ligand interactions [38]. Using a molecule that disrupts bacterial communication and virulence may help in circumventing known resistance mechanisms in bacterial communities, allowing for conventional treatments to be effective [39]. Although some small molecule testing has been carried out revealing some promising clinical candidates the clinical applications of this as a treatment are not yet certain [40, 41].
treatments may not be adequate to combat growing resistance. Based on the current literature combination therapies have proven to be more effective at combating MDR [42, 43]. Additionally these therapies can prolong the lifespan of drugs as well as restore efficacy to a drug for which resistance has previously been developed [44, 45]. There are a number of ways in which combination therapies can be utilised, they can either target different pathways, different targets in the same pathway or the same target in multiply ways [46].

Combination therapies can be used to bypass or block resistance mechanisms in which an antibiotic that is usually destroyed by a specific enzyme is co-administered with an inhibitor of said enzyme. The first example of this was an amoxicillin-clavulanic acid combination therapy, patented in the late 70’s [47]. Amoxicillin is a β-lactam antibiotic, comprising of a β-lactam moiety that is hydrolysed by β-lactamase, the clavulanic acid acts to prevent this hydrolysis allowing the amoxicillin to exert its antibiotic activity. A number of combination therapies are currently under clinical development in order to circumvent β-lactam resistance [48]. Research is also being done into combination therapies to counter the effects of increased efflux, which is a particularly big problem in Gram-negative bacterial strains. This can be targeted by another form of combination therapy in which an efflux pump inhibitor is co-administered with an antibiotic that is usually effluxed from the cell [49]. This allows the drug to enter the cell and remain in the cell and accumulate to an inhibitory dose, exerting its antibacterial activity and overcoming resistance [50]. Despite the discovery of much efflux pump inhibitors no such treatments have yet reached the clinic.

**Biologics**

The use of biologics is another method that can be used to overcome drug resistance examples of biologics are antimicrobials peptides (AMP) and bacteriophages. Four classes of antimicrobial peptide exist in nature, these are; anionic peptides, cationic peptides containing high percentage of glycine, tryptophan, proline, arginine or phenylalanine, linear cationic alpha-helical peptides and anionic and cationic peptides containing cysteine [51]. AMP’s have a number of physiological roles in cell membrane disruption and inhibition of nucleic acid and protein synthesis [51, 52]. AMP’s are potent and broad spectrum antibiotics which carry minimal risk of resistance [53]. However, they have a number of disadvantages such as the high cost, low stability and antigenic effects [54]. Neither the less they are a promising resource for future drug development [55], with a number of molecules approved for use by the FDA.

Bacteriophages are bacterial virus designed to recognise only bacteria by detection of a specific bacterial receptor, is one of the most attractive alternative ways of combating resistance [54]. Bacteriophages are unlike traditional therapies in that they titer and the efficacy change is directly proportional to bacterial populations and can increase to combat the re-emergence of pathogenic bacteria [56]. The use of bacteriophages has been reported to be effective in a number of Gram positive and negative bacteria although there are some limitations to the use of bacteriophages. These limitations include the development of resistance due to changes in cell surface receptors, failure due to restrictive specificity and the integration of the bacteriophage DNA into the bacterial genome [56, 57]. Use of a ‘cocktail’ of phages coupled with an antibiotic or using components of the bacteriophage such as AMP’s may help in alleviating the potential disadvantages of this therapy [58].

**Pro-drugs**

Use of pro-drugs is another strategy that can be used to treat MDR antibiotic infections [12]. A pro-drug is a molecule that is administered in an inactive form; it is then metabolized within the cells in some way converting it into the active drug molecule. This is becoming a very fashionable strategy in drug discovery. By administering an inactive form of the drug this can help to avoid toxic side effects and lead to directed therapies which will target only certain cells in the body. There are several examples in which this strategy has been applied using a variety of enzymes such as Nitroreductase (NTR) [59-62], amino peptidases [63] and carboxylases [64].

NTR has raised much interest in recent years due to the potential applications in biomedicine, bioremediation, biocatalysis and in particular pro-drug activation [65]. The use of NTR has been utilised in a number of fields such as anticancer, anti-parasitic and antibacterial research. NTR is of particular interest for pro-drug therapy in the treatments of bacterial and parasite infections due to the fact that these enzymes are widely spread in prokaryotic cells [66]. A number of different NTR enzymes have been identified and characterised from a variety of microorganisms, in particular, those from enteric bacteria have been well studied. There are a number of examples in which nitroaromatic/nitro-heterocyclic compounds are used as pro-drugs for the treatment of various diseases. For example nitrofurans, such as macrobid, have been used for the treatment of bacterial infections since the mid 1940’s [67]. Two of these derivatives are nitrofurazone and nitrofurantoin; these molecules have shown wide spectrum activity against a range of Gram-positive and negative bacterial infections [68]. More recently a bicyclic nitroimidazole, PA-824, has been developed for the treatment of TB [69].

**Drug Delivery**

Drug delivery is another key area in combating drug resistance; traditionally clinical antibiotic therapies have been administered systemically. However, the systemic administration can in some cases lead to a low local tissue concentration in the infected tissue causing the development of MDR bacteria and the possibility of systemic toxicity. Administration of sub-lethal concentrations of a drug is known to promote drug resistance in bacteria [10]. Locally released antibiotic therapies will allow a much more efficient and effective treatment regime, avoiding the consequences of systemically administered antibiotics. By doing so we can cause an antibiotic release in a local area above the minimum inhibitory concentration (MIC) but still below a toxic threshold allowing us to treat the infection with little or no side effects [54]. It is thought that the local delivery of antibiotics as well as biologics may help to prevent resistance and/or extend their efficacy. There are several strategies which have been studied in order to deliver antibiotics such as the use of antimicrobial polymers [70], nano particles [71, 72] and liposomes [71]. There is currently limited success to these strategies, however, it is hoped that with more
research these strategies can successfully be developed and used in the clinic in order to combat MDR [54].

CONCLUSION

Antimicrobial resistance is a complex problem and addressing this requires an active engagement of stakeholders at all levels, including the academic community, healthcare professionals, clinicians, industry and the general public. In addition to developing new antibiotics, it is important to develop technologies to detect drug-resistant pathogens, evaluating antibiotics, a better understanding of what conditions drive antibiotic resistance, markers and trends in the development of antibiotic resistance and tolerance. The academic research community have to play a bigger role in tackling this crisis due to the reluctance of the industry to invest in the antimicrobial research, but this is only possible with the active support from the governments.

REFERENCES


