Editorial Overview

The Molecular Basis of Antibiotic Action and Resistance

There is no doubt that one of the biggest crises facing mankind in the 21st century is antimicrobial resistance (AMR), that is, the ability of human pathogenic bacteria to resist the action of antibiotics [1]. This crisis is a consequence of numerous issues. Overpopulation has meant that bacterial diseases can spread readily among humans, and between animals and humans, and this is exacerbated by the ease of international travel. Our existing armory of antibiotics is becoming less effective as resistance to these agents increases, fuelled by over-use and misuse of antibiotics. Moreover, most large pharmaceutical companies have withdrawn from primary antibiotic research and development due to the inability to delivery sufficient return on investment for the treatment of acute bacterial infections, in comparison to chronic medical indications, for example, cancer [2,3]. As a result, the number of skilled researchers in the sector has collapsed. The consequence is that fewer new antibiotics are coming onto the market.

Against this background, it is important that the academic community makes a more significant contribution to the antibiotic research and development process. This Special Issue illustrates current research in the antibiotic area that will improve our understanding of antibiotics and their modes of action and targets, and promote the development of new antibiotics in the future. The particular focus of this Special Issue is the molecular basis of antibiotic interactions with their targets, and their resistance mechanisms.

Natural products have been a major source of antibiotics, with the majority of antibiotics in current clinical use being natural products or natural-product derived [4]. Wencewicz [5] provides an overview of the origins of antibiotic resistance, highlighting the crossroads of antibiotic biosynthesis and producer self-protection, which can result in clinically relevant resistance mechanisms. Widespread antibiotic use amplifies environmental pools of antibiotic resistance genes and thus increases the likelihood for the selection of a resistance event in human pathogens. He points out that resistance is something that should be considered at the earliest stages of antibiotic development and is an issue that should be managed, not avoided. The work of Atkinson and colleagues [7] illustrates these concepts in the context of ribosome-acting agents. More antibiotic classes inhibit bacterial protein synthesis than act upon any other target, resulting in a correspondingly rich diversity in mechanisms of ribosome protection. These authors focus on subgroup F of the ABC ATPase family, individual members of which have been shown to confer resistance to various antibiotics. A combination of phylogenetic and biochemical analysis identifies diverse and previously unrecognized candidate ABCF antibiotic resistance (ARE) determinants in both producer organisms and human pathogens, and demonstrates interaction of representative proteins with the bacterial ribosome.

Penicillin and other β-lactams are examples of natural product/natural product-derived antibiotics that inhibit bacterial cell wall (peptidoglycan) biosynthesis and, since their discovery just over 90 years ago, remain clinically the most widely used class of antibiotics. Resistance to these compounds has, in many bacterial species, largely arisen due the acquisition of various β-lactamases that have evolved and spread to counter each new generation of β-lactam antibiotic and β-lactamase inhibitor that has been developed. The interactions of β-lactams with different classes of β-lactamases and approaches to inhibition of these resistance enzymes are here reviewed by Tooke *et al*. [8], focusing on the new mechanistic and structurally informed insights that have emerged in recent years. A structurally informed approach has also been taken by Bellini *et al*. [9], who use new and improved structures of the β-lactam target penicillin-binding-protein 3 (PBP3) from a range of Gram-negative pathogens to develop some insight into target-mediated resistance in this essential PBP. PBP3 remains little understood, both in terms of its catalytic activity at the divisome (the macromolecular complex that coordinates peptidoglycan biosynthesis and cell division) nor the mechanism by which resistance develops. This is important as new generations of β-lactams, or PBP inhibitors with alternative warheads, need to be developed that can evade this mechanism of resistance. An alternative approach to disrupting assembly of the bacterial cell wall involves targeting lipid II, the final precursor in peptidoglycan biosynthesis, and has produced clinically important antibiotics such as vancomycin with activity against Gram-positive bacteria, including β-lactam-resistant organisms such as methicillin-resistant *Staphylococcus aureus*. Lipid II remains an attractive target for new antibiotics, in part because of the length of time taken for the global emergence of vancomycin

resistance; Grein *et al*. [10] examine the docking of inhibitors to lipid II to aid discovery of such agents.

Aside from cell wall biosynthesis, DNA topoisomerases, responsible for managing DNA topology during replication and bacterial cell division, have also become key targets for antibacterial agents, including the highly successful fluoroquinolones [6]. However, of the many natural product and synthetic agents identified as topoisomerase inhibitors, only a minority have been systematically studied. An example of a natural product that has yet to be exploited as a clinical antibiotic is microcin B17 (MccB17). Collin and Maxwell [11] review >40 years work on this bacterial toxin: summarizing current understanding of the molecular basis of its action and its biosynthesis, which reveals fundamental features likely to be applicable to the biosynthesis of other natural products. The prospects for developing new antibacterial agents based on MccB17 are discussed. MccB17 targets bacterial DNA gyrase, a DNA topoisomerase found in all bacteria and the primary target of fluoroquinolones in Gram-negative pathogens. Bax *et al*. [12] discuss the ways in which the "cleavage complex" between gyrase (and other type II topoisomerases) and DNA can be stabilized and how this knowledge can be utilized in the development of novel antibiotics. Crystal structures of complexes between gyrase, DNA and a range of inhibitors show that there are various ways in which this can occur. These structures also shed light on fundamental aspects of topoisomerase mechanism.

Antibiotic resistance also threatens to undermine much of the recent progress in the fight against tuberculosis (TB). Isoniazid (INH) is a key drug in TB treatment regimens and an example of an antibiotic that is a synthetic molecule rather than a natural product. Vilchèze and Jacobs [13] review the discovery and use of INH, and its mode of action and the molecular basis of resistance. They also discuss the phenomenon of persistence, which they describe as the single greatest impediment to TB control. The topic of persistence, which challenges the effectiveness of antibiotics against a wide range of bacterial pathogens, is discussed in detail in the article by Tchinda *et al*. [14]. The authors focus on the relationship between persistence, wherein a fraction of the organisms present becomes transiently tolerant to antibiotic exposure, and toxin–antitoxin systems that may control this process by increasing phenotypic diversity in a bacterial population. Relating recent advances in mechanistic understanding of toxin–antitoxin systems to survival and virulence in an infection scenario is now a key goal and necessary if rational approaches to avoiding or overcoming persistence are to be achieved.

Of the many scientific challenges that have impended development of new antibiotics, achieving drug entry into impermeable and resistant pathogens has been among the least tractable. Reviewing methods to quantify antibiotic penetration into bacteria, Cama *et al*. [15] discuss experimental approaches that will help generate the permeation data that, until recently, have been largely unavailable and, even at best, have not been well integrated into early-stage antibiotic discovery. This is a situation that needs to change as the research community struggles to identify new antibiotic classes effective against multi-drug resistant Gram-negative bacteria, in which issues of permeation across the outer membrane and, depending on compound size and structure, subsequent drug efflux present the greatest difficulty. New rules to inform the synthesis of compounds that are potent, retain activity against their desired target(s) and yet can still penetrate this barrier may or may not be generic. Development of such rules, along with further approaches, is necessary to build upon recent progress in understanding of compound accumulation, with a requirement to enable these to be undertaken at scale to permit artificial intelligence to be effectively deployed as part of the antibiotic discovery process.

Successfully countering AMR will likely require a combination of approaches extending beyond conventional chemotherapies. Development of antimicrobial peptides (AMPs) for therapeutic use presents one such alternative; AMPs represent promising alternatives to conventional antibiotics. Torres *et al.* [16] review the physicochemical properties of AMPs and consider strategies for their design and evaluation, focusing on features (specificity, cytotoxicity and stability) whose optimization will permit therapeutic application of AMPs as antibacterials or immunomodulatory agents or in materials and medical devices.

The breadth and depth of research presented in this Special Issue illustrates both the remarkable sophistication and diversity of antibiotic action and resistance mechanisms and our continuing progress in understanding them. Applying this new knowledge offers real hope for the future development of new and innovative therapies for bacterial infections that, when combined with parallel developments in areas such as diagnostics, personalized medicine and infection control, promise to transform our approach to treating bacterial infections. It is to be hoped that the combination of these scientific advances with similarly innovative approaches in the political and economic arenas will make possible timely and effective translation of such new discoveries into clinically useful antimicrobials that expand treatment options for the growing numbers of resistant bacterial pathogens.

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