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Overcoming drug resistance, the natural way

Matthew I. Hutchings[1] and Katherine R. Duncan[2*]

[1]Department of Molecular Microbiology, John Innes Centre, Norwich Research Park, Norwich, NR4 7TJ, UK.
[2]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, G4 0RE, UK. *katherine.duncan@strath.ac.uk

Summary. Colistin is an antibiotic of last resort for treating Gram-negative bacterial infections but resistance is spreading rapidly. In a recent issue of Nature, Wang et al (2022) use genome mining to identify and synthesise a natural variant that bypasses colistin resistance and offers new hope for tackling antimicrobial resistance.

The majority of clinically used anti-infectives are derived from microbial natural products but the alarming rise in antimicrobial resistance (AMR) means many of these drugs are no longer effective in treating bacterial and fungal infections (Hutchings, Truman and Wilkinson, 2019). Indeed, a recent review estimates that drug-resistant bacterial infections caused 1.27 million deaths worldwide in 2019 (Murray *et al.*, 2022). Infections caused by Gram-negative or diderm bacteria are harder to treat than Gram-positive infections because they have a protective outer membrane (OM) which likely evolved to confer intrinsic antibiotic resistance (May and Grabowicz, 2018). This OM is covalently attached to the peptidoglycan cell wall and, with the cytoplasmic (inner) membrane, these protective layers make up the essential cell envelope. Antibiotics target essential cellular processes such as peptidoglycan biosynthesis, protein synthesis and DNA and RNA synthesis. To inhibit the growth of diderm bacteria using these mechanisms, they must first cross the OM (Hutchings, Truman and Wilkinson, 2019). In Gram-negative bacteria the OM is an asymmetric bilayer, consisting of an inner phospholipid leaflet and an outer leaflet of lipopolysaccharide (LPS) (May and Grabowicz, 2018). One of the most effective last lines of defence against life-threatening Gram-negative bacterial infections is the antibiotic colistin, a non-ribosomal peptide which binds to the lipid A moiety of LPS and disrupts the OM. However, widespread use of colistin in agriculture and medicine has inevitably led to an alarming rise in colistin resistance, often conferred by the plasmid-borne mobilised colistin resistance (*mcr-1*) gene (identified in 2015, and relatives), which encodes an enzyme that modifies lipid A and prevents colistin from binding to the LPS (Arcilla *et al.*, 2016; Gogry *et al.*, 2021).

Colistin, also known as polymyxins B and E, was discovered in 1947, during a golden age of antibiotic discovery that lasted from 1940 to 1960 and peaked in 1955 (Hutchings, Truman and Wilkinson, 2019). Although colistin use was superseded by aminoglycosides in the 1970s, the last two decades have seen a resurgence in use to tackle multidrug-resistant Gram-negative bacterial infections (Nation and Li, 2009). Polymyxins are a large, structurally diverse family of antibiotics, that differ in peptide sequence and in their attached lipid moiety. These metabolites are biosynthesised by non-ribosomal peptide synthetases (NRPSs) encoded by biosynthetic gene clusters (BGCs) found in species of the Gram-positive bacterial genus *Paenibacillus* (Choi *et al.*, 2009). In their recent study, Wang and co-authors (Wang *et al.*, 2022) reasoned that the structural diversity of polymyxins is likely driven by the need to circumvent natural resistance mechanisms. As such, they hypothesised that natural

congeners that bypass *mcr-1*-mediated colistin immunity should exist. This also relies on the theory that antibiotic resistance genes evolved prior to the clinical use of antibiotics but spread from environmental isolates to human pathogens under the selective pressure of antibiotic therapy, which was initiated just over 100 years ago (Nation and Li, 2009).

The initial step in an approach termed 'BGC-guided chemical synthesis', was to screen 10,858 bacterial genomes to identify BGCs that were predicted to encode antibiotics belonging to the polymyxin family. Amongst the 35 BGCs identified (based on gene content and gene organisation) was the *mac* BGC. This differs from colistin at three of its 10 amino acids, more than any other known polymyxin. The approach had two key advantages. First, the polymyxin family are non-ribosomal peptides (NRPs) and as such, the modular nature of their biosynthetic pathway can enable sufficient chemical prediction. Second, the characterised members of the family showed the congeners do not differ significantly from the consensus peptide, enabling a certainty in chemical prediction. Multi-modular biosynthetic enzymes, such as NRPSs and polyketide synthases (PKSs) are ubiquitous in bacteria, often resulting in natural products with antibiotic activity. As such, the structurally-related analogues and predictive-certainty this affords, could in theory, be broadly applied. Expansion beyond modular biosynthesis or navigating the impact of post-translational structural modification will provide challenges in identifying both BGCs and predicting structural analogues, however the application to NRPs and polyketides (PKs) alone would be significant.

On identification of the *mac* BGC, the product, named macolacin (*mcr-1* active colistin-like antibiotic), was synthesised. The chemical synthesis of a bioinformatically predicted BGC product is termed a synthetic bioinformatic natural product (syn-BNP). This can provide a solution to challenges around bacterial gene expression in the laboratory (so-called cryptic natural products). Yet, it is important to recognise that feasibility in chemical synthesis, especially in the generation of synthetic libraries for structure-activity-relationship (SAR) studies has previously been problematic for some natural products. The authors screened macolacin for antibacterial activity against the ESKAPE pathogens, a group of six highly virulent and multidrug resistant pathogens. Like colistin, macolacin has a narrow spectrum of activity against Gram-negative bacteria, which is consistent with it targeting LPS in the OM. Although slightly less potent than colistin against *Pseudomonas aeruginosa* and *Enterobacter cloacae*, macolacin is considerably more potent against bacteria carrying the *mcr-1* resistance gene and other common mechanisms of colistin resistance. To investigate which of the three amino acids (that differ from between macolacin and colistin) were critical to overcome *mcr-1* encoded resistance, a series of SAR studies were conducted using a library of structures with two-amino acid changes. In particular, a library of differentially N-acylated macolacin analogues showed the biphenyl lipid analogue (biphenyl-macolacin) to be active against highly-resistant carbapenem-resistant *A. baumannii* (CRAB), a panel of extensively drug-resistant (XDR) *A. baumannii* clinical isolates and a number of intrinsically colistin-resistant pathogens, including *Neisseria gonorrhoeae*. The efficacy of biphenyl-macolacin was evaluated using a neutropenic thigh infection model in mice and showed that this naturally-inspired congener was active against both *A. baumannii* strains tested, reducing the bacterial burden compared with the colistin treatment group.

This study beautifully exemplifies the utilisation of environmental BGCs to guide chemical synthesis for tacking bacterial antibiotic resistance mechanisms. It illustrates the impact of structural changes, which are often incremental, and the impact they can have on observed

biology. As such, this bioinformatically-guided approach for determining an informed (BGC/chemistry) starting point will be fundamental in identifying candidates for SAR studies of the next generation of effective antibiotics. Ultimately, understanding how bacteria evolve in nature to overcome antibiotic resistance to enable nature-inspired chemistry will not only accelerate our understanding of AMR, but have a real impact in a healthcare setting.

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