A metabolic perspective into antimicrobial tolerance and resistance

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Bacterial infections that were once easily cured have become increasingly difficult to treat due to the emergence of antimicrobial resistance (AMR) and the substantial threat it poses to public health. A report on the global estimate of the burden of AMR[1] suggests that bacterial infections kill 17 million people worldwide. However, these data are largely reported by high-income countries and the actual death rate is likely to be much higher. For example, in 2017, there were 11 million deaths worldwide associated with sepsis alone,[2] a total which doubles the number of deaths so far due to the COVID-19 pandemic. Despite global antibiotic stewardship and surveillance programmes, AMR in bacteria is rising and we can expect the burden of disease to rise with it. A question of great concern is how long we can preserve the effectiveness of our existing antibiotics while driving forward research and translation of alternative therapies that could prevent AMR infections.

With AMR, opportunistic nosocomial infections, specifically infections caused by ESKAPE group pathogens (ie, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp) are of urgent concern. These pathogenic bacteria represent a major problem in health-care-related settings due to their incredible ability to survive in a wide range of conditions, including oxidative, chemical, and physiological stressors, and growth-limiting conditions. Survival of these bacteria under such harsh environments is made possible, in part, by their versatile metabolisms.

Under nutrition-deprived conditions, bacteria can utilise non-glycogenic carbon sources (eg, acetate and fatty acids) to form glucose by gluconeogenesis. The glyoxylate pathway, an alternative of the tricarboxylic acid cycle, is essential for converting acetyl-CoA to oxaloacetate so that they can use fatty acids as the starting material for gluconeogenesis. The glyoxylate cycle is upregulated during bacterial infections, antibiotic treatment, iron-limiting conditions, and persistent cells formations.[3,4] Importantly, this metabolic pathway is absent in mammals, which suggests that targeting this pathway could be a promising strategy to develop new antimicrobial interventions, specifically targeting the molecular mechanism that facilitates bacteria to switch from tricarboxylic acid to glyoxylate cycle, and developing direct inhibitors of key enzymes within the glyoxylate cycle.

The first enzyme of the glyoxylate cycle (appendix), isocitrate lyase, competes with isocitrate dehydrogenase for its substrate isocitrate. When isocitrate dehydrogenase is inactivated, isocitrate is directed into the glyoxylate cycle. However, the actual regulatory mechanism is much more complex with insufficient understanding of what controls the diversion through glyoxylate shunt in bacteria. In *Escherichia coli*, the metabolic flux directs the glyoxylate cycle through kinase—phosphatase Acek mediated phosphorylation and inactivation of isocitrate dehydrogenase.[5] In contrast, *Mycobacterium tuberculosis* does not have Acek, and glyoxylate itself regulates the catalytic activity of isocitrate dehydrogenase allosterically.[6] Furthermore, *P aeruginosa* possesses two isoforms of isocitrate dehydrogenase—AceK sensitive and insensitive, making the regulation process more complicated. A study reported that oxaloacetate and pyruvate (gluconeogenic precursors) regulate flux between glyoxylate and tricarboxylic acid cycle such that high supply of oxaloacetate and pyruvate activates isocitrate dehydrogenase (and inhibit isocitrate lyase) and vice versa.[7] Moreover, acetyl-CoA substantially increases the kinase activity of AceK, resulting in phosphorylation and inhibition of isocitrate dehydrogenase.[7] Nevertheless, to date, molecular and structural understanding of enzymes involved in this bifurcation mechanism across the ESKAPE pathogens is not sufficient.

Isocitrate lyase, which is a key enzyme of the glyoxylate pathway, represents an attractive avenue for antimicrobial development. The full druggable potential of isocitrate lyase has not been explored yet, partly due to the difficulty in designing inhibitors targeting isocitrate lyase's small, polar substrate-binding pocket.[8] In the past two decades, advances in the structural and molecular understanding of the isocitrate lyases from several bacteria have substantially shaped our understanding of substrate binding and structural rearrangement upon binding.[7] The shared feature of

isocitrate lyases throughout the bacterial species is the presence of cysteine amino acid in the substrate-binding pocket. Thus, targeting highly reactive free thiol (-SH) of cysteine in the isocitrate-binding pocket for the generation of covalent and mechanism-based inhibitors can facilitate the development of new lead compounds. Studies[9,10] have utilised these approaches to generate inhibitors and have shown, using in vitro assay, that these inhibitors are effective against *M. tuberculosis* isocitrate lyases.

Understanding the metabolic pathways associated with antibiotic resistance and pathogenesis would provide new knowledge on specific targets for developing antimicrobials. Although the glyoxylate cycle is a promising metabolic pathway in this regard, the complex regulatory mechanism and the little knowledge on the flux control mechanism of tricarboxylic acid to glyoxylate cycle reflect the substantial challenges associated with therapeutic development. However, recent advances have made isocitrate lyase an attractive druggable target against AMR bacteria.

We declare no competing interests.

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