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The role of bacterial metabolism in antimicrobial resistance

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Abstract

The relationship between bacterial metabolism and antibiotic treatment is complex. On the one hand, antibiotics leverage cell metabolism to function. On the other hand, increasing research has highlighted that the metabolic state of the cell also impacts all aspects of antibiotic biology, from drug efficacy to the evolution of antimicrobial resistance (AMR). Given that AMR is a growing threat to the current global antibiotic arsenal and ability to treat infectious diseases, understanding these relationships is key to improving both public and human health. However, quantifying the contribution of metabolism to antibiotic activity and subsequent bacterial evolution has often proven challenging. In this Review, we discuss the complex and often bidirectional relationships between metabolism and the various facets of antibiotic treatment and response. We first summarize how antibiotics leverage metabolism for their function. We then focus on the converse of this relationship by specifically delineating the unique contribution of metabolism to three distinct but related arms of antibiotic biology: antibiotic efficacy, AMR evolution and AMR mechanisms. Finally, we note the relevance of metabolism in clinical contexts and explore the future of metabolic-based strategies for personalized antimicrobial therapies. A deeper understanding of these connections is crucial for the broader scientific community to address the growing crisis of AMR and develop future effective therapeutics.

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Author contributions

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Introduction

The discovery of antibiotics is arguably one of the most transformative achievements in modern medicine, extending the human lifespan by over 20 years[1]. Antibiotics have revolutionized the treatment and cure of bacterial infections, ensuring sterility and infection control in complex medical procedures such as chemotherapy and organ transplantation, as well as in routine care such as tooth extractions and childbirth[1]. They are also often the first line of defence against common acute everyday infections, making conditions such as sore throats (for example, *Streptococcus pyogenes*), skin infections (for example, *Staphylococcus aureus*) and sexually transmitted diseases (for example, *Neisseria gonorrhoeae*) readily curable[2].

The widespread use of antibiotics to combat diverse bacterial infections has also driven the emergence of antimicrobial resistance (AMR), a phenomenon where bacteria can survive and grow in the presence of clinical antibiotic concentrations, rendering treatments ineffective. Misuse and overuse of antibiotics in clinical and agricultural settings have significantly exacerbated the prevalence of AMR. Now, AMR is recognized by clinicians, scientists and politicians alike as a growing global crisis. With the ever-increasing number of multidrug resistant organisms and fewer effective antibiotics, forecasts estimate that between 2025 and 2050, upwards of 169 million global deaths will be associated with AMR[3].

Bacterial AMR is primarily driven by genetic mechanisms. AMR arises either through natural genetic variation and subsequent clonal expansion, or via horizontal transfer of mobile genetic elements between cells. Regardless of the mode of acquisition, AMR mechanisms can generally be grouped into three broad categories: altered drug transport dynamics, which reduce intracellular drug concentrations; direct antibiotic inactivation, often mediated by enzymatic hydrolysis; and modification of antibiotic target sites, such as mutations in penicillin-binding proteins that prevent drug binding[4]. Collectively, these resistance mechanisms threaten our current arsenal of available antibiotics, especially as drug development has failed to keep pace with the inexorable march of bacterial evolution.

Understanding the bacterial response to antibiotic treatment remains a major area of research. Recent findings have indicated that bacterial metabolism — the collective sum of all catabolic and anabolic processes involving diverse genes, pathways, metabolites and interactions — not only is influenced by AMR, but is also profoundly shaped by it. As the engine driving all cellular functions, metabolism provides the energy and resources essential for the operation of highly coordinated and interconnected processes. This complexity enables metabolism to influence a wide spectrum of cellular activities, from gene expression and protein synthesis to defence mechanisms and cooperative behaviours. Consequently, metabolism lies at the crux of nearly every aspect of antibiotic treatment and response.

Studies investigating the role of metabolism in all aspects of the bacterial response to antibiotics have revealed a bidirectional relationship between the two: bacterial metabolism both influences and is influenced by antibiotic treatment. This interplay highlights the immense therapeutic potential of targeting the bacterial metabolic state to combat AMR. For instance, numerous studies have shown that boosting cellular metabolism — by

introducing external metabolites and/or upregulating diverse metabolic pathways — can sensitize resistant cells to antibiotic treatment[5–7]. These exciting findings offer a promising new perspective on tackling AMR and underscore the previously underappreciated role of metabolism.

In light of the growing body of research on both bacterial metabolism and the cellular response to antibiotics, the purpose of this Review is to rigorously delineate the distinct and bidirectional ways in which all aspects of cellular metabolism affect, and are affected by, antibiotics (Fig. 1). We first summarize the current understanding of how antibiotics affect metabolism. Then, we explore the corresponding influence of metabolism on antibiotics, with a specific focus on drug efficacy, associated evolutionary dynamics and specific AMR mechanisms. Finally, we briefly examine the relevance of metabolism in the context of mammalian hosts and consider the potential of metabolic-based therapies to combat AMR. The studies, insights and open questions discussed herein highlight the need for further investigation of these complex relationships and a more personalized approach to next-generation antimicrobial therapies.

Influence of antibiotics on metabolism

Antibiotics are a diverse class of small molecules that target bacterial cellular processes, ultimately compromising their growth or survival[8]. These agents are generally categorized as either bactericidal, inducing active cell death, or bacteriostatic, suppressing cell growth. While the growth-inhibitory effects of both antibiotic types have been well-documented, recent studies have revealed distinct ways in which each class disrupts cellular metabolism, providing deeper insights into their mechanisms of action (Fig. 2).

Generally, bactericidal antibiotics induce cell death by inhibiting essential cellular targets that, with some exceptions, are commonly related to DNA replication (for example, fluoroquinolones), protein production (for example, aminoglycosides) and cell wall synthesis (for example, β-lactams, cephalosporins). In each case, the resulting intracellular damage typically aligns with the mode of action of the drug. For example, fluoroquinolones inhibit DNA gyrase and topoisomerase IV, leading to toxic double-stranded DNA breaks[9,10]; aminoglycosides target the 30S ribosomal subunit, causing error-prone translation and proteotoxic stress[11,12]. Beyond these direct effects, studies have increasingly revealed multiple pathways by which metabolic homeostasis is disrupted, highlighting a critical secondary layer in bactericidal-induced cell death[5,8,13–19].

Bactericidal exposure triggers the immediate upregulation of stress response networks, including the SOS and stringent responses[20,21]. In parallel, and probably due to increased energy demands that are driven by multiple repair systems, bactericidal treatment often leads to dysregulation of core energy generation pathways, such as the electron transport chain, tricarboxylic acid (TCA) cycle, central carbon oxidation and cellular redox balance[22–29]. Consistent with these effects, increased ATP levels, oxygen consumption rates and reactive oxygen species have been observed in diverse bacterial species following bactericidal exposure, as shown by a variety complementary measurements[27,30]. For example, single-cell RNA sequencing of *Escherichia coli* exposed to ampicillin has shown clear signs of

dysregulated TCA cycle, glycine metabolism and glycolysis gene expression[31], consistent with population-level measurements[32]. Similarly, multiple studies report that treatment of *Mycobacterium bovis* with a range of antibiotics increases intracellular ATP levels[29,33,34]. Interestingly, this can be seen as early as 1.5 hours following drug treatment[29] and ATP levels can increase up to fourfold to fivefold[33]. Ultimately, this metabolic dysregulation causes toxicity to the cell that, combined with initial target inhibition, culminates in cell death[24]. We note the degree to which each aspect described contributes to cell death has been historically controversial and is likely to vary depending on the conditions.

Depending on the metabolic constraints at play, cell damage occurs via different pathways. For quinolones, aminoglycosides and β-lactam treatment in Gram-positive and Gram-negative bacteria, treatment under aerobic conditions is typically associated with oxidative damage caused by alterations to the TCA cycle and NADH depletion[24]. By contrast, under anaerobic conditions, the same antibiotics drive increased glycolysis — accumulating central carbon metabolites (for example, glucose and pyruvate) — and generate different reactive metabolic byproducts (for example, reactive electrophilic species)[35]. Moreover, although altogether diverse bactericidal classes ultimately dysregulate central metabolism, intermediate pathways vary widely, and this is influenced by antibiotic-specific mechanisms of action. For instance, β-lactam treatment induces a futile cycle of cell wall synthesis and degradation, thereby depleting cell resources via specific cell-wall synthesis mechanisms and enhancing -cidality[19]. Additionally, for ciprofloxacin and ampicillin toxicity, increased demand on purine biosynthesis was found to be critical[36]. This was not demonstrated to be the case for gentamicin[36], suggesting that nucleotide limitation may be specific for some drugs but not others[37]. A deeper understanding of drug-specific metabolic pathways, and their interplay with primary drug targets, is still needed.

While bactericidal drugs upregulate central metabolic processes related to energy generation, bacteriostatic drugs act in an opposing manner[38], typically decreasing metabolic activity by limiting energy utilization, macromolecule biosynthesis and protein translation[8,30,39]. This growth suppression also leads to unique metabolic states. For example, recent metabolic profiling revealed that bacteriostatic treatment with chloramphenicol and linezolid leads to an excess build-up of energy metabolites[30]. Similarly, proteomic analysis has shown that the antibiotic chlortetracycline downregulates glycolysis, gluconeogenesis, pyruvate metabolism and the TCA cycle[38]. Interestingly, bacteriostatic drugs can also behave in a bactericidal manner and vice versa, depending on the drug concentrations used[40,41], blurring the functional distinctions between these classes under certain conditions. To our knowledge, the literature has not yet reached a consensus on whether metabolic suppression directly precedes cell death in these cases or whether only specific circumstances trigger a cidal-like activation process; further research is needed to fully understand these dynamics.

A potential consequence of treatment with any antibiotic is the formation of antibiotic tolerant persister cells, often seen in chronic infections[42] (Box 1). Traditionally, persister cell formation is thought to occur either stochastically or in response to environmental stress, leading a bacterial subpopulation to enter a metabolically inactive state with high drug tolerance[43,44]. While the specific mechanisms of persister formation remain an active

area of study, they are generally associated with the SOS and stringent response, (p)ppGpp signalling and ATP depletion[44]. Additionally, changes in the expression of toxin–antitoxin systems[45], *trans*-translation[46,47], RNA methylation[48] and protein degradation[49] have been implicated in the formation of persister cells. Further, antibiotic treatment has been shown to increase the frequency of persister cell formation, which is likely to be related to the inhibition of key cellular processes, including transcription and translation[50]. Sublethal antibiotic concentrations can also activate general stress responses, secondary messenger pathways and virulence factors that are implicated in persister cell formation[51,52]. A detailed discussion of the mechanisms that lead to the formation of persister cells has been reviewed elsewhere[53].

Overall, antibiotics distinctly shift cellular metabolism. In turn, these varied metabolic states can complement and/or potentiate drug action, translating into diverse consequences for bacterial growth, antibiotic selection and long-term evolutionary survival. Thus, beyond studying how antibiotics alter cellular metabolism after treatment, it is crucial to characterize the metabolic state of the cell both prior to and during drug exposure, to better understand microbial survival and subsequent antimicrobial resistance.

Influence of metabolism on antibiotics

Given the diverse effects of antibiotic treatment on bacterial metabolism, the metabolic state of the cell at the time of treatment itself may also contribute to, and/or direct, the antibiotic response. This may allow metabolic pathways to modulate the intended mechanism of action of the antibiotic and downstream metabolic dysregulation. Indeed, this interconnectedness is well established in the notorious pathogen *Mycobacterium tuberculosis*, given its unique metabolic characteristics and distinct antibiotic treatment regimen[54,55] (Fig. 3). However, these concepts have only recently been applied to other environments, pathogens and antibiotics. In this section, we review how metabolism specifically impacts diverse aspects of the bacterial response to antibiotics by dissecting the three main contributions: antibiotic efficacy, resistance evolution and resistance mechanisms.

Antibiotic efficacy

Establishing the role of metabolism in antibiotic lethality has historically been challenging. As metabolism is tightly coupled to growth, conditions that modify metabolic activity will often influence growth rates as well. Furthermore, the relationship between growth and antibiotic efficacy is more readily quantifiable and, as such, has previously been a major focus. Most commonly, it has been reported that bacterial growth rate is directly proportional with bactericidal antibiotic efficacy[56–58]. That is, bactericidal antibiotics are often shown to be most effective against rapidly growing bacterial populations. However, growth rates and metabolic activity are not universally correlated[59–61], and prior work has often conflated growth effects with metabolic ones. Recent studies have uncovered greater complexity in this relationship. For example, the linear relationship between rates of cell growth and cell death depends on environmental conditions, highlighting a physiological constraint to the relationship; changing the environment by altering the antibiotic type, carbon source and salt concentration can change the magnitude of the slope or intercept[62].

Further, sufficient metabolic stimulation has been shown to promote antibiotic killing of non-growing persister cells, thereby indicating the unique effects of metabolism[7,63]. These findings suggest that while growth may serve as a proxy for antibiotic efficacy under certain conditions, the unique effects of metabolism on antibiotic efficacy warrant independent investigation.

Potentiating antibiotic activity. Multiple lines of evidence now demonstrate that the metabolic state of the cell, independent of growth, directly influences bactericidal antibiotic efficacy[6,8,17,30,64–68]. Indeed, under a wide range of conditions, antibiotic activity was shown to better correlate with the cellular metabolic state, rather than the growth rate[17]. Similarly, the successful eradication of *E. coli* and *S. aureus* has been shown to require metabolome remodelling[8,69]. Such metabolic effects also explain established antibiotic dose–response relationships. For example, the inoculum effect — a well-known phenomenon describing reduced bactericidal efficacy with increasing cell density — is also grounded in metabolism and, more specifically, the tradeoff between ATP concentration and density inhibition[70]. Given that the inoculum effect has been observed for diverse antibiotic classes and in clinical contexts[71–79], this study highlights the potential of the metabolism in driving antibiotic response in vivo.

Consistent with these findings, stimulating metabolism with specific metabolites has been shown to enhance antibiotic lethality in a drug-specific manner. In some cases, these effects may be partially explained by metabolic stimulation of the proton motive force (PMF). Particularly for positively charged aminoglycosides, the PMF increases intracellular drug uptake and thus boosts antibiotic lethality. For example, the addition of upper glycolysis metabolites (for example, glucose, mannitol and fructose) increased the efficacy of gentamicin against antibiotic tolerant *E. coli* and *S. aureus*[8,63]. Remarkably, these observations were reproducible in vivo, where gentamicin combined with mannitol reduced the viability of catheter biofilms by nearly 1.5 orders of magnitude and inhibited spread of infection to the kidneys[63]. While these results illustrate the benefits of metabolic stimulation in pathogen treatment, they also underscore the necessity of studying metabolism in isolation. For instance, the relative contributions of cellular respiration versus PMF-driven drug transport in aminoglycoside lethality remain incompletely understood[80].

Beyond enhancing drug uptake, stimulating metabolic activity can also potentiate antibiotic efficacy through secondary metabolic effects. For example, combining the TCA cycle intermediate fumarate with the antibiotic tobramycin killed antibiotic tolerant *Pseudomonas aeruginosa*, an opportunistic pathogen associated with cystic fibrosis[8,81]. Mechanistically, fumarate activated cellular respiration and stimulated a proton motive force via the TCA cycle[8,81]. Notably, inhibiting the TCA cycle restored antibiotic tolerance even in the presence of drug import, demonstrating that the TCA cycle activity was essential for both metabolite supplementation and tobramycin to exert their effects. These findings underscore the critical role of downstream metabolic processes — independent of drug uptake — in driving antibiotic lethality. Similarly, activating respiration in *M. tuberculosis* has been shown to prevent the emergence of tolerance and resistance. The addition of small thiol groups maintained *M. tuberculosis* in an active metabolic state that generated an ‘oxidative

burst,' increasing oxygen consumption and reactive oxygen species production, promoting cell death[82–85].

Reducing antibiotic efficacy. While high metabolic activity can potentiate antibiotic treatment, low metabolic activity can protect against antibiotic exposure. These protective effects have been observed in numerous pathogens and contexts and can occur via multiple mechanisms. For example, whereas fumarate increased aminoglycoside efficacy in *P. aeruginosa* by activating the TCA cycle, the metabolite glyoxylate induced antibiotic tolerance[81]. Protection was attributed to inhibited cellular respiration by acetyl-coenzyme A diversion through the glyoxylate shunt[81]. Likewise, antibiotic tolerance in *M. tuberculosis* is mediated by suppression of the TCA cycle metabolism[6,86]. Additionally, proteomics indicates that depressed central carbon metabolism promotes levofloxacin tolerance in the waterborne pathogen *Vibrio alginolyticus*[6,87].

Low metabolic states can arise in response to environmental stimuli, such as nutrient deprivation, which reduces antibiotic efficacy. For example, restriction of specific amino acids protects *Piscirickettsia salmonis* from ampicillin by downregulating energy production, and pentose phosphate and nucleotide metabolism — without affecting growth rate or resistance gene expression[88]. These effects are further exacerbated in biofilms[89,90], where bacteria exist in various heterogeneous states of metabolic dormancy (Fig. 4). Within biofilms, bacteria exhibit antibiotic tolerance at concentrations hundreds to thousands of times higher than otherwise required for treatment[91–93]. While the biofilm structure itself offers protection from antibiotic penetration, reduced aerobic metabolism from nutrient limitation majorly contributes to antibiotic tolerance[94–97]. Nutrient restoration can reverse this effect, sensitizing biofilm cells to antibiotics[63,98].

Population-level behaviours that confer protective phenotypes against antibiotics are also consistently associated with unique metabolic states. For example, swarming bacteria such as *P. aeruginosa* exhibit temporary tolerance[99], with metabolic genes such as *cbrA* implicated in virulence and resistance phenotypes[100]. Swarming imposes a high metabolic burden[101,102], leading to overall downregulation in pathways implicated in antibiotic efficacy, including glycolysis and pyruvate dissimilation, the TCA cycle and amino acid biosynthesis[101]. These findings highlight that in some complex environments, the complex interplay between metabolism and AMR has yet to be fully delineated.

Tolerance is also linked to density-dependent signalling, such as quorum sensing (QS). QS allows bacteria to adopt a cooperative state and impacts biofilm formation, virulence factor synthesis and swarming[103–105]. QS also regulates central metabolism by downregulating glucose uptake, the pentose phosphate pathway and de novo nucleotide biosynthesis[106,107]. This raises similar questions about the scope of impact of metabolism on both resistance and grouped bacterial states. The broad influence of metabolism on cell function and antibiotic efficacy underscores the need for its further exploration, particularly in complex behaviours that affect multiple pathways.

The evolution of new antimicrobial resistance

Following antibiotic exposure, accumulating evidence suggests that metabolism plays both a direct and indirect role in the evolution of AMR. Indeed, the generation of de novo AMR mutants[4,108–111] provides a clear example of this: bactericidal antibiotics increase the rate of mutagenesis by stimulating oxidative stress, SOS response activity and consequent DNA repair mechanisms[30,112,113]. This increased rate of mutagenesis following bactericidal treatment also increases the likelihood of a resistance-conferring mutation occurring by chance[114–118]. For example, treatment with ampicillin, ciprofloxacin or kanamycin, but not other drug types such as antimicrobial peptides, increases mutagenesis rates by threefold to fourfold[119]. SOS-mediated mutagenesis has also been crucial for AMR development in clinical strains of *P. aeruginosa* and *M. tuberculosis* and even in biofilms[6,117,120,121].

Separate from de novo mutation, AMR also develops by horizontal gene transfer (HGT), where mobile genetic elements spread between cells[122]. HGT occurs through conjugation, transformation and transduction, with plasmid conjugation (transfer between cells via direct contact) being the most significant mechanism for resistance spread. Conjugative plasmids encode all machinery to self-propagate and can encode for multiple AMR genes[123]. As plasmids encode a variety of mechanisms to maintain themselves and thus their resistance genes, they can promote AMR even in the absence of antibiotic selection[124,125]. Notably, conjugation is thought to be prevalent within biofilms, facilitating the spread of resistance genes[126–128].

The efficiency of conjugation (kinetic transfer rate) is influenced by the metabolic state of the cell, as conjugation is energy-intensive[129] (Fig. 5). High carbon concentrations and active physiological states can significantly enhance conjugation efficiencies[130,131]. Post-transfer, the energetic requirements associated with plasmid recircularization and host establishment impose transient metabolic costs, known as plasmid acquisition costs[132–137], which are greater in metabolically efficient cells, probably due to energy limitations[138]. Importantly, these costs do not correlate with growth rate without the plasmid[138]. Thus, separate from growth, metabolism impacts the extent to which antibiotic resistance can invade into new environments[138]. Plasmids impose long-term energy demands (maintenance, copy number, etc.) on their hosts. This fitness cost is primarily derived from the metabolic burden associated with increased protein production and constrains the extent and trajectory of plasmid spread[139–142]. Both these transient (acquisition) and prolonged (fitness) costs lead to potential competitive advantages or disadvantages in microbial communities, thus contributing to evolutionary outcomes[137].

Beyond conjugation, metabolism may be likely to influence other HGT modes as well. For example, QS, which regulates metabolism, also modulates bacterial competence. In this way, metabolism may be implicated in providing bacteria with mechanisms to repair damaged genes or obtain new advantageous traits[143,144]. Similarly, transduction depends on bacterial growth; this raises questions regarding how different bacterial metabolic states may impact the ability of a transducing phage to infect a bacterium[145]. Additionally, metabolic states can also act as phenotypic bridges between drug tolerance and genetic resistance. Dormancy, often associated with antibiotic tolerant states, has been shown to foster subsequent bona fide resistance and precede resistance evolution[146–149]. Moreover, prolonged survival may

also favour subsequent gene transfer events, although direct connections of this remain to be seen.

Due to the stochastic nature of resistance, it has been challenging to establish concrete relationships between antibiotic-driven metabolic dysregulation and AMR emergence. Nonetheless, increasing evidence highlights the role of metabolism in shaping AMR evolution. For example, the presence of metabolic genes on transferrable plasmids was shown to protect against antibiotic treatment, broadening the context by which plasmids and metabolic genes under direct antibiotic selection may confer AMR[150]. Whether this occurs by modulation of plasmid-related metabolic effects or directly via gene function itself remains to be seen. Further, carbon and energy metabolism was found to constrain AMR evolution in two different nutritional conditions against three antibiotics with distinct mechanisms of action. Comprehensive profiling of metabolites in 190 evolved populations showed that carbon and energy metabolism strongly constrained evolution of antibiotic resistance, both in terms of speed and mode of acquisition[66]. More comprehensive studies like these will continue to reveal the relevance of diverse metabolic pathways in the larger AMR context.

Primary and secondary antimicrobial resistance mechanisms

It is well established that antibiotics select for resistant bacteria that grow in the presence of otherwise lethal drug concentrations. The mechanisms by which this phenomenon occurs include primary target modification, drug inactivation and drug transport alteration. More recently, the role of metabolism in AMR has emerged, revealing that antibiotics can directly select for metabolic determinants of resistance while also driving metabolic effects that complement and amplify canonical resistance mechanisms (Fig. 6).

Mutating and/or altering core metabolic gene expression levels can result in AMR in vitro[30,32,36,150,151]. In some cases, the mechanism by which resistance occurs is clear, as impacted metabolic genes are directly related to a specific drug type. For example, as trimethoprim and sulfamethoxazole (TMP–SMX) collectively inhibit the synthesis of folate, a key precursor to nucleotide production, resistance to this common drug combination directly affects the metabolism of the strain[152]. This resistance can arise in multiple ways. For example, in *S. aureus, Streptococcus pneumoniae* and *Haemophilus influenzae*, mutation of the *dhfr* gene that encodes for dihydrofolate reductase can lead to trimethoprim resistance[153]. Alarmingly, the most prevalent *dhfr* variants allow growth in drug concentrations 1,000-fold greater than those needed to inhibit susceptible isolates. TMP–SMX resistance can also occur by overproducing either enzymatic drug targets of dihydrofolate reductase (DHFR) or dihydropteroic acid synthase (DHPS); this ‘metabolic bypass’ has been shown to occur through DNA promoter mutations and is thought to overwhelm the ability of TMP–SFX to inhibit folate production[154].

Whereas the primary effects of bacterial metabolism directly interfere with drug–target interactions, its secondary effects can also protect against antibiotic treatment, albeit indirectly. As the effects of diverse antibiotics often converge on common downstream metabolic pathways, some of these mechanisms can defend against multiple drug classes. For example, genes relevant to central carbon and energy metabolism were implicated in

E. coli resistance against three bactericidal antibiotics[32]. Indeed, overexpression of these genes increased drug resistance and the mutations themselves were highly prevalent in pathogenic genomes, highlighting their clinical relevance[32]. Other drug-agnostic approaches have resulted in similar conclusions. For example, highly accurate resistance predictions from *Salmonella* genomes identified the potential role of several previously unappreciated metabolic genes in resistance, including those involved in oxidative stress and electron transport[155]. Similarly, metabolic resistance that was specific to glucose utilization conferred resistance against both carbenicillin and ciprofloxacin[156].

In addition to metabolic-specific changes, canonical AMR mechanisms can also alter the metabolic state of the cell. In these cases, metabolic changes are assumed to be secondary consequences of canonical AMR. However, the direct role of concurrent metabolic changes in AMR phenotypes is largely uncharacterized; thus, their contribution to AMR is still unknown. For example, mutations in regulatory elements such as in CbrAB and envZ–ompR reduce antibiotic uptake by minimizing cell membrane porin expression, leading to reduced efficacy of polymyxin B, ciprofloxacin, tobramycin, cephalosporin and β-lactam antibiotics[100,157]. Resistance obtained through altered porin expression levels also alters metabolism, as porins transport amino acids, sugars and other essential ions[100]. Yet, to our knowledge, the effect of drug import separate from nutrient dynamics has never been tested. Additionally, specific resistance mechanisms have shown unexpected metabolic tradeoffs. For example, mutations in the RNA polymerase β subunit gene *rpoB* confer rifampicin resistance and promote growth on β-glucosides[158]. Overall, the full scope of the role metabolism of in resistance, including its potential as a direct modulator, remains conflated with other AMR mechanisms.

Although some intriguing metabolic mechanisms of AMR have been identified, these relationships are full of unanswered questions; metabolic networks are notoriously complex, redundant and compensatory. As such, simplifying rules of thumb — for example, the current literature consensus that ‘low respiration and low metabolic activity protect against antibiotic treatment’ — are likely to be insufficient to explain mechanisms by which these changes impart resistance. Given the numerous upstream and downstream implications of lowered metabolism, it is unlikely that such a generalization fully accounts for all bacterial responses during antibiotic treatment. For example, in populations of *E. coli*, *P. aeruginosa*, *Burkholderia cenocepacia* and *S. aureus*, faster growing bacteria were protected from macrolide antibiotic treatment, contrasting the aforementioned current consensus[159]. Of interest is that this protection occurred without genetic mutations[159]. Certainly, these apparent complexities reflect limitations in both fundamental understanding and current analytical methods. For example, current conventional microbiology techniques, such as fluctuation analyses, are unable to capture antibiotic-induced mutagenesis, the evolution of antibiotic-resistance mutations and cell-level and population-level behaviours in tandem. This is because such methods operate as end point assays and have indirect read-outs[117]. These shortcomings demonstrate that much methodological work remains to be done in the decades to come.

Clinical consequences in the mammalian host

Antibiotic efficacy and bacterial response to antibiotic treatment appear increasingly complex when considering the host context. We direct the reader to other articles describing in vivo pathologies in greater detail[160–175], but note that bacterial metabolism can vary significantly due to distinct metabolic host tissue environments[176–189]. For instance, abscesses are typically hypoxic and nutrient-limited; this may force bacteria to adopt anaerobic respiration or fermentation pathways, altering their susceptibility to antibiotics such as aminoglycosides that require oxygen-dependent uptake[190–192]. Similarly, in urinary tract infections caused by pathogens such as *E. coli*, the availability of urea and other nitrogen sources within urine of diverse pH can promote metabolic adaptations. These changes have potential consequences on the efficacy of antibiotics such as TMP–SMX that target folate synthesis pathways[193–198]. By contrast, lung infections in individuals with cystic fibrosis often feature mucus-rich environments that provide a unique carbon source (for example, mucin) for pathogens such as *P. aeruginosa*, enabling metabolic shifts that promote antibiotic tolerance[148,199–204]. These site-specific metabolic adaptations underscore the need to consider the metabolic state of both the pathogen and host when designing antibiotic therapies, as metabolic and spatial heterogeneity across infection sites can significantly alter drug effectiveness. Consistent with these findings, one study identified a wide diversity of metabolic gene mutations in over 3,500 clinical *E. coli* pathogens, underscoring the expansive metabolic landscape available for evolution[32]. Another recent study leveraging whole-genome CRISPR interference screens, transcriptomics and metabolomics revealed that pathways of respiration, ribosome biogenesis and nucleotide and amino acid metabolism represent druggable vulnerabilities that could be exploited in an isoniazid-resistant *M. tuberculosis* strain[205]. Determining which mutations are associated with specific environments and what benefits they confer remains an active area of investigation.

Beyond the metabolic characteristics of the local host environment, interactions with resident microbiota add another layer of complexity, as these microbial communities actively influence bacterial behaviour, antibiotic concentrations and local biochemistries. For example, the gut microbiota performs vital roles such as carbohydrate fermentation, vitamin synthesis, immune regulation and production of bioactive metabolites[206,207]. These microbial activities directly affect drug metabolism, altering antibiotic effectiveness and toxicity. Several antibiotics are known to be co-metabolized by gut microbiota, including metronidazole and chloramphenicol[208]. Importantly, the variability in the composition of the gut microbiota between individuals contributes to differing responses to identical antibiotic treatments, highlighting a major challenge for personalized medicine[208]. For example, changes in bile acid concentrations in the gut alter the efficacy of ciprofloxacin and meropenem against *E. coli*, and promote biofilm formation in *P. aeruginosa*[209]. Thus, unravelling in vivo metabolic factors influencing antibiotic treatment, response and subsequent evolution is key to devising therapeutics tailored to individual metabolic and microbial profiles.

Future outlooks

Metabolism is implicated in nearly every facet of the bacterial response to antibiotics. As such, it must be accounted for in the development of new antibiotics and therapeutic strategies. This new direction holds great potential, but is complicated by the numerous interactions between metabolism and antibiotic treatment. Although growing evidence indicates that there is a bidirectionality inherent between the two, our current understanding overwhelmingly focuses on one aspect of this relationship. Much is known about how antibiotics exploit cell metabolism to function; however, how metabolism impacts antibiotic efficacy and emergent evolutionary dynamics and mechanisms is not as thoroughly described.

A more thorough understanding of these relationships in the context of their larger interaction networks and environmental contexts will help uncover new strategies to combat AMR and/or maximize antibiotic utility. In the case of biofilms, a greater understanding of the role of metabolism has provided new therapeutic outlooks. For example, studies indicate that nutrient limitation in biofilms increases tolerance to antibiotics through either the SOS or stringent responses[120,210]. These studies suggest that promoting metabolic activity via nutrient supplementation may limit both tolerance and resistance, as has already been seen in murine models[63]. Exploiting metabolic states also shows promise in high-density environments that are historically difficult to treat. For example, density-dependent tolerance to ciprofloxacin could be overcome by stimulating respiratory activity with glucose for *E. coli, S. aureus* and *Mycobacterium smegmatis* pathogens[10].

Adopting new strategies to upregulate specific metabolic pathways is another promising way to counter both antibiotic tolerance and AMR. For example, leveraging metabolites such as amino acids, the TCA cycle intermediates and nucleotides can restore antibiotic lethality[36,63,81,211–218]. Other agents may also be designed to mimic antibiotic activity and upregulate bacterial metabolism. For example, diverse nanoparticle structures are being explored for their ability to generate reactive oxygen species and oxidative stress[219].

Separate from supplementing antibiotics with adjuvants, independently exploiting metabolism may also serve as an antimicrobial strategy. For example, synthetically engineered iron-chelating agents have been used to lower iron bioavailability, leading to growth inhibition[220–222]. Initial results using such innovatively designed compounds have yielded moderate to promising antimicrobial activity against organisms such as *S. aureus* and *Acinetobacter baumanii*[223–226]. Other strategies leveraging iron metabolism include using competing metals such as gallium to limit iron uptake[227,228]. Gallium compounds have demonstrated effectiveness against multidrug resistant and opportunistic pathogens relevant to cystic fibrosis, pneumonia, chronic obstructive pulmonary disease and general wound healing. Interestingly, these findings have been observed in numerous settings, including in vitro models, in vivo murine models, clinical isolates and human clinical trials[229–239]. Although concerns regarding the toxic effects of such agents remain, several of these and similar compounds are under clinical development[240].

The utility of direct metabolite interventions is likely to be context-dependent, as it is becoming increasingly clear that AMR emergence and its reversal depends not only on the current metabolic state of a population, but also on the historical conditions under which that emergence occurred. For example, in previously evolved antibiotic-resistant strains, sub-inhibitory bactericidal antibiotic concentrations selected for enhanced metabolic efficiency[156]. However, AMR was only observed in environments either identical or closely related to the original evolution[156]. Further, resistant cells could be re-sensitized to antibiotics. This occurred when nutrient sources were changed to restore original cell metabolic efficiency levels[156]. In this way, different metabolites that enable the treatment of AMR strains may require a high degree of strain specificity to be effective. In the long term, these theoretical advances suggest that environmental and phylogenetic features will probably inform the breadth and efficacy of potential metabolic strategies in real-world settings.

In addition to strain specificity, leveraging the host metabolism presents a largely untapped opportunity to personalize antibiotic therapeutics, potentially improving treatment outcomes with greater precision. Current in vitro antibiotic susceptibility testing often fails to fully align with in vivo clinical effectiveness[241–243], highlighting that a one-size-fits-all model for treating infectious diseases overlooks critical host–pathogen, pharmacokinetic and pharmacodynamic factors. Metabolic-specific factors, arising from distinct infection sites and person-to-person variability, likely contribute further to these discrepancies. However, these factors remain underexplored in clinical practice. Additionally, specific microbiome environments can modulate both antibiotic activity and pathogen behaviour. Indeed, studies have highlighted that the gut microbiome could serve as a reservoir for identifying new pathogen-specific therapeutics tailored to unique metabolic contexts. For instance, microbiome-derived agents such as lantibiotics, bacteriocins and microcins show promise as precision antimicrobials[244,245]. Bacteriocins, in particular, have demonstrated success in preclinical models; they have treated *P. aeruginosa* lung infections in mice[207,246] and mastitis in both bovines and lactating women[207,247,248]. Bridging the gap between basic research and personalized medicine requires explicit studies linking host-mediated and microbiome-mediated metabolic effects to observed clinical discrepancies.

The intricate role of metabolism in bacterial responses to antibiotics remains incompletely understood, partly due to a lack of optimized tools that can disentangle metabolic effects from those that are growth-specific or drug-specific. As such, methods that isolate metabolic effects must be designed. For example, the role of metabolism in canonical efflux AMR mechanisms should be extracted from that of metabolite-specific and drug-specific transporters. Additionally, future technologies, including simulations and informatic approaches, will be of great utility in revealing the growth-independent effects of metabolism. These approaches are often more tenable than physical quantification methods and include flux-based analysis and dynamic whole-genome modelling, along with coarse-grained approaches[249]. Critically, ecological-evolutionary models should also be utilized to explore all possible evolutionary consequences of new antimicrobial strategies. These models will likely heavily rely on whole-genome sequencing, machine learning, mass spectrometry and predictive modelling, enabling new understandings for complex microbiology communities[250].

Countering AMR is inherently complex, requiring solutions tailored to specific pathogens, environments and clinical contexts. Many of the current knowledge gaps lie in our limited ability to delineate the widespread impact of metabolism across host, pathogen and drug interfaces. However, incorporating metabolism into the AMR framework provides a great deal of promise. Unique patient biochemistries, infection sites, pathogen types and even local environmental characteristics are likely to all impose distinct metabolic constraints in vivo. Developing an integrated metabolic framework could conceivably facilitate personalized approaches to infectious disease treatment. Ultimately, these relationships will be crucial to advance ongoing and future fundamental research, design imminently needed therapeutics and better empower scientists, engineers and physicians to combat AMR.

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Box 1 |

Differentiating between resistance, tolerance and persistence

An overview of the unique phenotypes of resistance, tolerance and persistence is provided. In each case, the general accepted definition is provided, along with the method of quantification and molecular mechanisms[42].

Resistance

- Definition: The ability of bacterial populations to grow at high concentrations of an antibiotic, regardless of treatment duration
- Resistance may be extrinsic (for example, acquired genetically) or intrinsic (for example, due to inherent bacterial abilities)
- Quantification: Measured by changes in the minimum inhibitory concentration (MIC), which is the minimum antibiotic concentration necessary to prevent net culture growth
- Molecular mechanisms
  - Inherited genetic changes: drug target mutations, enzymatic inactivation, altered transport dynamics, altered metabolic state
  - Increased copy numbers of genes or plasmids
  - Induced or constitutive expression

Tolerance

- Definition: The ability of bacterial populations to survive exposure to bactericidal antibiotic concentrations that would otherwise be lethal (at concentrations far exceeding the MIC)
- Often occurs in settings characterized by generally lowered metabolic states
- Quantification: Measured by quantifying the minimum duration for killing, the typical duration of antibiotic treatment that kills a proportion of the bacterial population
- Molecular mechanisms
  - Inherited abilities (mutations)
  - Un-inherited environmental stressors: nutrient limitations, antibiotic exposure, location within a biofilm, lag-phase specific metabolic adaptation

Persistence

- Definition: The ability of a subpopulation within a clonal culture to survive antibiotic exposure, despite drug treatment otherwise killing a majority of said clonal population
- Also attributed to lowered metabolic states (for example, biofilms)

- Quantification: Measured by a bimodal (or multimodal) time–kill curve, which quantifies a change in bacterial population over time with the addition of an antibiotic
- Molecular mechanisms
  - Toxin–antitoxin system induction
  - Stress response activation
  - Transient efflux pumps overexpression
  - Transient changes in enzymatic expression
  - Inherent cell stochasticity and heterogeneity

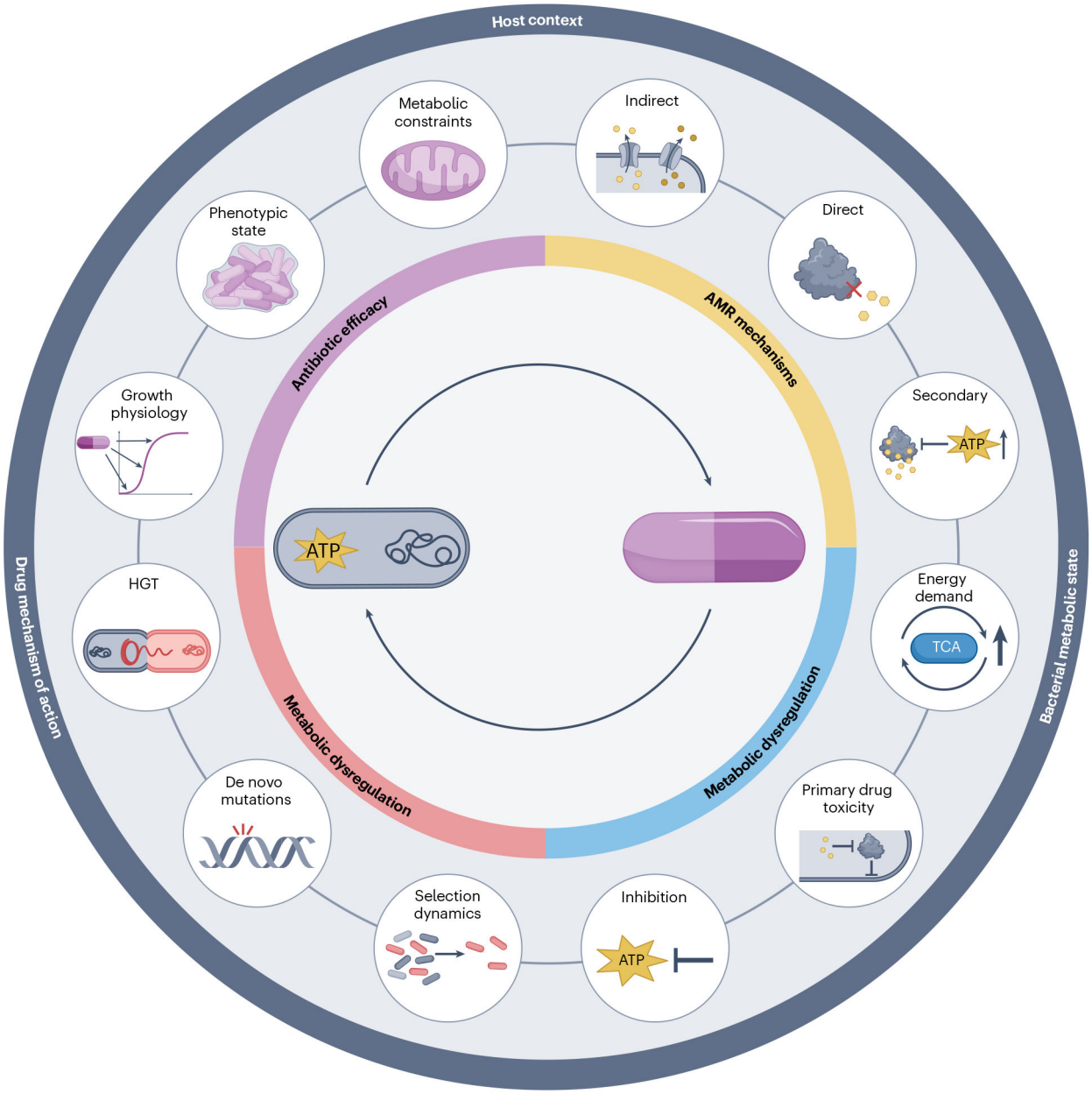

Fig. 1 |. Interactions between antibiotics and metabolism.

The bacterial metabolic state, host context and the drug mechanism of action collectively impact the bacterial response to antibiotics. In turn, antibiotics can directly alter the metabolic state of a bacterium, further shaping this interaction. This bidirectional relationship can be understood through four main aspects: antibiotic efficacy, antimicrobial resistance (AMR) mechanisms, AMR evolution and metabolic dysregulation. Modulation of antibiotic efficacy may manifest in bacteria that adopt distinct metabolic growth physiologies or phenotypically metabolically dormant states (for example, biofilms). Antibiotics may also demonstrate different efficacies depending on the metabolic environment of the pathogen or host. AMR mechanisms impact metabolism via primary (for example, metabolic bypass), secondary (for example, altered nutrient preferences with

target mutations), or indirect (for example, altered metabolism gene expression levels) routes. Metabolic dysregulation may occur by altering ATP levels, the tricarboxylic acid cycle (TCA) or other metabolic cell processes (for example, cell wall biosynthesis). AMR evolution may be observed via altered selection dynamics, processes of horizontal gene transfer (HGT), or de novo mutations.

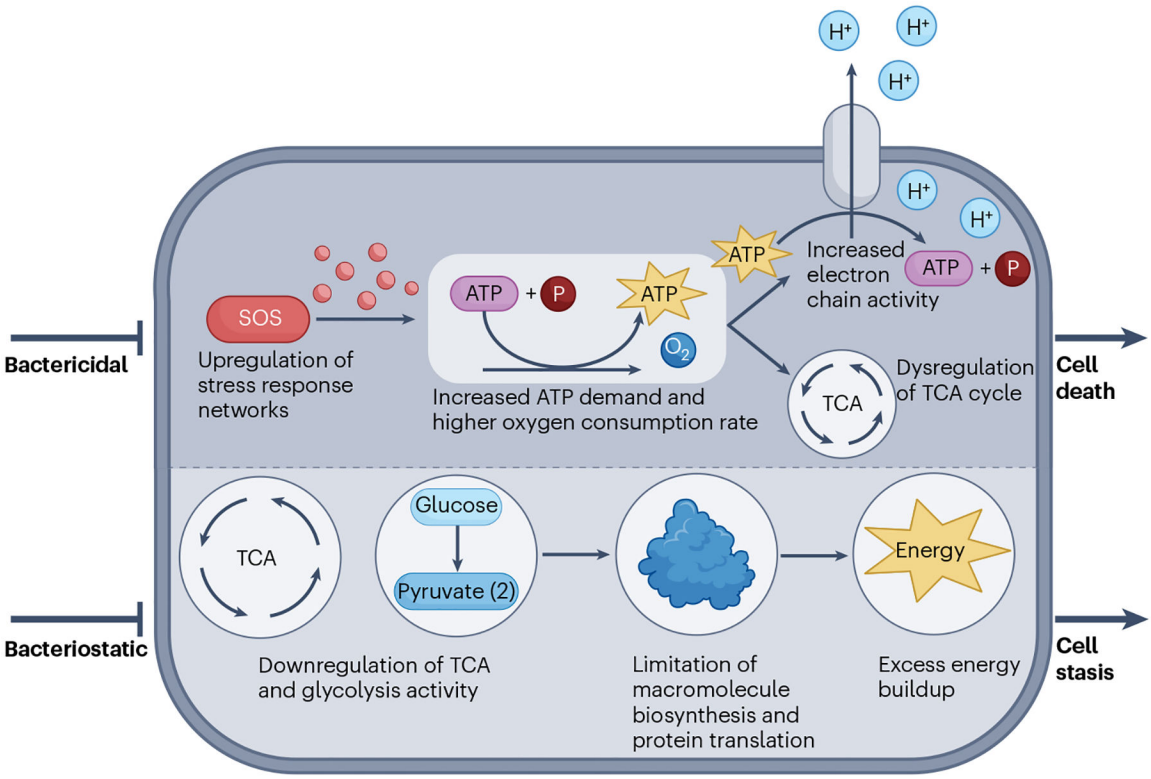

Fig. 2 |. Bactericidal and bacteriostatic antibiotics leverage metabolism for their function.
Bactericidal antibiotics (upper cell panel) induce cell death through various mechanisms that, following initial target inhibition (not shown), primarily stimulate cell metabolism, including upregulating stress response networks and electron chain and tricarboxylic acid cycle (TCA) cycle activity. Bacteriostatic antibiotics (lower cell panel) inhibit cell growth. Classically, following initial target inhibition (not shown), growth inhibition occurs via the downregulation of key cell processes, including the glycolysis pathway and the TCA cycle. Macromolecular biosynthesis and protein translation processes may also be limited, resulting in a build-up of energy within the cell and ultimately cell stasis.

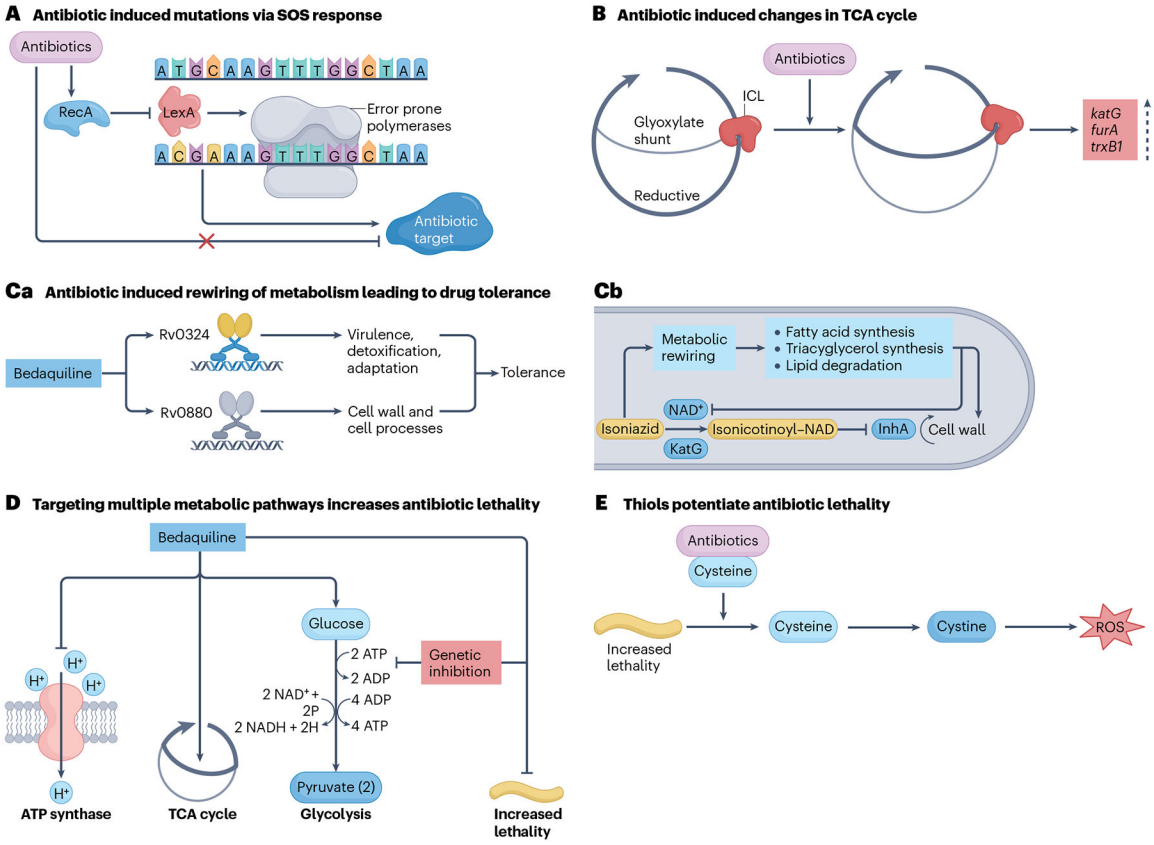

Fig. 3 |. The unique impact of metabolism on antibiotic resistance, tolerance and lethality in *Mycobacterium tuberculosis*.

A, Bactericidal antibiotics can induce the SOS response pathway. Antibiotics activate the RecA protein. Activated RecA represses LexA, which allows multiple genes in the SOS pathway to be expressed, including error-prone polymerases. This can result in mutations, including those to the antibiotic target, which confer resistance. **B**, Antibiotics can alter tricarboxylic acid cycle (TCA) cycle activity. During antibiotic treatment, isocitrate lyase (ICL) activates the glyoxylate shunt, reducing activity through the reductive steps of the TCA. This coincides with an overall reduction in metabolism and the expression of genes (for example, *katG, furA, trxB1*) involved in detoxifying reactive oxygen species (ROS). Together, this confers tolerance. **C**, Antibiotic-specific mechanisms that promote *M. tuberculosis* resistance and tolerance. Treatment of *M. tuberculosis* with the antibiotic bedaquiline activates two transcription factors, Rv0324 and Rv0880, increasing the expression of genes involved in virulence, detoxification, adaptation and cell wall processes, resulting in tolerance (part **Ca**). Treatment of *M. tuberculosis* with the antibiotic isoniazid may either promote metabolic rewiring, induce tolerance or successfully kill susceptible cells (part **Cb**). Isoniazid treatment, combined with intracellular KatG and NAD^+, inhibits the enzyme isonicotinoyl–NAD (InhA), which is required for mycolic acid biosynthesis. Inhibition of InhA prevents cell wall synthesis in *M. tuberculosis*, resulting in cell death. **D**, Targeting multiple metabolic pathways can increase antibiotic lethality. Bedaquiline represses the activity of ATP synthase and the TCA cycle by increasing activity through the glyoxylate shunt. This forces *M. tuberculosis* to use glycolysis and

substrate-level phosphorylation for ATP production. However, inhibiting substrate-level phosphorylation can increase bedaquiline lethality. **E**, Supplementing antibiotics with thiols, including cysteine, potentiates antibiotic lethality. When *M. tuberculosis* is treated with cysteine, it enters the cell and is converted to cystine, generating reactive oxygen species. Thus, when cysteine is provided in conjunction with an antibiotic, the increased ROS potentiate antibiotic lethality.

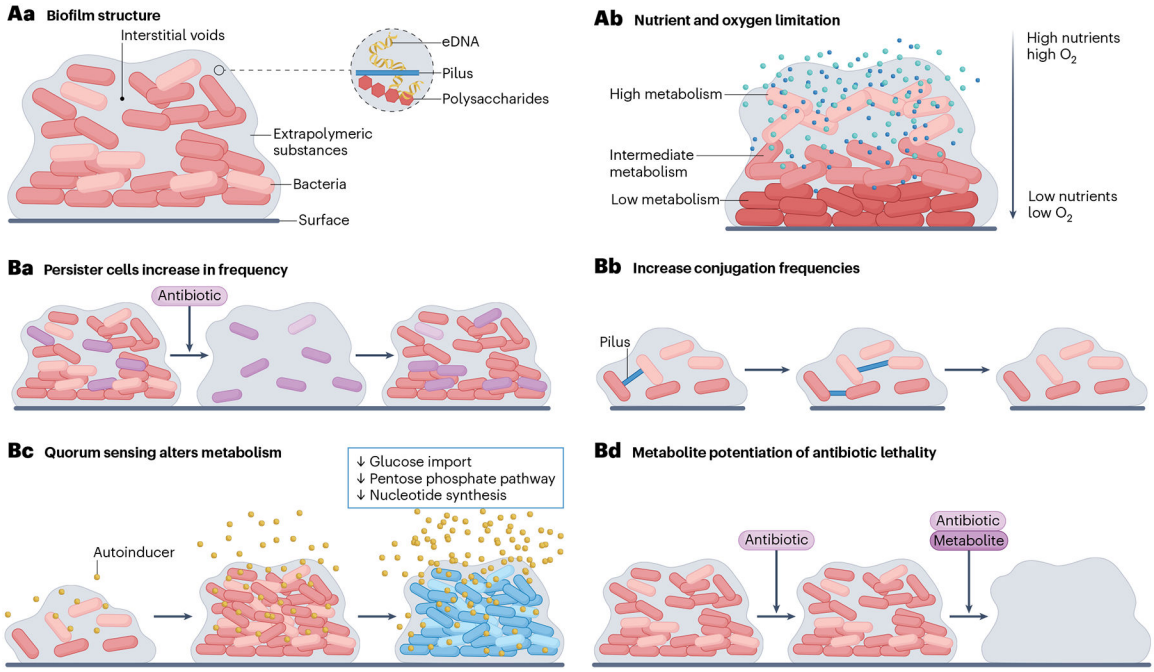

Fig. 4 |. Unique properties of biofilms confer antibiotic tolerance via altered metabolism.

A, Biochemical characteristics of biofilms relevant to antibiotic treatment. Biofilms are communities of bacteria affixed to a surface surrounded by a layer of extrapolymeric substances (EPS). EPS is primarily comprised of extracellular DNA (eDNA), pili and polysaccharides. Although interstitial voids carrying nutrients and oxygen into the inner layers of the biofilm can form, overall nutrient and oxygen transport is limited by EPS (part **Aa**). Bacteria closer to the surface of the biofilm experience more nutrients and oxygen, whereas those towards the base have limited access to both, and consequently, exhibit increased tolerance to antibiotics (part **Ab**). **B**, Antibiotic effects on biofilm communities. The presence of metabolically dormant persister cells is increased in biofilms following antibiotic treatment (part **Ba**; purple); these cells are more tolerant to antibiotics. The frequency and efficiency of conjugation is increased between biofilm members, allowing for genes that confer resistance to quickly increase in abundance (part **Bb**). Quorum sensing is enhanced in biofilms (part **Bc**). This can result in a rewiring of metabolic pathways, including glucose import, the pentose phosphate pathway and nucleotide synthesis. These changes may increase antibiotic tolerance. Increasing metabolism in biofilms using a metabolite can increase antibiotic lethality (part **Bd**).

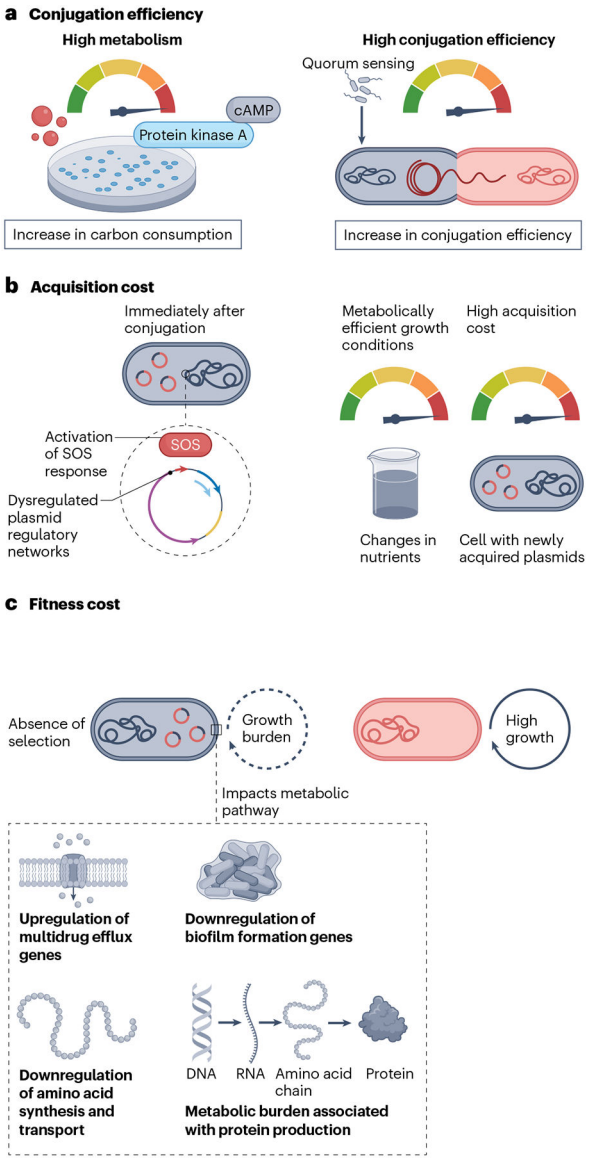

Fig. 5 |. Metabolism promotes antimicrobial resistance evolution via its effect on conjugation. **a**, Manipulating the metabolic state of a cell modulates conjugation efficiency. Conjugation is a mechanism of horizontal gene transfer by which bacteria transfer their circular components of DNA (that is, plasmids) from one cell to another through direct contact. Conjugation efficiencies (that is, the kinetic rate of plasmid transfer) can be promoted by activating metabolism (for example, increasing carbon consumption). Increased conjugation efficiency increases plasmid spread. High conjugation efficiency may also be sustained by quorum sensing, which regulates cell metabolism to encourage bacteria to adopt a cooperative state. Often, this cooperative state is characterized by factors that increase bacterial virulence. **b**, Transient acquisition costs depend on the metabolic efficiency of the cell. Immediately after conjugation, newly acquired plasmids impose a short-term disruption to the cell by activating the SOS response and dysregulating plasmid regulatory networks. This intracellular perturbation results in a growth defect on the brand-new plasmid-carrying

host strain, referred to as an acquisition cost. Manipulation of the metabolic state of the cell via changing the nutrient composition (beaker) can modulate acquisition costs. **c**, The long-term implications of metabolism on the competitiveness of conjugative plasmids. Compared with a plasmid-free host (right cell), hosts carrying a plasmid (left cell) may also experience a long-term growth defect or burden (that is, plasmid fitness cost), especially in the absence of antibiotic selection. The fitness cost impacts diverse pathways implicated in metabolism.

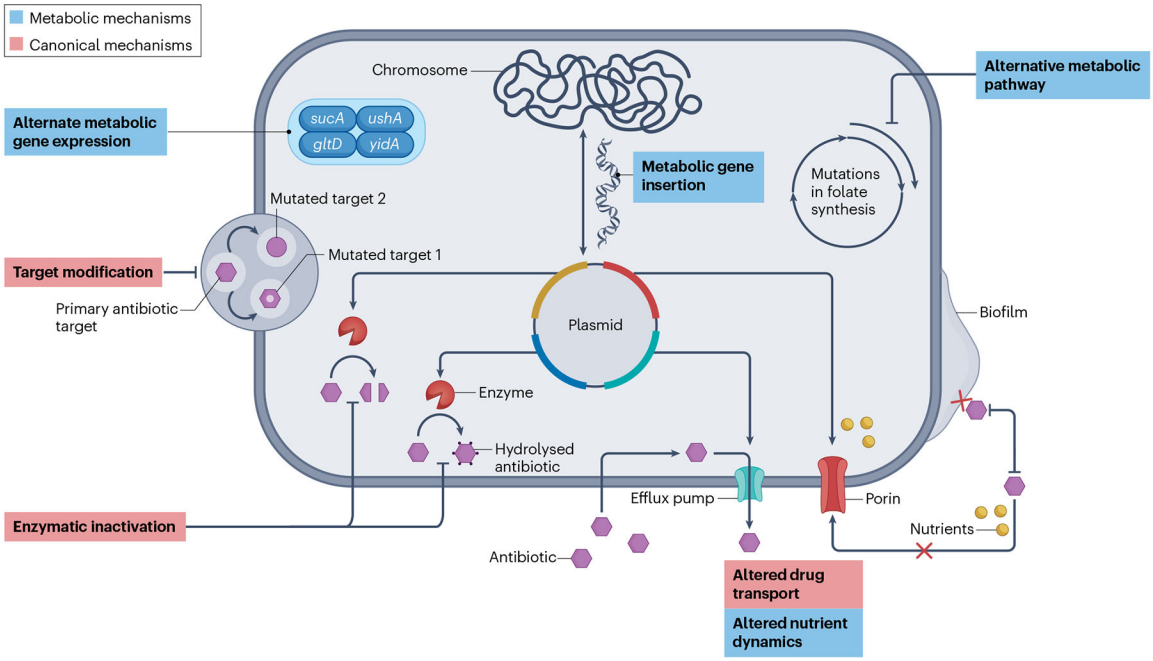

Fig. 6 |. Classic and metabolic mechanisms of antimicrobial resistance.

A bacterial cell exhibiting antimicrobial resistance (AMR) will have a chromosome and, in some cases, one or more circular plasmids that encode for antibiotic resistance genes and/or mutations. The most common canonical mechanisms of antimicrobial resistance (red) include target modification, enzymatic inactivation and reduced antibiotic intake. These may also have indirect metabolic effects (for example, porins that alter nutrient uptake). Direct mechanisms (blue) by which metabolism promotes AMR include differential metabolic gene expression, activation of alternative metabolic pathways and insertion of metabolic genes. Other cellular characteristics which may also potentially impact AMR through indirect metabolic effects include the presence of biofilms and porins. Biofilms may structurally protect the cell from antibiotic treatment and often demonstrate unique and heterogenic metabolic states. Porins may also modulate both antibiotic resistance levels and cell metabolism. Minimizing cell membrane porin expression reduces both nutrient and antibiotic uptake.