Engineering Microbiology 2 (2022) 100021

Contents lists available at ScienceDirect

Engineering Microbiology

journal homepage: www.elsevier.com/locate/engmic

Review

Genome-scale metabolic modeling in antimicrobial pharmacology☆

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ARTICLE INFO

Keywords:
Genome-scale metabolic model
Flux balance analysis
Antimicrobial pharmacology
Antimicrobial resistance
Toxicity
Pharmacokinetic/pharmacodynamic model

ABSTRACT

The increasing antimicrobial resistance has seriously threatened human health worldwide over the last three decades. This severe medical crisis and the dwindling antibiotic discovery pipeline require the development of novel antimicrobial treatments to combat life-threatening infections caused by multidrug-resistant microbial pathogens. However, the detailed mechanisms of action, resistance, and toxicity of many antimicrobials remain uncertain, significantly hampering the development of novel antimicrobials. Genome-scale metabolic model (GSMM) has been increasingly employed to investigate microbial metabolism. In this review, we discuss the latest progress of GSMM in antimicrobial pharmacology, particularly in elucidating the complex interplays of multiple metabolic pathways involved in antimicrobial activity, resistance, and toxicity. We also highlight the emerging areas of GSMM applications in modeling non-metabolic cellular activities (e.g., gene expression), identification of potential drug targets, and integration with machine learning and pharmacokinetic/pharmacodynamic modeling. Overall, GSMM has significant potential in elucidating the critical role of metabolic changes in antimicrobial pharmacology, providing mechanistic insights that will guide the optimization of dosing regimens for the treatment of antimicrobial-resistant infections.

1. Introduction

The rapid emergence of multidrug-resistant (MDR) microbial pathogens represents a critical challenge to human health globally and has imposed significant economic and clinical burdens [54]. Considering the substantial challenges in the discovery of new classes of antimicrobials, the development of novel antimicrobial treatments to combat MDR bacterial pathogens is critical [35]. However, the exact mechanisms of activity and resistance of many antimicrobial agents remain uncertain and appear far more complex than the conventional 'one drug, one target, one mechanism' paradigm. These knowledge gaps have significantly hindered antimicrobial development.

Aided by the rapid development of systems biology, multi-omics approaches have been widely applied to elucidate the complicated mechanisms of antimicrobial activity, resistance, and toxicity. Notably, numerous studies have demonstrated that cellular metabolic changes may play critical roles in antimicrobial killing, resistance development, and host side effects. For example, beta-lactams may impair purine metabolism in *Escherichia coli* and reinforce a futile cycle of cell wall biosynthesis and degradation, adding to their already well characterized inhibition of cell wall biosynthesis and thereby contributing to beta-lactam lethality [20]. Also in *E. coli*, impairment of cellular respiration may contribute to the activity of bacteriostatic translation inhibitors such as tetracycline, spectinomycin, erythromycin, and chloramphenicol [50]. Exogenous feeding with alanine and/or glucose restores kanamycin susceptibility to resistant strains of *Edwardsiella tarda* by promoting metabolic flux through the tricarboxylic acid (TCA) cycle, increasing proton motive force and thereby enhancing antimicrobial uptake [66]. *Mycobacterium tuberculosis* may divert trehalose and maltose from biosynthesis of cell wall components toward synthesis of central carbon metabolism intermediates, thus maintaining intracellular levels of ATP and antioxidants [43]. Interestingly, this metabolic shift was present in clinical isolates of MDR *M. tuberculosis*, suggesting that it has an essential role in the development of resistance. Human lung and kidney cells are known to undergo severe metabolic changes and oxidative stress following polymyxin treatment, indicating that antimicrobial-induced metabolic alterations contribute to host side effects [4,5]. While effects on cellular metabolism are now known to be critical in antimicrobial pharmacology, this has long been a neglected area of study. Accurate assessments of these effects will be important for the development of novel and effective antimicrobial treatments.

First developed ~30 years ago, genome-scale metabolic model (GSMM) together with flux balance analysis (FBA) approaches have been widely employed to study microbial evolution, metabolic engineering, physiology, host–pathogen interactions, and antimicrobial pharmacology [27,57,79]. A GSMM is a comprehensive representation of a metabolic network that incorporates most enzymatic biochemical reactions occurring in a cell, with these reactions interconnected via partici-

☆ Given his role as editorial board member, Dr. Jian Li, had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Dr. Shengying Li.

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https://doi.org/10.1016/j.engmic.2022.100021
Received 6 January 2022; Received in revised form 12 April 2022; Accepted 13 April 2022
Available online 23 April 2022

Table 1
Representative genome-scale metabolic models applied in antimicrobial pharmacological studies.

Organism	Model ID	Brief summary	Refs.
Escherichia coli K12 substr. MG1655	iML1515	Understanding metabolic adaptations related to antimicrobial resistance	[65]
Klebsiella pneumoniae MGH 7857	iYL1228	Identifying novel drug targets	[16]
Acinetobacter baumannii AYE	AbyMBEL891	Identifying novel drug targets	[39]
Acinetobacter baumannii ATCC 19606	iATCC19606	Elucidating mechanism of metabolic changes to colistin treatment	[90]
Acinetobacter baumannii ATCC 19606	iLP844	Elucidating mechanism of metabolic changes to colistin treatment	[67]
Pseudomonas aeruginosa PAO1	iPAO1	Elucidating mechanism of metabolic changes to polymyxin B treatment	[89]
Pseudomonas aeruginosa UCBPP-PA14	iPau1129	Understanding metabolic adjustments related to antimicrobial resistance	[25]
Chromobacterium violaceum ATCC 12472	iDB858	Understanding metabolic adjustments related to antimicrobial resistance	[7]
Chromobacterium violaceum ATCC 12472	iDB149	Identification of strategies to restore antibiotic susceptibility	[6]
Mycobacterium tuberculosis H37Rv	iNJ661	Identifying novel drug targets	[32]
Rattus norvegicus	iRno	Understanding metabolism related to antimicrobial toxicity	[12]
Homo sapiens	iHsa		
Plasmodium falciparum	iPfal17	Identifying novel drug targets	[15]
Plasmodium falciparum		Dissecting the mechanism of action of chloroquine	[77]

pating metabolites [59]. With appropriate constraints such as substrate utilization and metabolic activity, a GSMM can be used to calculate the reaction flux distribution, allowing quantification of metabolic shifts in a pathogen or host cell following antimicrobial treatments [21,56].

This review discusses the current application of GSMM in antimicrobial pharmacology, with particular emphasis given to the integration of GSMM with multi-omics and machine learning to elucidate the mechanisms of antimicrobial activity, resistance, and toxicity. The insights presented here provide useful information for the development of novel and safer antimicrobial treatments to combat MDR microbial pathogens.

2. Genome-scale metabolic modeling and flux balance analysis

Since the first GSMM for *E. coli* was published in 1993, over 6000 models have been constructed for a broad range of organisms, including prokaryotes, eukaryotes, and archaea [28,40]. Many of these models have been applied in antimicrobial pharmacological studies (**Table 1**). Previously, construction of a GSMM required extensive searching of the literature and biochemical databases, manual curation, and iterative checking of genome annotation [64]. However, recent developments in automatic reconstruction procedures have substantially shortened this tedious process to a few minutes to hours [53,92]. In GSMM, a typical biochemical reaction is linked with the associated enzyme(s) and encoding genes (Fig. 1). The number of genes, metabolites, and reactions in a GSMM may range from hundreds to thousands depending on the scale of study and the complexity of the metabolic network of an organism (**Table 1**). A metabolite may also have multiple isoforms in different organelles; this is particularly important for eukaryotic cells which have many more specialized organelles than prokaryotic cells [50].

With GSMM, flux balance analysis (FBA) is used to calculate metabolic fluxes. FBA assumes a pseudo steady state where the overall production of each intracellular metabolite is balanced with the overall consumption (Fig. 2**A**). Therefore, intracellular metabolite levels are considered invariant. The metabolic flux distribution $\mathbf{v}$ can be calculated by solving a set of linear equations (Eq. (1)) [61]:

$$\mathbf{S}\cdot\mathbf{v}=0,\ a_i\le v_i\le b_i,\ i=1,2,\cdots,n \tag{1}$$

where the number of metabolites m (number of rows in stoichiometric matrix $\mathbf{S}$) is smaller than the number of reactions n (number of columns in stoichiometric matrix $\mathbf{S}$); v_i is the flux through reaction i; and a_i and b_i represent the lower and upper bounds, respectively, of reaction i. Given an objective function such as maximizing biomass accumulation (v_{biomass}), the metabolic fluxes can be calculated by solving a linear programming problem (Eq. (2)), Fig. 2**B**) [61]:

$$\begin{aligned}&\text{Max } v_{\text{biomass}}=\mathbf{c}^{\mathrm{T}}\mathbf{v}\\ &s.t.\ \mathbf{S}\cdot\mathbf{v}=0,\ a_i\le v_i\le b_i,\ i=1,2,\cdots,n\end{aligned} \tag{2}$$

The optimal solution is $\mathbf{v}^*$. Flux variability analysis can be implemented by calculating the maximum and minimum bounds of each reaction flux at $\mathbf{v}^*$, or by characterizing the entire metabolic solution space using hit-and-run Monte Carlo sampling [45]. Moreover, the gene essentiality can be assessed by calculating $\mathbf{v}^*$ after manually shutting down all the reactions associated with the specific gene. This method is often used to predict potential drug targets in a cell [18].

In Eq. (2), most of the fluxes are only constrained by reaction reversibility, and the optimal solution varies within a vast range. Multi-omics data can be integrated to constrain the flux variability and enable accurate predictions of metabolic fluxes under specific conditions [69]. Many methods have been developed for integration including MADE (Metabolic Adjustment by Differential Expression) [34], REMI (Relative Expression and Metabolomic Integrations) [63], INIT (Integrative Network Inference for Tissues) [2], RIPTiDe (Reaction Inclusion by Parsimony and Transcript Distribution) [33], and ETFL (Expression and Thermodynamics FLux) [74].

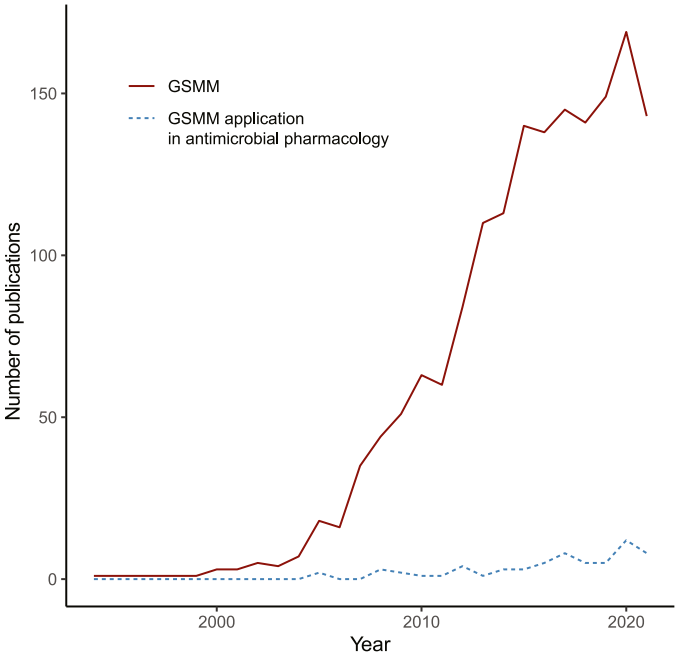

Fig. 1. The number of publications describing genome-scale metabolic model (GSMM) and emerging applications of GSMM in antimicrobial pharmacology have rapidly increased. The data shown are based on PubMed searches with the keywords “genome-scale metabolic model” OR “constraint-based model” OR “flux balance analysis” for GSMM, and (“genome-scale metabolic model” OR “constraint-based model” OR “flux balance analysis”) AND (“antibiotic” OR “antimicrobial”) for GSMM applications in antimicrobial pharmacology.

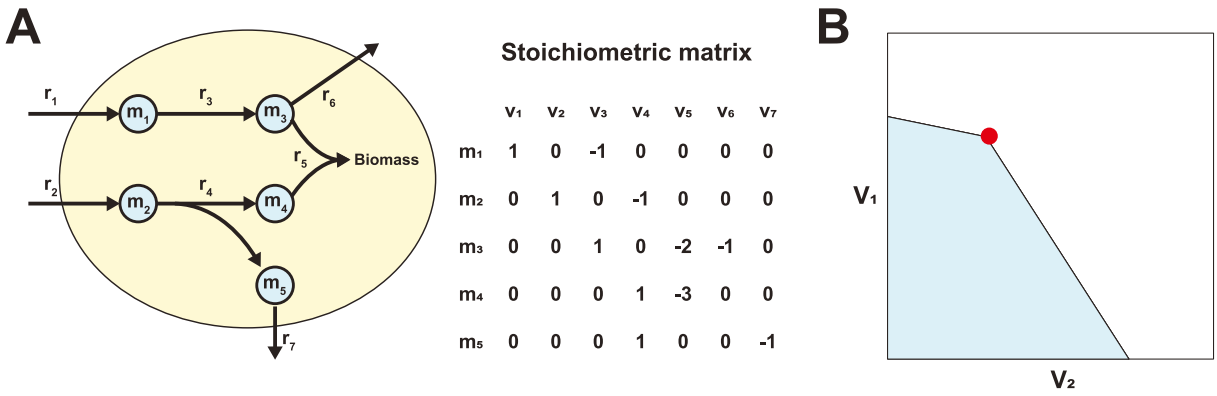

Fig. 2. A. Diagram of flux balance analysis showing the conversion of a simple metabolic network to a stoichiometric matrix. **B.** The optimal solution (red node) of biomass formation (V5) is shown in a metabolic solution space, with two variables of substrate uptake (V1 and V2).

Recently, the conventional GSMM (metabolic model, or M model) has been extended to a genome-scale model of gene expression and metabolism (ME model) by incorporating data related to protein translation, RNA transcription and processing, protein complexation and subcellular localization, and DNA replication [58]. The production of cellular components such as peptides, protein complexes, RNA molecules, and metabolites is constrained together by biomass dilution and consumption [58]. The ME model has been applied to investigate mechanisms of nutrient preference [85], overflow metabolism [48], and responses to oxidative stress [86].

With the rapid development of sequencing techniques, GSMM has also been expanded to model metabolism in multiple strains of the same species (e.g., *E. coli, Klebsiella pneumoniae, Salmonella enterica*, and *Staphylococcus aureus*) [9,47,60,78,82]. These models have been used to calculate strain-specific metabolic flux distributions for *in silico* growth, which are then used as a surrogate for metabolic phenotypes to determine metabolically unique phylogenetic clades [9,47,60,78,82]. Importantly, these models can also be used to study the association between metabolism and antimicrobial susceptibility [38]. Given sufficient constraints, GSMM can accurately predict cellular metabolic status. These models therefore serve as a common tool in biomedical studies (e.g., antimicrobial pharmacology), as discussed below.

3. Genome-scale metabolic modeling in antimicrobial pharmacology

3.1. Elucidation of the mechanisms of metabolic changes contributing to antimicrobial activity

The impact of antimicrobial treatments extend far beyond their effects on their initial targets, with numerous studies having demonstrated that metabolic alterations in microbial pathogens are closely related to antimicrobial efficacy [49,50,87]. Perturbations to intracellular metabolic homeostasis are critical aspects of antimicrobial lethality; such perturbations include impairment of energy production, hyperactivity of the electron transfer chain to produce excessive free radicals, and general metabolic stasis [51].

GSMM and FBA are employed to understand how metabolic changes contribute to antimicrobial activity (Fig. 2) [14,36,67,71,77,84,89]. Chloroquine is a first-line antimalarial agent against the unicellular protozoan parasite *Plasmodium falciparum*. This organism invades red blood cells and catabolizes hemoglobin, producing heme as a byproduct which is toxic to the parasite. While the parasite can detoxify heme by crystallization into inert hemozoin, chloroquine inhibits hemozoin crystal growth and leads to the build-up of heme, eventually resulting in death of the parasite [36]. Using transcriptomic data as constraints, metabolic modeling of *Pl. falciparum* following chloroquine treatment suggests that excessive heme may inhibit DNA synthesis via inhibition of redox metabolism [77]. Similarly, constrained by transcriptomic data, GSMM was used to delineate the metabolic changes of *M. tuberculosis* in response to anti-tuberculosis agents [71]. Of the 11 metabolically active drugs examined, TMC207 was predicted to have the greatest effect on metabolism owing to its inhibition of ATP synthase and consequent indirect effects on a broad range of metabolic pathways. Polymyxins (i.e., polymyxin B and colistin) are lipopeptide antibiotics increasingly used as a last resort to treat MDR Gram-negative infections [55]. They initially target lipopolysaccharides of the Gram-negative outer membrane, but also significantly perturb cellular metabolism [46]. Combining GSMM with transcriptomic constraints, significant metabolic changes were detected in *Acinetobacter baumannii* ATCC 19606 following treatment with 1 mg/L colistin for 1 h, including increased fluxes through gluconeogenesis, biosynthesis of amino acid and nucleotide, and pentose phosphate pathway; decreased fluxes through tricarboxylic acid (TCA) cycle and cell envelope biogenesis; and altered fluxes in the respiratory chain [67]. Different metabolic shifts were detected in *P. aeruginosa* PAO1 following a similar treatment with polymyxin B (1 mg/L for 1 h), which included upregulated amino acid catabolism, induction of the TCA cycle, and accelerated redox turnover.

Cellular metabolism may affect bacterial antimicrobial susceptibility [14]. In a recent large-scale pharmacodynamic study, Biolog phenotype microarrays were used to examine the antimicrobial susceptibility of *E. coli* grown on 206 unique nutrients (Fig. 3**A**) [84]. GSMM was used to calculate metabolic fluxomes under specific nutrient conditions, and the results were combined with antimicrobial IC_{50} data to train a machine leaning model. Enrichment analysis of the resulting regression coefficients indicated that metabolic reactions associated with purine biosynthesis, driven by antibiotic-induced adenine limitation, contributed to antibiotic lethality. This was further demonstrated by the enhanced antimicrobial susceptibility of mutants deficient in the early enzymatic steps of purine biosynthesis, and by the reduced antimicrobial killing by exogenous feeding of adenine. It has been suggested that a common effect of antibiotics is perturbation of energy metabolism, such as purine biosynthesis, particularly the biosynthesis of the ATP precursor adenine, an important bacterial defense mechanism against antimicrobial stress [84]. Therefore, GSMM is a powerful tool to convert conventional 'machine learning' to 'machine reasoning' and will significantly increase our understanding of metabolic changes associated with bacterial killing by antimicrobials [84].

3.2. Revealing the mechanisms of metabolic reprogramming in antimicrobial-resistant pathogens

Microbial pathogens acquire antimicrobial resistance via spontaneous mutations or horizontal gene transfer [38,87], and significant metabolic shifts often occur concomitantly with resistance acquisition [24]. A GSMM of *M. tuberculosis* H37Rv named iEK1011 was

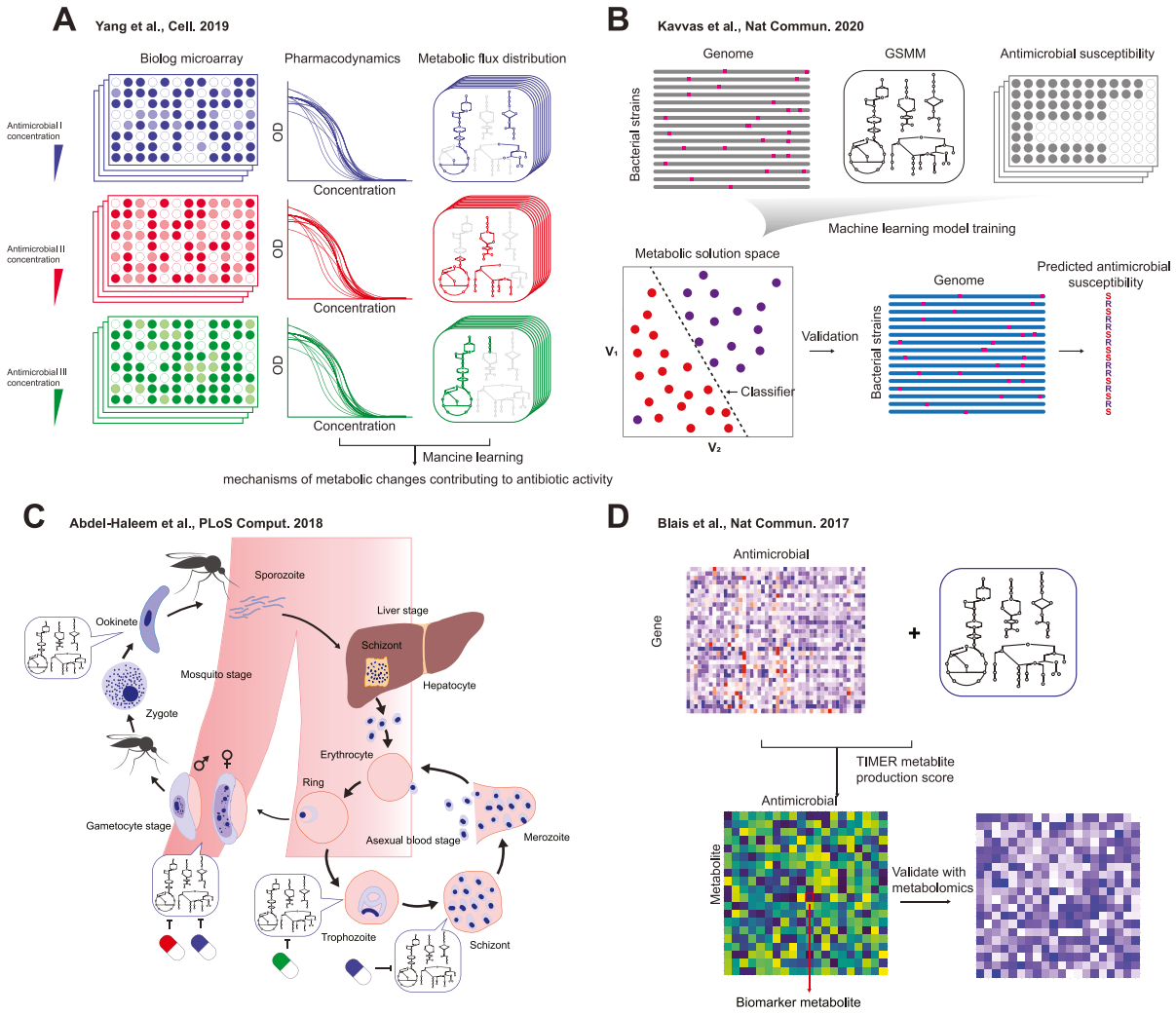

Fig. 3. Applications of genome-scale metabolic models (GSMMs) in antimicrobial pharmacology. **A**. A machine learning model was trained with high-throughput antimicrobial pharmacodynamics of *E. coli* combined with metabolic flux values calculated using GSMM to unravel the mechanisms of metabolic perturbation in antimicrobial killing [84]. **B**. Genetic variations in the *M. tuberculosis* genome were translated to metabolic changes through modeling. The calculated metabolic fluxomes were combined with strain-specific antimicrobial susceptibility (minimum inhibitory concentration) data to train a support vector machine-based model to identify the superplane (Metabolic Allele Classifier, dotted line) that best separates antimicrobial-susceptible and -resistant strains in the metabolic solution space [38]. **C**. Schematic workflow showing the use of GSMM to decipher the mechanisms of antimicrobial activity, resistance, and toxicity. GSMMs were constructed for the *Pl. falciparum* life cycle to predict life stage-specific antimalarial drug targets. After validation, the classifier was used to predict antimicrobial resistance of > 1000 *M. tuberculosis* strains and to analyze the metabolic features contributing to resistance. **D**. The TIMBR algorithm was employed to integrate transcriptomic data with the model *i*Hsa to predict biomarker metabolites in human hepatocytes in response to antimicrobial treatments. The results were further validated with metabolomics [12].

employed to study the metabolic adjustments associated with antibiotic resistance [37]. The reported resistance-conferring mutations were mapped to metabolic pathways, and the fluxes through these pathways were maximized or minimized to interpret the potential contribution to resistance. For example, the antimycobacterial agent ethambutol inhibits the function of three membrane-embedded arabinosyltransferases (EmbA, EmbB, and EmbC), which are required for the biosynthesis of the cell wall component arabinogalactan [88]. However, mutations in the gene *ubiA* may confer ethambutol resistance [73]. *ubiA* encodes 5-phospho-α-D-ribose-1-diphosphate:decaprenyl-phosphate 5-phosphoribosyltransferase, the first enzyme in the biosynthetic pathway for the arabinogalactan precursor decaprenylphosphoryl-β-D-arabinose (DPA). GSMM showed that flux-increasing mutations in *ubiA* could increase DPA production and overcome ethambutol inhibition of arabinosyltransferases [37]. Similarly, minimizing production of mycothiol was predicted to lead to ethionamide resistance, and maximizing production of tetrahydrofolate and L-alanine could counteract the antimicrobial effects of *para*-aminosalicylic acid and D-cycloserine, respectively. Comparative analysis of the uptake differences driving the objective functions across different antibiotic-resistant conditions revealed external L-alanine availability as a key environmental factor. Previous studies have demonstrated that internalized *M. tuberculosis* can utilize alanine from macrophage cytosol for growth [11]; hence, it has been suggested that D-cycloserine may be less effective *in vivo* owing to the increased availability of L-alanine [37].

GSMM has been used in conjunction with metabolomics and genomics to unravel the effects of genetic variation on strain-specific metabolism in *M. tuberculosis*, specifically metabolic vulnerabilities or carbon-source dependencies [62]. Double gene knockout was conducted *in silico* to predict all synthetic lethal gene pairs, with one gene containing a resistance-conferring single nucleotide polymorphism (SNP). The predicted epistatic interactions of SNP-affected enzymes varied across *M. tuberculosis* lineages and were enriched in glycolysis, amino acid biosynthesis, and metabolite transport. Model predictions also correctly

classified SNP effects in pyruvate kinase, and suggested a genetic basis for strain-specific inherent susceptibility to *para*-aminosalicylic acid [62].

P. aeruginosa can develop resistance to polymyxins via modification of lipid A with positively charged 4-deoxy-4-amino-L-arabibnose (L-Ara4N) or deacylation (removal of R-3-hydroxydcanoate from the 3 position of lipid A). Using GSMM, we discovered that these lipid A modifications could significantly alter the electrostatic status of the cellular surface without significant impacts on cellular growth or metabolism [89]. Another study combining transcriptomics and GSMM revealed that aztreonam-resistant *P. aeruginosa* diverted flux from the Entner-Doudoroff (ED) pathway to the pentose phosphate and peptidoglycan biosynthesis pathways, and that this diversion did not have a significant fitness cost in the absence of aztreonam [83]. In another biochemical screening of antibiotic-resistant *P. aeruginosa* mutants, GSMM demonstrated that a piperacillin-resistant mutant showed defects in leucine catabolism due to mutations that abolished catabolism of isovaleryl-coenzyme A (CoA) to the TCA cycle substrate acetyl-CoA [25].

Machine learning has been integrated with GSMM to unravel the metabolic basis for development of antimicrobial resistance. For example, a recent high-throughput study developed a GSMM-based machine learning classifier, the Metabolic Association Classifier (MAC), to discriminate between 1595 antibiotic-resistant and -susceptible *M. tuberculosis* strains based on their genetic variations and potential biochemical differences (Fig. 3**B**) [38]. The MAC identified sequence variations in *pncA/ppsA, thyA*, and *katG* as major predictors of resistance to pyrazinamide, *para*-aminosalicylic acid, and isoniazid, respectively. The metabolic pathways most associated with antimicrobial resistance to pyrazinamide were nicotinamide metabolism, CoA biosynthesis, and phthiocerol metabolism. *Para*-aminosalicylic acid resistance was most associated with cysteine and methionine metabolism, and resistance to isoniazid was most associated with the respiratory chain, TCA cycle, and mycolic acid biosynthesis. Pyrazinamide-resistant *ansP2* mutants may have a large pool of CoA due to increased production of L-asparagine.

In another study, *E. coli* was evolved in the lab to gain resistance to three antibiotics on two carbon sources [87]. Metabolomic analysis was conducted in parallel to identify significant changes in metabolite abundance, with the assumption that altered metabolite concentrations in evolved strains would reflect an attempt to rewire metabolic networks to compensate for resistance. GSMM was employed to calculate “shadow prices” (i.e., the sensitivity of biomass accumulation to changes in a specific metabolite) for each metabolite involved in the model. Metabolites with negative shadow prices likely limit biomass production during antibiotic treatment. Reactions with many limiting metabolites are therefore critical for *E. coli* to develop resistance. The association analysis revealed that anhydromuropeptide transport in cell wall recycling may mediate ampicillin resistance. In parallel to developing resistance to chloramphenicol, cells downregulated TCA fluxes and diverted resources to fermentative metabolism; pure respiration-dependent acetate growth was more affected by chloramphenicol compared to glucose growth, which used both respiration and fermentation. As demonstrated in these studies, GSMM allows a more detailed understanding of the mechanism(s) underlying metabolic adaptation in antimicrobial-resistant strains. GSMM can therefore significantly facilitate the development of novel metabolism-targeted approaches to address antimicrobial resistance.

3.3. Identification of novel drug targets

In silico gene knockout is used to predict essential genes that, if deleted, would abolish bacterial growth under specific nutrient conditions [45]. Antimicrobial agents can then be designed to target candidate essential gene products. Due to the structural and functional homology of certain conserved proteins, it is important to filter out essential microbial genes with highly conserved human homologs. A recent study used multiple-tiered filters of network vulnerability, sequence similarity, and structural accessibility to predict drug targets in *M. tuberculosis* [68]. The hub nodes of a protein–protein interactome network and essential genes identified by GSMM were considered the first tier of filtration. Candidates were then further filtered by removing targets with high similarity to the human proteome or proteins in the gut microbiome. Finally, 186 potential broad-spectrum antibacterial targets (those with high similarity to other pathogenic proteomes) and 66 targets unique to mycobacteria were identified. GSMM-based screening of antimicrobial targets has been applied to a broad range of pathogens, including *A. baumannii* [39,67], *K. pneumoniae* [16], *Yersinia pestis* [17], *S. aureus* [42], *E. coli* [3], *P. aeruginosa* [8], and *Pl. falciparum* [1]. Notably, integration with transcriptomic and physiological data allowed the construction of GSMMs specific for five different stages of the *Pl. falciparum* life cycle (trophozoite, schizont, early gametocyte, late gametocyte, and ookinete) (Fig. 3**C**). *In silico* single gene deletion was then conducted to identify stage-specific antimicrobial targets [1]. That study highlights the differences in gene essentiality between life cycle stages as a result of metabolic variation. In general, GSMMs of microbial pathogens can guide faster identification of potential targets for drug discovery compared with the traditional time-consuming, labor-intensive experimental trial-and-error.

3.4. Elucidating the mechanisms of antimicrobial-induced toxicity

GSMM has additionally been used to decipher the mechanisms underpinning drug side effects in hosts [12,70,91]. Using 6040 gene expression profiles as transcriptomic constraints, a human GSMM was employed to analyze the metabolic impact of exposure to each of 1221 drugs individually in three human cell lines (MCF-7, PC-3, and HL-60). Machine learning was implemented to select discriminating metabolite production perturbations based on side effect frequency data from the Side Effect Resource (SIDER) database [91]. The results suggested that drug-induced non-pharmacokinetic metabolic dysregulation is a common mechanism underlying host side effects, and that targeted nutrient supplementation may be an effective approach to reduce side effect incidence [91].

Antimicrobials often have adverse effects. GSMM has therefore been used in human and rat systems to elucidate the mechanisms of antibiotic toxicity [12,70]. An algorithm named Transcriptionally Inferred Metabolite Biomarker Response (TIMBR) was developed to predict potential biomarkers for diagnosis of renal dysfunction (Fig. 3**D**) [12]. TIMBR predicted a decrease in fatty acids in rat renal proximal tubule epithelial cells after 24 h of gentamycin treatment, suggesting that fatty acids were employed as an energy source during stress [70]. Another large study using human and rat GSMMs predicted biomarker metabolites that could indicate hepatotoxicity for 76 drugs, including 11 antimicrobials (e.g., ciprofloxacin, chloramphenicol, rifampin, tetracycline, erythromycin ethylsuccinate, ethambutol, isoniazid, and rifampin) [12]. The results were validated with high sensitivity and specificity using metabolomics. It is important to note that all of the GSMMs discussed above were limited to one type of cell or organ. With the development of whole-body human GSMM [79], a comprehensive understanding of the mechanisms of antimicrobial toxicity in multiple organs, and the discovery of novel metabolite biomarkers, will be more feasible (Fig. 4).

4. Future perspectives

Comprehensive considerations of antimicrobial pharmacokinetics, pharmacodynamics, and toxicodynamics (PK/PD/TD) in patients are required to optimize antimicrobial treatments for maximum activity and minimal toxicity and resistance. Conventional PK/PD modeling describes the relationship between drug exposure and antimicrobial efficacy, with parameters usually estimated by data fitting. These conventional models lack specificity of antimicrobial pharmacology at the network level. Thus far, GSMMs have been integrated with PK models to elucidate the mechanisms of drug–drug interactions between

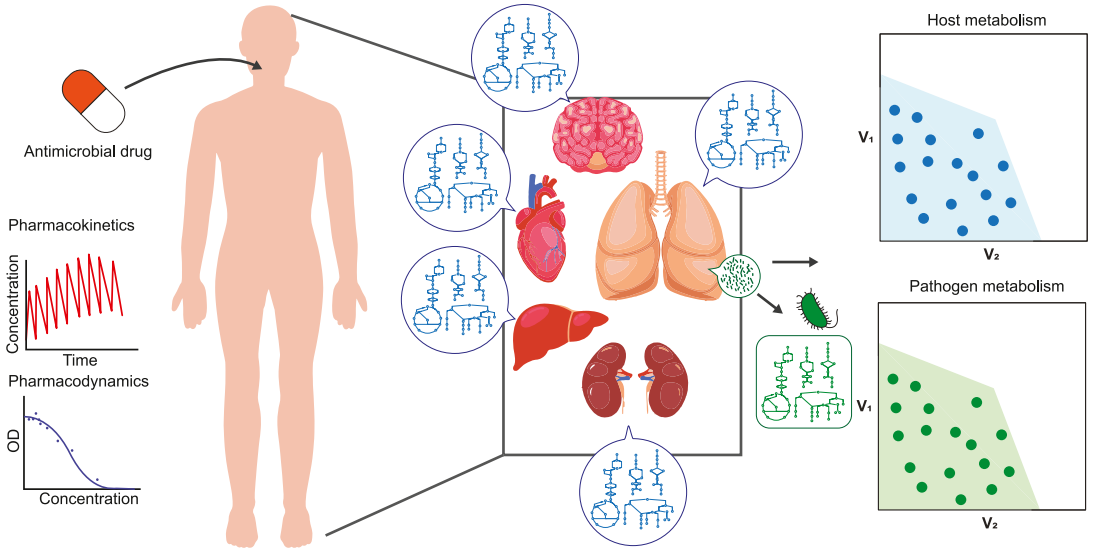

Fig. 4. Integration of pharmacokinetics/pharmacodynamics with genome-scale metabolic modeling to elucidate the mechanisms underlying metabolic changes in both host and pathogen in response to antimicrobial treatment for pulmonary infection.

phenytoin and estradiol [76] and to investigate ammonia detoxification, hyperuricemia therapy, and acetaminophen-induced toxification [41]. They have also been used to study the relationship between amino acid metabolism and levodopa in Parkinson's disease patients [29], and to simulate blood glucose regulation in Type-I diabetes [81]. However, only one study has thus far integrated GSMM with a PK model to study an antimicrobial: an investigation into the metabolic responses to idiosyncratic drug-induced liver injuries caused by isoniazid treatment [22]. This integrated model delineated isoniazid-induced metabolic alterations in the liver and identified several significantly changed metabolites, such as cholesterol, amino acids, and fatty acids. These metabolites can therefore be used as liver physiology biomarkers during isoniazid treatment. Although integration of GSMM with antimicrobial PK/PD/TD is still in its infancy, we envision that this approach will be critical to future antimicrobial pharmacology (Fig. 4).

Microbial cellular responses to antimicrobial treatments are not limited to metabolism. Rather, they involve a broad range of activities, including transcription, translation, and cell envelope assembly. Conventional GSMM focuses primarily on metabolism, limiting the ability to describe the complex mechanisms underlying responses to antimicrobial treatments. Recently, significant progress has been made by developing ME models that incorporate gene expression, protein structure information, protein complexation, and enzyme activity. Of particular interest is the modeling of bacterial responses to reactive oxygen species (ROS) using a specific ME model of *E. coli* (called OxidizeME) [86]. That model correctly predicted amino acid auxotrophy under ROS stress, candidate carbon sources with differing ROS sensitivity, and ROS-specific differential gene expression. This modeling strategy can be used to investigate cellular responses to antimicrobial stress, facilitating a better understanding of the mechanisms underpinning antimicrobial activity and assisting in the development of novel antimicrobial treatments.

With the surge in high-throughput generation of myriad types of biological data, machine learning has become a popular approach to discover critical cellular components contributing to antimicrobial activity, resistance, and toxicity. However, machine learning methods often lack mechanistic interpretability due to their 'black box' nature, (i.e., the functions between input and output are so complicated that it is difficult to show how a final prediction is achieved) [72]. Because GSMM utilizes deterministic models based on the steady-state assumption and reaction stoichiometry, it provides a mechanistic understanding of cellular metabolism. Applying machine learning to GSMM studies can improve the prediction and data coverage of GSMM and, more importantly, increase the interpretability of machine learning via causality analysis. Recently, integration of machine learning and GSMM has been applied to examine antimicrobial activity [80], combination synergy, side effects, and resistance [23,26,44,52]. It is expected that machine learning will be increasingly employed in conjunction with GSMMs to further improve their use and capacities.

Although it is critical to validate the prediction accuracy of GSMM experimentally, most validations in the current literature are limited and qualitative. For example, although a previous study involving a GSMM of *E. coli* MG1655 identified 38 indispensable reactions as potential novel antimicrobial targets, only the type II fatty acid biosynthesis (FAS II) reactions were selected for virtual screening of inhibitors from a compound library, representing a severely limited validation [75]. Another study employed GSMM to predict gene deletions promoting endogenous ROS production, which may potentiate antibiotic activity against *E. coli* [13]. In that study, a deletion mutant with > 5% more ROS production than the wild-type control was predicted to have increased susceptibility to both oxidants and bactericidal antibiotics (ampicillin, ofloxacin, ciprofloxacin and gentamicin). Experimental validation showed that predictions of sensitivity to ampicillin and fluoroquinolones in 13 mutants achieved an overall accuracy of over 70%. Predictions of either increased or unchanged susceptibility compared to the wild-type strain were considered correct when they matched experimental results [13]. However, measurements of metabolic fluxes in the deletion mutants and subsequent comparisons with the predictions were not performed; the validation performed was therefore more qualitative than quantitative [13]. ^{13}C metabolic flux analysis can be used to quantitatively validate GSMM predictions [19]. Recently, significant efforts have been made to enhance the prediction accuracy of GSMM, including the use of tailored biomass formulations [10], constrained exchange fluxes based on exometabolomic data [93], and machine learning approaches [30,31]. A recent study employed an ensemble machine learning model to predict the maximum *in vivo* apparent turnover number of enzymes ($k_{app,max}$) based on biochemistry, protein structure, and network context [31]. The calculated $k_{app,max}$ was incorporated into two extended GSMM frameworks (metabolic modeling with enzyme kinetics [MOMENT] and ME model) to quantitatively predict proteomics data. The predictions showed a significantly higher accuracy compared to previous methods, with an average reduction in root mean squared error of 34% and 20% for the MOMENT and the ME model, respectively. These findings suggest that machine learning can significantly improve the predictive power of GSMM.

Overall, GSMM has arisen as a cutting-edge platform to investigate cellular metabolism, and has been widely applied in antimicrobial pharmacology. The integration of GSMM with antimicrobial PK/PD modeling and advanced machine learning techniques will shift the paradigm of antimicrobial pharmacology and facilitate the development of novel and effective antimicrobial therapies to combat antimicrobial resistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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