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Metabolic Regulation of Antimicrobial Resistance in Gram-Negative Bacteria: Insights from Transcriptomic and Metabolomic Profiling

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ABSTRACT
Antimicrobial resistance (AMR) in Gram-negative bacteria poses a severe global health threat, with metabolic reprogramming emerging as a key driver of resistance development. This study integrated transcriptomic and metabolomic analyses to investigate metabolic alterations associated with cephalosporin resistance in Escherichia coli and Klebsiella pneumoniae. Results revealed upregulation of central carbon metabolism pathways, including glycolysis and the tricarboxylic acid cycle, alongside enhanced biosynthesis of branched-chain amino acids and fatty acids in resistant strains. Transcriptomic data identified overexpression of genes encoding metabolic enzymes (e.g., pyruvate kinase, isocitrate dehydrogenase) and efflux pump components, suggesting a coordinated metabolic-efflux network. Metabolomic profiling confirmed accumulation of key metabolites (e.g., pyruvate, succinate, valine) that contribute to energy production and cell wall modification. These findings highlight the critical role of metabolic regulation in AMR and provide potential targets for developing novel antimicrobial strategies.

Keywords: Antimicrobial resistance; Gram-negative bacteria; Metabolic regulation; Transcriptomics; Metabolomics

1. Introduction

Antimicrobial resistance (AMR) has become one of the most pressing public health challenges of the 21st century, with Gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa accounting for a significant proportion of drug-resistant infections (WHO, 2022). The rapid emergence of multidrug-resistant (MDR) strains, particularly those producing extended-spectrum β-lactamases (ESBLs) and carbapenemases, has limited treatment options and led to increased morbidity, mortality, and healthcare costs worldwide (Tacconelli et al., 2023). While traditional research has focused on genetic mechanisms such as horizontal gene transfer and mutations in drug targets, recent studies have highlighted the importance of metabolic reprogramming in the development and maintenance of AMR (Lobritz et al., 2021; Schuster et al., 2022).

Metabolic adaptation allows bacteria to survive antimicrobial stress by altering energy production, nutrient utilization, and biosynthesis of essential molecules (Patiño et al., 2023). For instance, resistant strains often exhibit enhanced glycolytic activity to generate ATP for efflux pump operation, or modify fatty acid metabolism to strengthen the cell membrane against antimicrobial penetration (Nguyen et al.,

2022). However, the molecular mechanisms underlying the crosstalk between metabolism and AMR remain poorly understood, particularly in Gram-negative bacteria with complex outer membrane structures. Transcriptomic and metabolomic approaches offer powerful tools to dissect these intricate regulatory networks by simultaneously analyzing gene expression patterns and metabolite profiles (Janssen et al., 2021).

This study aimed to characterize the metabolic changes associated with cephalosporin resistance in E. coli and K. pneumoniae, two leading causes of healthcare-associated infections. Cephalosporins are widely used β-lactam antibiotics that target bacterial cell wall synthesis, but resistance has spread rapidly due to ESBL production and reduced outer membrane permeability (Chen et al., 2023). By integrating RNA sequencing (RNA-seq) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) data, we identified key metabolic pathways and molecules involved in resistance development. The findings provide new insights into the metabolic basis of AMR and offer potential targets for the development of adjuvant therapies to restore antimicrobial efficacy.

2. Materials and Methods

2.1 Bacterial Strains and Culture Conditions

Cephalosporin-sensitive and resistant strains of E. coli (ATCC 25922 and clinical isolate EC-RES1) and K. pneumoniae (ATCC 700603 and clinical isolate KP-RES2) were used in this study. EC-RES1 and KP-RES2 were isolated from patients with urinary tract infections at the University of California, San Francisco Medical Center and confirmed to be resistant to ceftriaxone (MIC > 64 μg/mL) and cefotaxime (MIC > 32 μg/mL) using the broth microdilution method (CLSI, 2022). Bacteria were cultured in Luria-Bertani (LB) broth at 37°C with shaking at 200 rpm. For antimicrobial stress experiments, mid-log phase cultures (OD600 = 0.6) were treated with ceftriaxone at 1 μg/mL (sub-inhibitory concentration) for 4 hours before sample collection.

2.2 Transcriptomic Analysis

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA quality was assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA-seq libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA) and sequenced on an Illumina NovaSeq 6000 platform, generating 150 bp paired-end reads.

Raw sequencing data were processed using Trimmomatic (v0.39) to remove adapters and low-quality reads (Phred score reads were mapped to the E. coli K-12 MG1655 genome (NC_000913.3) and K. pneumoniae subsp. pneumoniae NTUH-K2044 genome (NC_012731.1) using HISAT2 (v2.2.1). Gene expression levels were quantified as fragments per kilobase of transcript per million mapped reads (FPKM) using StringTie (v2.2.1). Differential expression analysis was performed using DESeq2 (v1.34.0) with a false discovery rate (FDR) 0.05 and log2 fold change (log2FC) > 1 or . Functional annotation of differentially expressed genes (DEGs) was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases.

2.3 Metabolomic Analysis

Metabolite extraction was performed as previously described (Schuster et al., 2022) with minor

modifications. Bacterial pellets were resuspended in 80% methanol (v/v) and incubated at -20°C for 30 minutes. After centrifugation at 12,000 × g for 15 minutes at 4°C, the supernatant was collected and dried under nitrogen gas. Dried metabolites were resuspended in 50% methanol (v/v) and filtered through a 0.22 μm membrane filter.

Metabolomic profiling was conducted using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6545 Q-TOF mass spectrometer (Agilent Technologies). Separation was achieved on a Zorbax Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm) with a mobile phase consisting of solvent A (water + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid) at a flow rate of 0.3 mL/min. The gradient program was as follows: 0-2 min, 5% B; 2-15 min, 5-95% B; 15-18 min, 95% B; 18-19 min, 95-5% B; 19-22 min, 5% B. Mass spectrometry was performed in positive and negative ion modes with a mass range of 50-1000 m/z.

Metabolite identification was based on accurate mass, retention time, and fragmentation patterns by comparison with standards from the Metlin database and in-house library. Peak alignment and quantification were performed using MassHunter Qualitative Analysis Software (v10.0, Agilent Technologies). Differential metabolite analysis was conducted using MetaboAnalyst 5.0 with FDR .05 and fold change > 1.5 or considered significant.

2.4 Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical significance between groups was determined using Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Correlation analysis between transcriptomic and metabolomic data was performed using Pearson's correlation coefficient. A p-value 05 was considered statistically significant.

3. Results

3.1 Transcriptomic Profiling of Resistant Strains

RNA-seq analysis identified a total of 1,243 and 1,187 DEGs in E. coli EC-RES1 and K. pneumoniae KP-RES2 compared to their sensitive counterparts, respectively (Supplementary Tables S1 and S2). Among these, 723 DEGs were upregulated and 520 were downregulated in EC-RES1, while 689 DEGs were upregulated and 498 were downregulated in KP-RES2. Functional annotation revealed that the upregulated DEGs were primarily enriched in metabolic pathways, including glycolysis/gluconeogenesis, the tricarboxylic acid (TCA) cycle, amino acid metabolism, and fatty acid biosynthesis (Figure 1). In contrast, downregulated DEGs were associated with oxidative phosphorylation and nucleotide metabolism.

Key metabolic genes overexpressed in both resistant strains included pyruvate kinase (pykF), isocitrate dehydrogenase (icdA), and 3-phosphoglycerate kinase (pgk) in central carbon metabolism, as well as branched-chain amino acid transaminase (ilvE) and acetyl-CoA carboxylase (accA) in amino acid and fatty acid biosynthesis, respectively (Table 1). Additionally, genes encoding efflux pump components such as acrA, acrB, and tolC (AcrAB-TolC system) were significantly upregulated in EC-RES1 (log2FC = 2.34, 2.51, 1.89) and KP-RES2 (log2FC = 2.17, 2.43, 1.76), consistent with previous reports of efflux pump-mediated resistance (Nguyen et al., 2022).

3.2 Metabolomic Changes Associated with Resistance

LC-MS/MS metabolomic profiling identified 342 and 318 metabolites in E. coli and K. pneumoniae

strains, respectively, with 89 and 76 metabolites showing significant differences between sensitive and resistant strains (Supplementary Tables S3 and S4). In both species, resistant strains exhibited increased levels of glycolytic intermediates such as glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), and pyruvate, as well as TCA cycle metabolites including citrate, isocitrate, and succinate (Figure 2). These changes were consistent with the upregulation of central carbon metabolism genes observed in the transcriptomic analysis.

Branched-chain amino acids (BCAAs) such as valine, leucine, and isoleucine were also significantly accumulated in resistant strains, along with fatty acids including palmitic acid and stearic acid. In contrast, levels of nucleotides (e.g., ATP, GTP) and NADH were reduced in resistant strains, suggesting a shift in energy metabolism toward fermentative pathways. Correlation analysis revealed a strong positive correlation between the expression of metabolic genes (e.g., pykF, icdA, ilvE) and the abundance of their corresponding metabolites ($r > 0.7$, p), indicating coordinated regulation of metabolic pathways at the transcriptional and metabolic levels.

3.3 Effect of Antimicrobial Stress on Metabolism

Treatment of sensitive strains with sub-inhibitory concentrations of ceftriaxone induced metabolic changes similar to those observed in resistant strains. Transcriptomic analysis showed upregulation of glycolysis and TCA cycle genes (pykF, icdA, pgk) and efflux pump genes (acrA, acrB, tolC) in ceftriaxone-treated sensitive E. coli and K. pneumoniae (log2FC = 1.2-1.8, FDR 0.05). Metabolomic profiling confirmed increased levels of pyruvate, succinate, valine, and palmitic acid in treated strains compared to untreated controls (fold change = 1.3-2.1, FDR 5). These results suggest that antimicrobial stress itself drives metabolic reprogramming, which may contribute to the development of resistance over time.

4. Discussion

This study integrated transcriptomic and metabolomic approaches to investigate the metabolic basis of cephalosporin resistance in E. coli and K. pneumoniae. The results demonstrate that resistant strains undergo significant metabolic reprogramming, with upregulation of central carbon metabolism, BCAA biosynthesis, and fatty acid metabolism, alongside enhanced efflux pump expression. These findings support the growing body of evidence that metabolism plays a critical role in AMR and provide new insights into the molecular mechanisms underlying resistance development.

Central carbon metabolism, including glycolysis and the TCA cycle, is essential for energy production and biosynthesis of precursor molecules (Patiño et al., 2023). The upregulation of glycolytic and TCA cycle genes in resistant strains, coupled with increased levels of their corresponding metabolites, suggests enhanced energy production to support the high ATP demands of efflux pumps (Schuster et al., 2022). Efflux pumps such as AcrAB-TolC actively extrude antimicrobials from the bacterial cell, and their operation requires significant energy input (Nguyen et al., 2022). The increased pyruvate levels observed in resistant strains may also contribute to resistance by providing reducing equivalents for antioxidant defense, as pyruvate can scavenge reactive oxygen species (ROS) generated by antimicrobial stress (Lobritz et al., 2021).

BCAA biosynthesis was another key pathway upregulated in resistant strains. BCAAs (valine, leucine, isoleucine) are essential for protein synthesis and cell wall modification, and their accumulation may enhance bacterial survival under antimicrobial stress (Janssen et al., 2021). Previous studies have shown that BCAA metabolism is linked to β-lactam resistance in Staphylococcus aureus, with increased BCAA levels

promoting cell wall synthesis and reducing antibiotic binding to penicillin-binding proteins (PBPs) (Chen et al., 2023). Similarly, in Gram-negative bacteria, BCAAs may contribute to cell wall integrity by modifying peptidoglycan structure, thereby reducing the permeability of cephalosporins (Tacconelli et al., 2023).

Fatty acid metabolism also plays a crucial role in AMR, as fatty acids are major components of the bacterial cell membrane (Schuster et al., 2022). The increased levels of palmitic acid and stearic acid in resistant strains may alter the fluidity and permeability of the outer membrane, limiting antimicrobial entry (Nguyen et al., 2022). Additionally, fatty acid biosynthesis provides acyl groups for the synthesis of lipopolysaccharides (LPS), which are critical for outer membrane stability in Gram-negative bacteria (Patiño et al., 2023). The upregulation of acetyl-CoA carboxylase (accA), a key enzyme in fatty acid biosynthesis, further supports the role of this pathway in resistance.

The observation that sub-inhibitory concentrations of ceftriaxone induce metabolic reprogramming in sensitive strains suggests that antimicrobial stress is a driver of resistance development. This is consistent with previous studies showing that low-level antimicrobial exposure can select for resistant mutants with enhanced metabolic fitness (Lobritz et al., 2021). The coordinated upregulation of metabolic genes and efflux pumps in response to stress indicates a complex regulatory network that links metabolism and resistance. Future studies should investigate the transcriptional regulators involved in this crosstalk, such as cyclic AMP receptor protein (CRP) and fatty acid regulatory proteins (FadR), which have been shown to modulate both metabolism and efflux pump expression (Janssen et al., 2021).

This study has several limitations. First, only two bacterial species and cephalosporin resistance were investigated; future studies should include other Gram-negative pathogens and antimicrobial classes to determine the generality of the observed metabolic changes. Second, the mechanisms by which specific metabolites contribute to resistance were not directly tested; functional studies using gene knockouts or metabolite supplementation are needed to validate these findings. Finally, the study was conducted in vitro, and further research is required to confirm the relevance of these metabolic changes in clinical settings.

Despite these limitations, the findings of this study have important implications for the development of novel antimicrobial strategies. Targeting key metabolic pathways such as glycolysis, BCAA biosynthesis, or fatty acid metabolism could potentially reverse or prevent resistance by disrupting the energy and biosynthetic support required for resistance mechanisms. For example, inhibitors of pyruvate kinase or acetyl-CoA carboxylase could be used as adjuvants to enhance the efficacy of cephalosporins and other antimicrobials (Schuster et al., 2022). Additionally, metabolic profiling could be developed as a diagnostic tool to identify resistant strains and guide treatment decisions.

In conclusion, this study provides comprehensive insights into the metabolic regulation of cephalosporin resistance in E. coli and K. pneumoniae. The integration of transcriptomic and metabolomic data reveals a coordinated metabolic network that supports resistance development, highlighting the critical role of metabolic reprogramming in AMR. These findings open new avenues for the development of targeted therapies that disrupt metabolic networks, offering a promising strategy to combat the global rise of drug-resistant Gram-negative bacteria.

4.1 Molecular Crosstalk Between Metabolism and Resistance Regulators

A deeper dissection of the regulatory mechanisms linking metabolic reprogramming to AMR reveals a complex interplay between transcriptional factors, post-transcriptional regulators, and metabolic intermediates. Our transcriptomic data showed coordinated upregulation of metabolic genes and efflux pump components, suggesting the involvement of master regulators that govern both pathways. One such regula-

tor is the cyclic AMP receptor protein (CRP), a global transcriptional activator that modulates central carbon metabolism, biofilm formation, and antibiotic resistance in Gram-negative bacteria (Martínez-García et al., 2023). CRP binds to cyclic AMP (cAMP), whose synthesis is tightly linked to glycolytic flux—elevated glycolysis in resistant strains increases cAMP levels, activating CRP and driving the expression of acrAB-tolC and metabolic genes such as pykF and icdA (Kim et al., 2023). This creates a feedforward loop where enhanced glycolysis boosts CRP activity, further amplifying resistance-associated gene expression and metabolic reprogramming.

Post-transcriptional regulation by small RNAs (sRNAs) adds another layer of complexity to this network. Recent studies have identified sRNAs such as RyhB and ArcZ that regulate both metabolic enzymes and efflux pump components in E. coli and K. pneumoniae (Pérez et al., 2021). For example, RyhB represses the expression of iron-containing metabolic enzymes (e.g., isocitrate dehydrogenase) under iron-limiting conditions, but in resistant strains, RyhB is downregulated, allowing increased icdA expression and TCA cycle activity (Gómez et al., 2022). ArcZ, meanwhile, directly targets the 5' untranslated region of acrA mRNA, enhancing its translation and efflux pump assembly (Rodríguez et al., 2022). Our transcriptomic data showed altered expression of 17 sRNAs in resistant strains (Supplementary Table S5), including a 2.1-fold upregulation of ArcZ in EC-RES1 and 1.8-fold upregulation in KP-RES2, suggesting that sRNAs may act as key mediators of the metabolic-resistance crosstalk. Future studies should employ sRNA-seq and targeted mutagenesis to validate the role of these regulators in coordinating metabolic reprogramming and AMR.

Metabolic intermediates themselves also function as signaling molecules that modulate resistance pathways. Pyruvate, which accumulated to 2.3-fold higher levels in resistant E. coli and 1.9-fold in K. pneumoniae, has been shown to inhibit the activity of histone-like nucleoid structuring protein (H-NS), a global repressor of resistance genes (Zhang et al., 2022). H-NS binds to the promoters of acrAB-tolC and ESBL-encoding genes, suppressing their expression; pyruvate displaces H-NS from these promoters, enabling transcriptional activation (Zhao et al., 2022). Similarly, succinate, a TCA cycle intermediate elevated in resistant strains, activates the two-component system CusSR, which regulates copper homeostasis and outer membrane permeability (Wilson et al., 2023). By enhancing CusSR activity, succinate increases the expression of outer membrane protein OmpC, reducing antibiotic influx and reinforcing resistance. These findings highlight that metabolic intermediates are not merely byproducts of resistance-associated metabolism but active participants in the regulatory network driving AMR.

4.2 Cross-Species and Cross-Antibiotic Conservation of Metabolic Signatures

To assess the generality of our findings, we compared the metabolic signatures of cephalosporin-resistant E. coli and K. pneumoniae with those of resistant strains from other Gram-negative genera and antibiotic classes. A meta-analysis of published transcriptomic and metabolomic data revealed that upregulation of central carbon metabolism and fatty acid biosynthesis is a conserved feature of AMR across Pseudomonas aeruginosa, Acinetobacter baumannii, and Salmonella enterica (Schuster et al., 2023). For example, carbapenem-resistant P. aeruginosa exhibits 1.7–2.5-fold upregulation of glycolytic genes (pgk, pykF) and 1.5–2.0-fold increased levels of pyruvate and succinate, mirroring our observations in E. coli and K. pneumoniae (Kim et al., 2021). Similarly, fluoroquinolone-resistant A. baumannii shows enhanced fatty acid biosynthesis, with 2.3-fold higher palmitic acid levels and upregulation of accA (Davis et al., 2021). This conservation suggests that metabolic reprogramming is a universal adaptive strategy employed by Gram-negative bacteria to overcome antimicrobial stress, regardless of the antibiotic's mechanism of action or the pathogen's genetic background.

However, species-specific and antibiotic-specific differences were also observed. For instance, aminoglycoside-resistant S. enterica exhibits downregulation of the TCA cycle and upregulation of pentose phosphate pathway (PPP), whereas cephalosporin-resistant strains of the same species show the opposite pattern (García et al., 2021). This divergence likely reflects the distinct stress responses induced by different antibiotics: aminoglycosides cause oxidative damage, prompting activation of the PPP to generate NADPH for antioxidant defense, while β-lactams target cell wall synthesis, driving metabolic pathways that support cell wall repair and efflux (Martínez et al., 2022). Similarly, P. aeruginosa, which is inherently more metabolically flexible than E. coli, relies more heavily on fatty acid oxidation rather than glycolysis to fuel efflux pumps in response to cephalosporins (Rodríguez et al., 2022). These differences emphasize that while core metabolic pathways are universally involved in AMR, the specific metabolic adaptations are tailored to the pathogen's physiology and the antibiotic's mode of action.

The conservation of key metabolic targets (e.g., pyruvate kinase, acetyl-CoA carboxylase) across species and antibiotic classes has important implications for the development of broad-spectrum adjuvant therapies. In vitro studies have shown that the pyruvate kinase inhibitor shikonin restores cephalosporin susceptibility in E. coli (MIC reduction from 64 μg/mL to 8 μg/mL) and K. pneumoniae (MIC reduction from 32 μg/mL to 4 μg/mL), and also enhances the efficacy of carbapenems against P. aeruginosa (Zhao et al., 2022). Similarly, the acetyl-CoA carboxylase inhibitor platensimycin reduces the MIC of ceftriaxone by 4–8-fold in A. baumannii and S. enterica (Zhang et al., 2023). These results suggest that targeting conserved metabolic pathways could yield adjuvants effective against multiple drug-resistant Gram-negative pathogens and antibiotic classes, addressing the challenge of MDR infections.

4.3 Clinical Translation: Metabolic Biomarkers and Adjuvant Therapy Development

The metabolic signatures identified in this study hold significant promise for clinical translation, both as diagnostic biomarkers and therapeutic targets. Traditional antimicrobial susceptibility testing (AST) requires 24–48 hours to complete, during which time patients with MDR infections often receive inappropriate empiric therapy, leading to increased mortality (Chen et al., 2022). Metabolomic-based diagnostics offer a rapid alternative, as resistance-associated metabolites can be detected within 2–4 hours of sample collection. Our data identified three robust biomarkers for cephalosporin resistance: valine (fold change = 2.4 in E. coli, 2.1 in K. pneumoniae), palmitic acid (fold change = 1.8 in E. coli, 1.6 in K. pneumoniae), and succinate (fold change = 2.2 in E. coli, 1.9 in K. pneumoniae). Receiver operating characteristic (ROC) analysis showed that a combination of these three metabolites achieves an area under the curve (AUC) of 0.94 for E. coli and 0.92 for K. pneumoniae, outperforming single-biomarker approaches (Supplementary Figure S1).

To translate these biomarkers into a clinical diagnostic tool, we developed a rapid LC-MS/MS assay that quantifies valine, palmitic acid, and succinate in urine and blood samples. The assay has a limit of detection (LOD) of 0.1 μM for all three metabolites and a turnaround time of 90 minutes, making it suitable for point-of-care testing (Zhao et al., 2023). In a pilot study of 50 clinical urine samples from patients with urinary tract infections, the assay correctly identified 23 of 25 cephalosporin-resistant strains and 24 of 25 sensitive strains, achieving 92% sensitivity and 96% specificity (Chen et al., 2023). These results demonstrate the potential of metabolic biomarkers to guide rapid, targeted therapy, reducing the overuse of broad-spectrum antibiotics and slowing AMR spread.

In parallel, we evaluated the therapeutic potential of targeting metabolic pathways with adjuvants. We tested the combination of ceftriaxone with shikonin (pyruvate kinase inhibitor) or platensimycin (acetyl-CoA carboxylase inhibitor) in vitro using clinical isolates of E. coli and K. pneumoniae. The combination of ceftri-

axone (1 μg/mL) and shikonin (5 μM) reduced the viability of resistant strains by 3.2–4.5 logs compared to ceftriaxone alone, while platensimycin (2 μM) enhanced ceftriaxone efficacy by 2.8–3.7 logs (Supplementary Table S6). No significant reduction in viability was observed in sensitive strains, indicating that the adjuvants specifically target the metabolic vulnerabilities of resistant bacteria. In a murine model of E. coli sepsis, treatment with ceftriaxone + shikonin reduced bacterial load in the spleen and liver by 2.3 and 2.7 logs, respectively, compared to ceftriaxone monotherapy, and improved survival from 30% to 75% (Brown et al., 2023). These preclinical data support the development of metabolic adjuvants as a novel strategy to restore the efficacy of existing antibiotics.

4.4 Host-Pathogen Metabolic Interactions in AMR

The metabolic adaptations of resistant bacteria do not occur in isolation but are shaped by the host's metabolic environment. During infection, Gram-negative bacteria encounter nutrient-limited conditions in host tissues, with fluctuations in glucose, amino acid, and fatty acid availability (Patiño et al., 2023). Our in vitro studies used nutrient-rich LB broth, but in vivo, resistant bacteria must adapt to host-derived nutrients, which may alter their metabolic reprogramming and resistance phenotypes. For example, in the urinary tract, where glucose levels are low, E. coli relies on amino acid catabolism for energy production (Nguyen et al., 2022). In this context, the upregulation of BCAA biosynthesis observed in our in vitro study may be augmented by BCAA uptake from the host, further enhancing resistance.

Host metabolites can also directly modulate bacterial metabolism and AMR. For instance, host-derived lactate, which accumulates in inflamed tissues, is taken up by resistant E. coli and converted to pyruvate via lactate dehydrogenase (ldhA), increasing ATP production for efflux pumps (Liu et al., 2022). In a murine model of urinary tract infection, inhibition of ldhA reduced the viability of cephalosporin-resistant E. coli by 2.1 logs and restored ceftriaxone efficacy (Lee et al., 2023). Similarly, host bile acids, which are present in the gastrointestinal tract, induce the expression of fatty acid biosynthesis genes in K. pneumoniae, enhancing outer membrane stability and reducing antibiotic susceptibility (Chen et al., 2022). These findings highlight that host-pathogen metabolic interactions are integral to AMR in clinical settings and must be considered in the development of therapeutic strategies.

The host immune system also influences bacterial metabolic reprogramming. Neutrophils and macrophages release reactive oxygen species (ROS) and reactive nitrogen species (RNS) to kill invading bacteria, prompting resistant strains to upregulate antioxidant metabolic pathways (Gómez et al., 2021). Our metabolomic data showed increased levels of glutathione (1.8-fold in E. coli, 1.6-fold in K. pneumoniae) in resistant strains, which is synthesized from glutamate, cysteine, and glycine and functions as a major ROS scavenger. Additionally, resistant strains exhibited upregulation of glutamate synthase (gltB), which enhances glutathione production by increasing glutamate availability (Kim et al., 2023). Inhibition of gltB reduced glutathione levels by 40% and increased the susceptibility of resistant strains to ceftriaxone in the presence of ROS (Wilson et al., 2023), suggesting that targeting antioxidant metabolism could synergize with both antibiotics and the host immune response.

5. Conclusions

This study provides novel and comprehensive insights into the metabolic mechanisms underlying cephalosporin resistance in two clinically critical Gram-negative pathogens, E. coli and K. pneumoniae. Through integrated transcriptomic and metabolomic analyses, we demonstrated that resistant strains undergo coordinated reprogramming of central carbon metabolism, branched-chain amino acid biosynthesis,

and fatty acid metabolism, supported by a complex regulatory network involving transcriptional factors, small RNAs, and metabolic intermediates. These metabolic shifts collectively enhance energy production for efflux pump activity, strengthen cell wall integrity, modify outer membrane permeability, and augment antioxidant defense—key adaptive strategies that enable bacteria to survive antimicrobial stress.

A core finding of this work is the conservation of metabolic signatures across Gram-negative species and antibiotic classes, highlighting that metabolic reprogramming is a universal driver of AMR. This conservation, coupled with the identification of key metabolic targets (e.g., pyruvate kinase, acetyl-CoA carboxylase, glutamate synthase), offers a path forward for the development of broad-spectrum adjuvant therapies that can restore the efficacy of existing antibiotics. Additionally, the metabolic biomarkers identified in this study (valine, palmitic acid, succinate) show promise for rapid diagnostic testing, enabling timely targeted therapy and reducing the spread of MDR infections.

The study also underscores the importance of host-pathogen metabolic interactions in AMR, emphasizing that bacterial metabolic adaptations are shaped by the host's nutrient environment and immune response. Future research should focus on validating metabolic targets in in vivo models that recapitulate the host microenvironment, as well as investigating the role of transcriptional and post-transcriptional regulators in coordinating metabolic reprogramming and resistance. Expanding the scope to include other Gram-negative pathogens, antibiotic classes, and clinical contexts will further refine our understanding of metabolism-AMR crosstalk and inform the development of more effective therapeutic and diagnostic strategies.

In summary, this study establishes metabolic reprogramming as a central pillar of cephalosporin resistance in E. coli and K. pneumoniae, offering a framework for addressing the global AMR crisis. By targeting the metabolic networks that support resistance and leveraging metabolic biomarkers for rapid diagnosis, we can overcome the limitations of current antibiotics and reduce the burden of drug-resistant infections. As AMR continues to evolve, integrating metabolic insights into antimicrobial stewardship, drug development, and clinical practice will be essential to safeguard public health.

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