



# Exogenous metabolite feeding on altering antibiotic susceptibility in Gram-negative bacteria through metabolic modulation: a review

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## Abstract

**Background** The rise of antimicrobial resistance at an alarming rate is outpacing the development of new antibiotics. The worrisome trends of multidrug-resistant Gram-negative bacteria have enormously diminished existing antibiotic activity. Antibiotic treatments may inhibit bacterial growth or lead to induce bacterial cell death through disruption of bacterial metabolism directly or indirectly. In light of this, it is imperative to have a thorough understanding of the relationship of bacterial metabolism with antimicrobial activity and leverage the underlying principle towards development of novel and effective antimicrobial therapies.

**Objective** Herein, we explore studies on metabolic analyses of Gram-negative pathogens upon antibiotic treatment. Metabolomic studies revealed that antibiotic therapy caused changes of metabolites abundance and perturbed the bacterial metabolism. Following this line of thought, addition of exogenous metabolite has been employed in *in vitro*, *in vivo* and *in silico* studies to activate the bacterial metabolism and thus potentiate the antibiotic activity.

**Key scientific concepts of review** Exogenous metabolites were discovered to cause metabolic modulation through activation of central carbon metabolism and cellular respiration, stimulation of proton motive force, increase of membrane potential, improvement of host immune protection, alteration of gut microbiome, and eventually facilitating antibiotic killing. The use of metabolites as antimicrobial adjuvants may be a promising approach in the fight against multidrug-resistant pathogens.

**Keywords** Antimicrobial resistance · Metabolism · Antibiotic susceptibility · Metabolic modulation · Gram-negative bacteria

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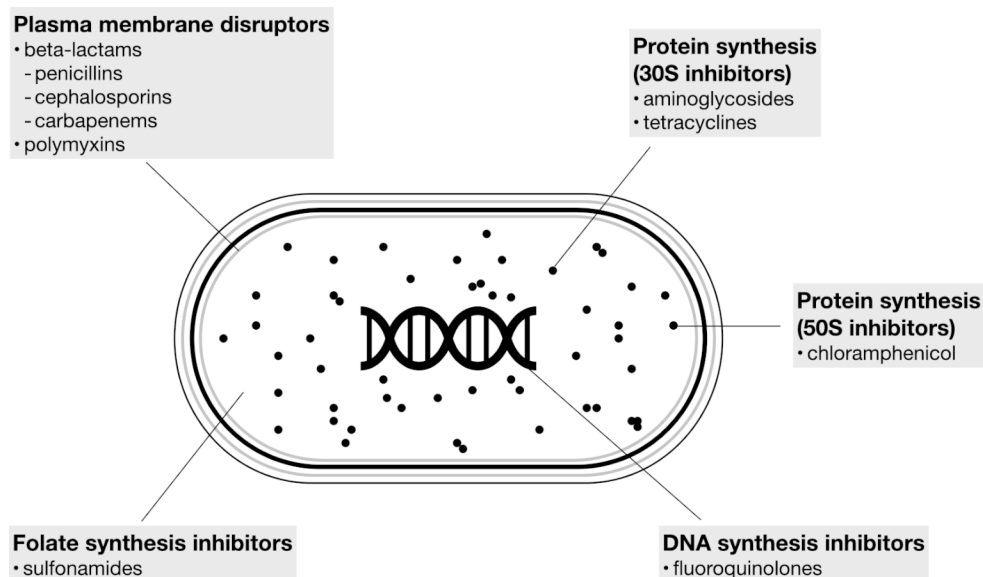
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## 1 Introduction

Gram-negative bacteria have developed resistance to all available antimicrobials, urging researchers to venture into antibiotic adjuvant therapy to counteract antibiotic resistance (Peterson & Kaur, 2018). In recent years, drug development via modulating bacterial metabolism is gaining broad interest (Zhang et al., 2019; Tang et al., 2020). The modern antibiotic efficacy is threatened by the rapid rise of antimicrobial drug resistance (Aslam et al., 2018; Betts et al., 2018). Conventional antimicrobial treatments are essential-target focused where they exert bactericidal activity on the basis of phenotypically inhibiting bacterial growth and targeting cellular processes (Fig. 1) (Cheah et al., 2016; Machuca et al., 2017; Annunziato, 2019). The discovery of new drug candidates targeting metabolism might be a promising approach in combating bacterial resistance (Thiele & Palsson, 2010; Yang et al., 2018a, b; Turi et al., 2018).

**Fig. 1 Modes of action of major antibiotics against Gram-negative pathogens**

Major antibiotics targeting at cellular processes including cell envelope, DNA and protein biosynthesis.



Cell metabolism is closely linked with cell viability, given that metabolism provides building blocks and energy for biomass formation (Alwarawrah et al., 2018). Drug-resistant bacteria are reported to have reduced cell metabolism whereas bacteria with enhanced metabolism are more susceptible to antibiotics (Baquero & Martínez, 2017). It is imperative for us to understand the interplay between bacterial fitness, bacterial metabolism and antibiotic efficacy. Metabolomic studies allow us to identify crucial metabolites in response to internal and external factors (Johnson et al., 2016). The significant changes in metabolites abundance suggest variations of metabolic pathways upon antimicrobial treatments (Vincent et al., 2016; Han et al., 2018). Hence, metabolites that significantly alter the metabolic fluxes are potential new drug candidates or antibiotic adjuvants. They may act by reverting the phenotypic antibiotic resistance of a drug-resistant strain and causing it to be susceptible to a drug (Cheng et al., 2019).

Metabolic profiling provides an instantaneous snapshot of cell physiology and thus metabolomics is used as an analytical approach for identification and quantification of small molecule metabolites that define the metabolic state of an organism (Nalbantoglu, 2019). Metabolomics approach provides mass screening of metabolite changes to discover the interrelation of microbial metabolic response with antibiotic activity (Campos & Zampieri, 2019; Fudyma et al., 2019; Hussein et al., 2020). On top of that, it can be also used to identify crucial metabolites from affected metabolic pathways following the antimicrobial treatment (Zampieri et al., 2018). Thereafter, feeding of the exogenous crucial metabolites can trigger metabolic modulation which may be possible to restore antibiotic susceptibility and enhance antibiotic efficacy.

## 2 Metabolic analyses of Gram-negative bacteria upon antibiotic treatment

Metabolomics is the study of metabolites involved in cellular biochemical processes under a specific physiological condition (Nalbantoglu, 2019). This provides a window for metabolic process delineation and identification of key metabolites involved in response to antibiotic treatment (Johnson et al., 2016; Vincent et al., 2016; Han et al., 2018). Metabolic analyses revealed that polymyxin combinations treatment in Gram-negative bacteria, including *Acinetobacter baumannii* (Maifiah et al., 2017), *Pseudomonas aeruginosa* (Hussein et al., 2018; Han et al., 2019) and *Klebsiella pneumoniae* (Abdul Rahim et al., 2021), led to perturbations in carbohydrate and nucleotide metabolism. Polymyxins exert bactericidal action as a membrane permeabilizer by initially targeting the bacterial outer membrane. In addition, metabolomics data revealed that, apart from membrane disruption, several biochemical pathways are perturbed by polymyxin treatment. The polymyxins treatment caused depletion in the levels of significant metabolites of pentose phosphate pathway including D-sedoheptulose-7-phosphate, D-erythrose 4-phosphate and D-ribose 5-phosphate (Maifiah et al., 2016, 2017; Zhu et al., 2019). These metabolites are the key precursors for biosynthesis of lipopolysaccharides (LPS), aromatic amino acids and nucleotides respectively.

The tricarboxylic acid (TCA) cycle exhibited downstream metabolism in response to polymyxin combination treatment against *A. baumannii* and *P. aeruginosa* (Maifiah et al., 2016, 2017; Hussein et al., 2018; Zhu et al., 2019). TCA cycle is another essential central metabolic pathway in bacterial cells, providing precursors for certain amino acids to yield energy. The altered TCA metabolites are

namely fumarate and *cis*-aconitate. Furthermore, decreased nucleotide metabolism was observed in *A. baumannii* following colistin and doripenem treatment (Maifiah et al., 2017). Treatment of kanamycin, ampicillin and norfloxacin also caused a reduction of nucleotide metabolites in *Escherichia coli* (Belenky et al., 2015a). These antibiotics treatment showed an impact on energy metabolism in terms of decreased nucleotide levels of ATP, NADP<sup>+</sup> and NAD<sup>+</sup> (Belenky et al., 2015a; Maifiah et al., 2017).

With the polymyxin treatment, the amino sugar metabolism was perturbed and led to low level metabolites for cell envelope biosynthesis. One of the essential intermediates for peptidoglycan biosynthesis, namely UDP-N-acetylglucosamine (UDP-GlcNAc) decreased significantly and reduced bacterial viability (Maifiah et al., 2017; Hussein et al., 2018; Zhu et al., 2019; Abdul Rahim et al., 2021). The combination treatment of polymyxin B with tamoxifen against *P. aeruginosa* altered the abundance of LPS biosynthesis precursor 3-deoxy-D-manno-octulosonate (KDO) and disrupted the LPS formation (Hussein et al., 2018).

The lipid metabolism in Gram-negative bacteria was greatly perturbed when treated with polymyxin monotherapy. Metabolomic results revealed profound alterations of lipids, predominantly the glycerophospholipids and fatty acids (Maifiah et al., 2017; Han et al., 2018; Hussein et al., 2018). These results were consistent with the proposed bactericidal mechanism of polymyxin through disruption of bacterial outer membranes (OM) (Henry et al., 2014; Berglund et al., 2015). The greatly reduced OM lipid levels attributed to polymyxin resistance as shown in previous findings (Henry et al., 2012; Han et al., 2018). Furthermore, polymyxin combination therapy caused a significant reduction of phospholipid metabolites, *sn*-glycero-3-phosphoethanolamine and *sn*-glycerol 3-phosphate (Maifiah et al., 2017; Hussein et al., 2018; Abdul Rahim et al., 2021). This induced a considerable impact to the lipid metabolism and thus decreased the bacteria cell survival.

A nontargeted metabolomics study by Zampieri et al., (2017) revealed that exposure of chloramphenicol to *E. coli* induced a rapid increase level of arginine and reduced level of *N*-acetyl-L-glutamate in amino acid metabolism (Zampieri et al., 2017). *N*-acetyl-glutamate plays a key role as the first intermediate in arginine biosynthesis. It was suggested that chloramphenicol treatment inhibited protein biosynthesis and actively induced arginine biosynthesis (Zampieri et al., 2017). This observation is in agreement with the metabolic response analysis to colistin treatment in *A. baumannii* (Zhu et al., 2019). The upregulated arginine biosynthesis might be an adaptive mechanism to increase cell survival during antibiotic action via production of excess ammonia and polyamines to attenuate the toxicity of hydroxyl radical

(Zampieri et al., 2017). High level of ammonia metabolism leads to an increase of oxidative stress in bacteria cells.

Chlortetracycline treatment against *E. coli* was determined to cause alteration in multiple metabolic pathways in *E. coli* due to its intrinsic resistance mechanism (Li et al., 2014). Chlortetracycline belongs to the family of tetracycline antibiotics that act as protein synthesis inhibitors (Chopra & Roberts, 2001). Metabolic regulation occurred in *E. coli* in response to chlortetracycline stress via upregulation of translation protein and downregulation of gluconeogenesis, pyruvate metabolism and TCA cycle (Li et al., 2014). Metabolite pyruvate as an end-product of gluconeogenesis pathway would be converted into acetyl-CoA via pyruvate metabolism (Li et al., 2014). Acetyl-CoA would drive the TCA cycle to generate NADH and proton motive force which may potentiate uptake of antibiotics (Su et al., 2015). Hence downregulated TCA cycle in this study showed that this may be attributed to the antibiotic resistance mechanism (Li et al., 2014). Furthermore, chlortetracycline treatment also affected in other pathways including purine metabolism and alanine, aspartate and glutamate metabolism (Li et al., 2014). This suggests that metabolite abundance level is vitally important in bacterial responses to environmental conditions. Based on this, it can be suggested that affected metabolites could be potential antibiotic adjuvants in antibiotic combination development to revert antibiotic-resistant metabolic pathways.

### 3 Exogenous metabolite feeding to restore bacterial susceptibility

Untargeted metabolomics has been established to investigate the metabolome changes of cells under different conditions, identify crucial biomarkers and understand impact of variations in metabolic pathways affected (Issa et al., 2017). The approach can be leveraged to identify crucial metabolites which can be used to revert the metabolic profile differences of a drug-resistant bacteria. This is termed as metabolome reprogramming where the host's phenotype can be reverted through the exogenous administration of crucial metabolites (Peng, Li Peng et al., 2015a, b). The metabolite abundance plays a significant role in metabolite biological activities in response to environmental factors. The significant changes in metabolite abundance alter the enzyme activity and metabolic pathway regulators and thus metabolic modulation occurs (Peng, Li Peng et al., 2015a, b).

#### 3.1 *In silico* coupled with *in vitro* study

From early studies, it is evident that antibiotic lethality is highly associated with central metabolism and

cellular respiration (Schrader et al., 2020). Bactericidal antibiotic treatment disrupts the cell homeostasis and results in increased ATP demand, increased metabolic burden and then gradually increased toxic metabolic by-products thus inducing cellular death (Bhargava & Collins, 2015; Yang et al., 2019) revealed exogenous supplementation of uracil would increase the antibiotic lethality including ampicillin, ciprofloxacin and gentamicin against *E. coli* through model prediction (41). Integration of *in vitro* biochemical screening with network modelling unveiled the antibiotic mechanism of action upon supplementation with uracil (Yang et al., 2019). Firstly, it was discovered that purine biosynthesis and cellular oxygen consumption rate was altered by the three antibiotics activities. Then, the addition of uracil was shown to promote purine biosynthesis activity. As a result of these changes, central metabolism activity was suppressed and there was inhibition in NAD production (Yang et al., 2019). These findings support that uracil addition induced metabolic changes and increased ATP demand to a disrupted nucleotide pool following antibiotic treatment and as a consequence, bacterial failure in maintaining its cellular homeostasis.

### 3.2 *In vitro* studies

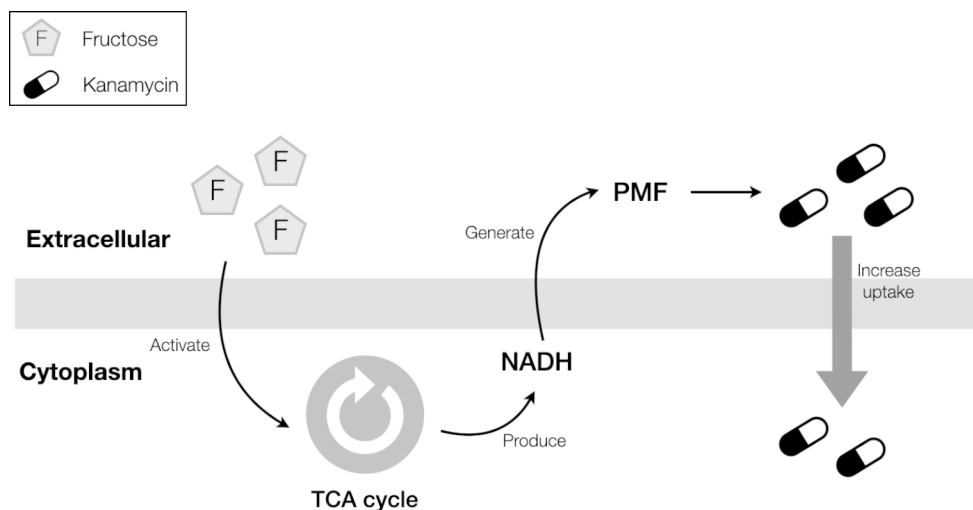
Addition of exogenous metabolites with antibiotics from two classes (aminoglycosides and quinolones) against Gram-negative bacteria have been investigated *in vitro*. Aminoglycoside antibiotics exhibit rapid concentration-dependent killing action through inhibition of protein synthesis. The increasing rate of resistance has hampered its effective usage on multidrug-resistant bacterial infections. The drug-resistant bacteria conferring mutations are often associated with a fitness cost. Drug resistance mechanisms including constant production of drug-inactivating enzymes, activation of drug efflux and modifications of membrane permeability will

result in metabolic cost for bacteria (Aghapour et al., 2019; Kakoullis et al., 2021). In the works of Su et al., (2015), they observed that metabolic deficiency is a characteristic feature in drug-resistant strains compared to drug-susceptible strains (Su et al., 2015). Lower abundance of fructose was detected in kanamycin-resistant strains. Exogenous feeding of fructose to *Edwardsiella tarda* resistant strains resulted in higher kanamycin efficacy against the resistant strains (Su et al., 2015). This finding suggested that exogenous fructose stimulated the TCA cycle which promoted NADH production and led to increase of proton motive force (PMF) (Fig. 2). The uptake of kanamycin to *E. tarda* was driven by the PMF (Su et al., 2015).

The aminoglycosides potentiation was also demonstrated in exogenous feeding of alanine and glucose with kanamycin to eliminate multidrug-resistant *E. tarda* (Peng et al., 2015a, b). In the presence of 40 mM alanine, 10 mM glucose and kanamycin, they achieved a synergistic effect on bacterial killing. Combination index (CI) theorem of Chou-Talalay was employed to measure the degree of drug combination interaction (Chou, 2010). The synergistic mechanism is attributed to the metabolic modulation that promotes TCA cycle flux (Peng et al., 2015a, b).

The potentiation of aminoglycosides activity dependent on PMF was already shown in year 2011 by Allison et al. where metabolites enabled eradication of bacterial persisters following gentamicin treatment against both *E. coli* and *Staphylococcus aureus* (Allison et al., 2011). Bacterial persisters are sub-populations of bacterial cells that are tolerant to antibiotic treatment due to a state of dormancy (Lewis, 2007). Feeding sugar and glycolysis intermediates such as glucose, mannitol, fructose, pyruvate would stimulate and awaken the metabolically inactive persister cells (Allison et al., 2011; Wood, 2017). The awakened persister cells would then be killed by gentamicin (Allison et al., 2011). In addition, basic amino acid metabolites such as L-arginine and

**Fig. 2 Exogenous fructose potentiated uptake of kanamycin** Exogenous fructose activates the TCA cycle to produce more NADH which generates PMF thus increasing uptake of kanamycin.



L-lysine were found to sensitise bacteria against aminoglycosides through a similar mechanism which promotes PMF (Lebeaux et al., 2014; Deng et al., 2020).

Tobramycin sensitivity on *P. aeruginosa* was affected due to the biofilm formation of *P. aeruginosa* which affects its elimination by the host immune defence system thus reducing antibiotic lethality. Studies revealed that fumarate (Meylan et al., 2017) and mannitol (Barraud et al., 2013) restored tobramycin sensitivity on *P. aeruginosa* biofilms. This was attributed to the increased TCA cycle activity by extracellular fumarate (Meylan et al., 2017). This hypothesis was supported by the elevated cellular respiration in conjunction with tobramycin uptake and downstream lethality. Similarly, exogenous addition of 5 to 40 mM mannitol reverted the persister phenotype of biofilm cells by inducing metabolism and thus enhancing the efficacy of tobramycin (Barraud et al., 2013).

Subsequent study by Su et al. in year 2018 showed that exogenous glutamate has potency in bactericidal effect against both *E. coli* and *E. tarda* when treated with kanamycin (Su et al., 2018). The increased pyruvate cycle flux triggered by 2.5 mM glutamate increased abundance of succinate, fumarate and malate and led to formation of oxaloacetate to pyruvate (Su et al., 2018). The preference of oxaloacetate to form pyruvate over citrate indicated that pyruvate cycle is a prevalent cycle in metabolite-mediated mechanisms initiated by glutamate. This suggests that the TCA cycle is part of the pyruvate cycle and is regulated by pyruvate cycle (Su et al., 2018). Pyruvate cycle was validated to produce one more NADH than TCA cycle, thus the higher build-up of proton resulted in elevated PMF which enhanced kanamycin efficacy in bacterial killing. The driven pyruvate cycle functioned as a respiratory energy source to the bacterial cell and hence facilitated the cell death of multidrug-resistant bacteria by kanamycin (Su et al., 2018).

Quinolone antibiotics induce bactericidal action through inhibition of DNA synthesis (Fàbrega et al., 2009). The mechanism of action of quinolone is highly affected at high density of bacterial populations (Zeiler, 1985). A strategy to eradicate this cell density-dependent persistence was demonstrated by Gutierrez et al. in year 2017 which was through supplementation of carbon source and appropriate terminal electron acceptor (Gutierrez et al., 2017). Both carbon and oxygen are limiting factors in density-dependent persistence to quinolones therefore exogenous addition of glucose and fumarate could restore ciprofloxacin susceptibility in stationary phase *E. coli* (Gutierrez et al., 2017). Upon the combination treatment, *E. coli* can utilise exogenous glucose as carbon source and fumarate as electron acceptor to stimulate cellular respiration, and then synergistically enhance ciprofloxacin activity in high cell-density setting. Similar synergistic effect was achieved using levofloxacin and

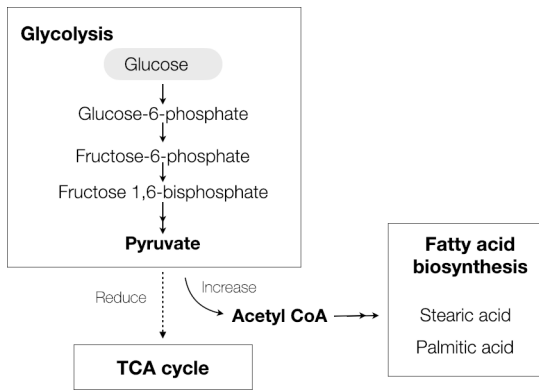
moxifloxacin in the same study. Furthermore, this hypothesis was also confirmed in Gram-positive bacteria, *S. aureus* and *Mycobacterium smegmatis* treated with ciprofloxacin (Gutierrez et al., 2017). These findings demonstrated that metabolism stimulation and cellular respiration were driven by feeding with exogenous metabolite and this approach may have broad applicability of quinolone antibiotic against a range of bacteria.

### 3.3 *In vivo* studies

In addition to *in vitro* and *in silico* studies, metabolic reprogramming has shown broad applications in *in vivo* studies of antibiotic-resistant infections treatment (Chen et al., 2017; M. Yang et al., 2018a, b, 2020). This approach demonstrated the metabolite-mediated potentiation to revert host's phenotype and make it susceptible to antibiotic killing. Using a similar approach, the crucial metabolites identified can be used to reprogram the host's immune system and improve its fitness. Metabolite feeding of mannitol and L-arginine increased effect of gentamicin in reduction of *E. coli* biofilm viability in a mouse model (Allison et al., 2011; Lebeaux et al., 2014). The gentamicin combination with mannitol treatment reduced biofilm viability by nearly 1.5 orders of magnitude and inhibited spread of bacterial infection to the kidneys of infected mice (Allison et al., 2011). Furthermore, the use of L-arginine combined with gentamicin showed an effective eradication of *E. coli* and *S. aureus* biofilms *in vivo*; it could constitute a clinically relevant therapeutical alternative to the existing catheter-removal treatment used in common biofilm infections (Mermel et al., 2009; Lebeaux et al., 2014; Olivares et al., 2020; Talapko & Škrlec, 2020).

Exogenous glucose has been discovered contributing to cell metabolism modulation in tilapias fish against *E. tarda* infection (Zeng et al., 2017). Addition of glucose attenuated the TCA cycle and increased biosynthesis of fatty acids (Fig. 3). The pathway analysis revealed the elevated enzyme activity of pyruvate dehydrogenase and reduced succinate dehydrogenase activity indicating the glycolytic flux was induced to fatty acid formation, rather than the TCA cycle. The conversion of glucose into stearic acid via pyruvate and acetyl-CoA enhanced fish immune system (Zeng et al., 2017). This finding was not similar with the aforementioned metabolite potentiation strategy, whereby TCA cycle flux activity was not promoted by the metabolite addition in this study. Instead, it enhanced tilapia anti-infectious ability via production of stearic acid redirected from glucose source (Zeng et al., 2017). The promotion of stearic acid by glucose may repay the reduced stearic acid level associated with *E. tarda* infection (Zeng et al., 2017). In addition, stearic acid demonstrated in enhanced interferon and Mx protein





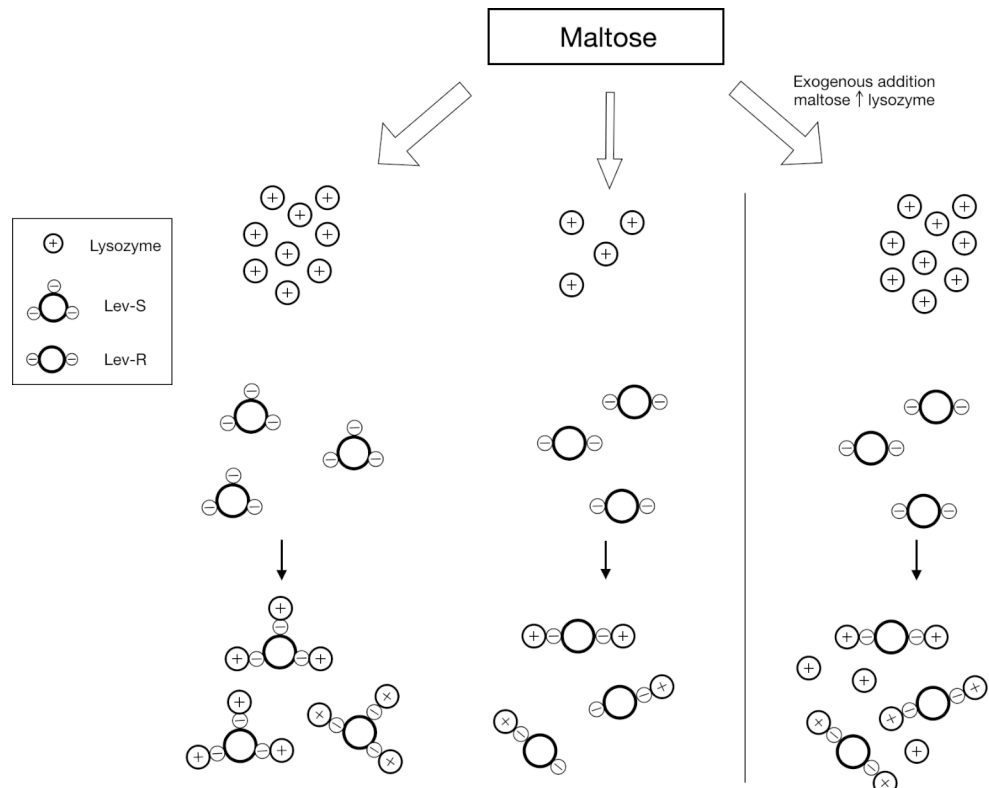
**Fig. 3 Exogenous glucose induced metabolic alteration in tilapia fish** Addition of glucose source was converted to produce fatty acids via pyruvate and acetyl-CoA. This caused reduction in TCA cycle activity but elevation in fatty acid biosynthesis in tilapia fish.

transcription where Mx protein possesses antiviral activity (Zeng et al., 2017). The Mx protein provides immune protection to tilapia against *E. tarda* but the underlying mechanism remains unclear (Zeng et al., 2017).

The interplay between the host immunity, antibiotic resistance and antimicrobial effectiveness has recently been integrated into antimicrobial development (Ankomah & Levin, 2014; Gjini & Brito, 2016; Ahmed et al., 2020). The innate system in zebrafish plays a key role in fighting against infections. Jiang et al., (2020) discovered that

different metabolic host responses to antibiotic-resistant and antibiotic-sensitive pathogens could reveal the host survival ability (Jiang et al., 2020). Levofloxacin-resistant (Lev-R) *Vibrio alginolyticus* was found to be less pathogenic than levofloxacin-susceptible (Lev-S) strains (Jiang et al., 2020). The metabolome in zebrafish induced by Lev-R strain displayed a lower bacterial elimination as well as weaker immune response compared to Lev-S strain. Upon exploring the metabolome difference of Lev-R and Lev-S *V. alginolyticus*-infected zebrafish, higher abundance of maltose was found in Lev-S *V. alginolyticus*-infected zebrafish due to higher regulation of alpha-amylase expression (Jiang et al., 2020). As a result, maltose was identified as the crucial biomarker in response to the different metabolomic strategies. Subsequently, exogenous 225 µg maltose was examined to regulate the metabolome in Lev-R *V. alginolyticus* infected zebrafish. It was evident that maltose increased the lysozyme activity which promoted Lev-R bacterial killing. The ability of exogenous maltose to regulate immune gene expression contributed to the higher lysozyme expression and activity. Binding of lysozyme with bacterial cell wall via electrostatic interactions led to elimination of pathogens from the host (Fig. 4) (Jiang et al., 2020). Lower membrane potential of Lev-R bacteria resulted in poorer lysozyme binding and hence poor clearance of bacteria. Metabolic modulation triggered by metabolite maltose strengthened the host innate immune system through promoted lysozyme

**Fig. 4 Exogenous maltose promoted Lev-S *V. alginolyticus* killing in zebrafish** The Lev-S *V. alginolyticus* induced a higher amount of lysozyme and have a higher electric charge which promote to better lysozyme binding.



activity, higher membrane polarisation and thus enhanced the elimination of Lev-R *V. alginolyticus* (Jiang et al., 2020).

In addition, a recent study also employed metabolite-enabled metabolic reprogramming to elucidate metabolic mechanisms of colistin-resistant *V. alginolyticus* in zebrafish

(Li et al., 2020). Reduction of central carbon and energy metabolism, attributing the disruption of pyruvate cycle are the key mechanisms associated with colistin resistance (Li et al., 2020). This study revealed that exogenous pyruvate activated the pyruvate cycle and thus promoted colistin

**Table 1** Recent studies published on metabolite-mediated metabolic modulation to potentiate antibiotic activity and improve anti-infection ability of host.

Study	Metabolite	Antibiotic	Gram-negative bacterium	Mechanism	Reference
<i>In silico coupled in vitro</i>	Uracil	Ampicillin, Ciprofloxacin, Gentamicin	<i>E. coli</i>	Increase purine biosynthesis and cellular energy consumption thus failure to restore bacterial homeostasis	(Yang et al., 2019)
<i>In vitro</i>	Fructose	Kanamycin	<i>E. tarda</i>	Increase of PMF thus stimulate antibiotic uptake	(Su et al., 2015)
	Alanine	Kanamycin	<i>E. tarda</i>		(Peng et al., 2015a, b)
	Glucose	Kanamycin	<i>E. tarda</i>		
	Glutamate	Kanamycin	<i>E. coli, E. tarda</i>		(Su et al., 2018)
	Glucose, mannitol, fructose, pyruvate	Gentamicin	<i>E. coli</i>		(Allison et al., 2011)
	L-arginine	Gentamicin	<i>E. coli, P. aeruginosa</i>		(Lebeaux et al., 2014)
	L-lysine	Kanamycin, Gentamicin, Amikacin	<i>A. baumannii, E. coli, K. pneumoniae</i>		(Deng et al., 2020)
	Fumarate	Tobramycin	<i>P. aeruginosa</i>		(Meylan et al., 2017)
<i>In vivo</i>	Mannitol	Tobramycin	<i>P. aeruginosa</i>		(Barraud et al., 2013)
	Glucose and fumarate	Ciprofloxacin	<i>E. coli</i>	Increase carbon and energy metabolism to sensitise bacteria to ciprofloxacin	(Gutierrez et al., 2017)
	Glucose	-	<i>E. tarda</i>	Increase host's immune protection	(Zeng et al., 2017)
	Maltose	Levofloxacin	<i>V. alginolyticus</i>	Increase lysozyme activity thus promote bacteria binding with levofloxacin	(Jiang et al., 2020)
	Pyruvate	Colistin	<i>V. alginolyticus</i>	Increase membrane potential thus promote bacteria binding with colistin	(Li et al., 2020)
	Glucose	Amoxicillin	<i>Bacteroides thetaiotaomicron</i>	Alter gut microbiota and promote carbohydrate metabolism	(Cabral et al., 2019)
	Indole-3-acetic acid (IAA)	Ciprofloxacin	<i>E. coli</i>	Alter gut microbiota and promote energy metabolism	(Liu et al., 2021)

killing *in vivo* in zebrafish and *in vitro* against multidrug-resistant *K. pneumoniae*, *E. coli* and *E. tarda*. Li et al., (2020) highlighted their key finding indicated lower binding of colistin to the outer membrane in resistant strains was due to the reduced Na(+)-NQR complex and membrane potential of colistin-resistant *V. alginolyticus* strains compared to colistin-susceptible *V. alginolyticus* strains due to attenuated central carbon and energy metabolism. Exogenous 5mM pyruvate increased membrane potential by elevating pyruvate cycle and hence promoted colistin binding to the bacterial membrane (Li et al., 2020).

The role of host diet in response to antibiotic treatment within the microbiome has been explored in recent studies. The gut microbiome is an ecosystem comprising a diverse array of microbes that utilise nutrient metabolism depending on environmental factors such as presence of oxygen, electron acceptor availability and nutrient condition (Faber et al., 2017; Hughes et al., 2017; Reese et al., 2018). Antibiotic treatments that resulted in disturbances in microbiome homeostatic and lead to dysbiosis have been well documented (Cho et al., 2012; Cox et al., 2014; Mahana et al., 2016). Using a combination of metagenomics and metatranscriptomics, addition of glucose elevated sensitivity to amoxicillin treatment within the murine gut microbiome (Cabral et al., 2019). Amoxicillin-treated mice displayed a significant reduction in carbohydrate metabolism, suggesting that the microbial community is less metabolically active (Cabral et al., 2019). The reduction changes could occur via two related mechanisms, amoxicillin *in vivo* might select sub-population of microbiota with reduced metabolic gene expression or microbiota might actively lower metabolism in response to antibiotic stress Adolfsen & Brynildsen 2015; Belenky et al., 2015b; Shan et al., 2017; Cabral et al., 2018; Cabral et al., 2019). Metatranscriptomics data revealed that the sensitivity of *Bacteroides thetaiotaomicron* to amoxicillin depends on carbohydrates availability (Cabral et al., 2019). Additionally, higher growth rate and susceptibility to amoxicillin were observed using *B. thetaiotaomicron* isolates obtained from murine cecum in the presence of glucose (Cabral et al., 2019). This indicates that the host diet plays an important role in modulating microbiota response to antibiotics.

Alterations of the microbiota diversity, antibiotic susceptibility and dietary composition are associated with various infectious diseases. Liu et al., (2021) demonstrated addition of indole-3-acetic acid (IAA) improved ciprofloxacin killing against *E. coli* in high-fat diet (HFD) fed mice. Dietary composition such as high-fat or low-fibre diets have a substantial impact on microbiota composition, diversity and metabolome (Wu et al., 2011; Simpson & Campbell, 2015). Metabolomics analysis revealed an alteration of gut microbiota such as *Bacteroidaceae* which mainly produces IAA

in HFD fed mice (Liu et al., 2021). The alteration resulted in reduced production of IAA where IAA is involved in tryptophan metabolism (Liu et al., 2021). The perturbed tryptophan metabolism impairs the efficacy of antibiotic treatment (Liu et al., 2021). Therefore, addition of IAA was found to promote energy metabolism and significantly potentiated ciprofloxacin activity in the eradication of methicillin resistant *S. aureus* persisters (Liu et al., 2021). These results highlight regulators of bacterial metabolism that may serve as potential antibiotic adjuvants to fight against antibiotic-resistant pathogens (Liu et al., 2021). *In vivo* studies enable the understanding of the interplay of host immunity, host diet, metabolism and antimicrobial susceptibility during metabolite feeding which are essential in the clinical translation of antibiotic-resistant infections management.

Taken together, these findings as summarised in Table 1 strongly suggest that metabolite feeding which alters metabolic pathways could serve as a potential strategy to treat antibiotic-resistant infections.

## 4 Conclusion and future directions

In summary, the present review demonstrates that exogenous metabolite-mediated metabolic reprogramming offers an attractive approach to overcome antibacterial resistance. Exogenous metabolites have the possibility to (i) drive central carbon metabolism (ii) elevate cellular respiration (iii) strengthen innate immune system and (iv) alter composition of gut microbiota and thereby potentiate uptake of antibiotics. Metabolic modelling approach has been increasingly employed to elucidate mechanism(s) of antibiotic killing and bacterial metabolic response during infection. Integrative modelling with multi-omics data such as metabolomics and transcriptomics data enabled identification of the significantly lowered levels of metabolites as a consequence of metabolic suppression by antibiotics. Inspired by this notion, exogenous addition of metabolites could trigger elevation of metabolic activity and thus enhanced effectiveness of antibiotic treatment.

Future research focusing on the translation of bioinformatics findings, analysis and integration of omics data are warranted to further explore other potential regulating molecules (Wang et al., 2021). Regulation of metabolites offer an inexpensive and non-antibiotic to address the problem of antimicrobial resistance (Brunner et al., 2016; M. Yang et al., 2018a, b; Annunziato, 2019>; Rosenberg et al., 2020). Machine learning approach can provide mechanistic insight on how metabolic mechanism interfaces with antibiotic lethality and enable further exploration into the underlying principle of metabolic reprogramming (Yang et al., 2019). Promisingly, genome-scale metabolic modelling



which serves as an *in silico* platform for metabolic analyses at network level to compare with *in vitro* and *in vivo* for validation will shed light on the translatability of bioinformatics research. This approach provides a broad scope of application in drug discovery as metabolomics is a universal language to elucidate metabolism mechanisms in various species. The similarity of basic metabolic pathways among organisms enables metabolic modulation application not only in bacteria but other organism species.

Nonetheless, there are challenges in bringing this exogenous metabolite feeding approach to the clinical setting. The *in silico* metabolic studies were conducted under preserved conditions where the enzyme stoichiometry is controlled, without considering the pharmacokinetics and pharmacodynamics of antibiotics. Interestingly, the approach of metabolic network modelling coupled with *in vitro* or *in vivo* experiments enables the construction of detailed mechanistic models to provide precise understanding of metabolic changes. On this notion, modelling a predictive and comprehensive model requires interdisciplinary knowledge and advanced computational tools (Vasilakou et al., 2016). Furthermore, there is still a lack of *in vivo* studies on metabolite feeding induced metabolic modulation to enhance antibiotic activity. The translation of *in vitro* to *in vivo* experiments remains highly challenging due to the need for precise understanding of biological parameters and identification of relevant metabolite concentrations. Determining an optimal metabolite concentration is a major concern to facilitate the development of effective and safe antimicrobial combination therapy approaches. Overall, a deeper understanding of the complex bacterial metabolic networks would facilitate metabolite feeding in becoming a promising metabolic-based adjuvant therapy to enhance existing antibiotic's activity against multidrug-resistant Gram-negative pathogens.

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## Declarations

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