Metabolic Vulnerability of ESKAPE Pathogens to Exogenous L-Cysteine in M9 Media: Restoring Meropenem Susceptibility

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Abstract

The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) represent a critical threat to global health due to their multidrug resistance, virulence, and limited therapeutic options. The escalating failure of conventional antibiotics necessitates the identification of novel metabolic vulnerabilities that can be exploited for therapeutic intervention. Among metabolic targets, sulphur and redox metabolisms have gained increasing attention, as intracellular redox balance is essential for bacterial survival under stress and nutrient limitation. L-cysteine, a sulphur-containing amino acid, occupies a central position in bacterial metabolism as a precursor of glutathione and a regulator of cellular redox homeostasis. However, excess cysteine can induce oxidative stress through thiol auto-oxidation and the generation of reactive oxygen species, suggesting that cysteine availability may differentially impact bacterial viability. This study provides a comparative analysis of dose-dependent, species-specific growth responses to exogenous L-cysteine across ESKAPE pathogens under nutritionally defined 0.5X M9 minimal medium conditions. Experiments were conducted over 24 hours with technical triplicates. Results demonstrate that cysteine exerts divergent effects across species. In *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Enterobacter* spp., exogenous cysteine significantly inhibits growth in a dose-dependent manner, while in *Acinetobacter baumannii* and *Staphylococcus aureus*, it enhances growth. The inhibitory effect of L-Cysteine on susceptible ESKAPE pathogens prompted further investigation into its potential as an antibiotic adjuvant. Meropenem (Carbapenem class antibiotic) resistant *Klebsiella pneumoniae* JIIT/NS72/I4 strain present in the lab was used for validating the above hypothesis. Disc diffusion assays demonstrated that while meropenem-resistant JIIT/NS72/I4 showed no zones of inhibition with meropenem alone, co-supplementation with 10 mM L-Cysteine exhibited some growth inhibition across all tested concentrations of Meropenem. Notably, Meropenem susceptibility could be restored with 10 mM L-Cysteine under nutrient-limited 0.5X M9 conditions, suggesting cysteine's adjuvant potential with antibiotic under stress conditions. These findings reveal a previously unrecognised metabolic bifurcation within the ESKAPE group, highlighting species-specific differences in sulphur assimilation, redox buffering capacity, and metabolic wiring under nutrient-restricted conditions. The study advances understanding of ESKAPE pathogen physiology and identifies cysteine-induced redox imbalance as a tractable, species-selective vulnerability with potential for pathogen-selective therapeutic intervention.

Keywords: multi drug resistance; cysteine; Antibiotics; Cysteine Metabolism; Meropenem

1. Introduction

The ESKAPE pathogens *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species represent the most clinically significant multidrug-resistant bacterial threats worldwide. These organisms are responsible for the majority of nosocomial infections and exhibit remarkable abilities to evade antimicrobial therapies through diverse resistance mechanisms [1]. Recent projections from the Global Burden of Disease study 2021 suggest that by 2050, AMR-associated mortality could escalate to 8.22 million deaths annually [2]. A substantial proportion of this burden is driven by ESKAPE pathogens, which are characterised by high mortality rates due to limited treatment options and frequent treatment failure. Consequently, the World Health Organisation has designated several

ESKAPE members, particularly carbapenem-resistant *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*, as "critical priority" pathogens in its 2024 update, highlighting the urgent need for novel therapeutic strategies [3-4]. Traditional antibiotic development has struggled to keep pace with the rapid evolution of resistance in these species, necessitating the exploration of alternative antimicrobial approaches that exploit bacterial physiological weaknesses.

Metabolic vulnerabilities represent an emerging frontier in antimicrobial research. Unlike conventional antibiotics that target specific cellular processes such as cell wall synthesis or protein translation, metabolic interventions exploit the fundamental biochemical requirements and constraints of bacterial physiology. Recent studies have demonstrated that bacterial metabolism is highly sensitive to growth conditions, with minimal media revealing phenotypic and transcriptomic profiles distinct from those observed in nutrient-rich environments [5]. Importantly, nutritionally starved conditions can mimic aspects of *in vivo* dormancy or persistence, states in which bacteria reduce metabolic activity, alter energy utilisation pathways, and display heightened tolerance to antibiotics. Understanding metabolic behaviour under such nutrient-limited conditions is therefore critical for identifying vulnerabilities relevant to chronic and persistent infections. Amino acids are essential for bacterial survival and growth in nutrient-limited conditions. Numerous amino acids serve as supplementary sources of carbon and nitrogen in addition to their core function as building blocks for protein synthesis. This allows for metabolic flexibility in situations where favoured substrates, like glucose, are in short supply. For instance, in glucose-limiting conditions, organisms like *Campylobacter jejuni* and a number of ESKAPE pathogens can use glutamate and aspartate as their primary energy substrates [6]. Moreover, amino acids like glutamate and proline function as compatible solutes and build up inside cells as Osmo protectants, which protect cells from external stress and osmotic shock [7]. These varied functions highlight how crucial amino acid metabolism is to maintaining bacterial physiology, especially in low-resource settings where catabolic and biosynthetic efficiency are crucial. However, the boundary between nutrient and toxin can be narrow. The growth suppression in minimum media brought on by an excess of exogenous amino acids is an established example of amino acid-induced metabolic sensitivity. By preventing the synthesis of isoleucine or by blocking vital enzymes like threonine deaminase, serine has been demonstrated to prevent the growth of a variety of bacteria [8]. When given exogenously in low-nutrient conditions, other amino acids, such as leucine and glycine, can also result in serious nutritional imbalances or pathway disruptions, implying that even common proteinogenic amino acids can change from being necessary nutrients to strong metabolic inhibitors. In contrast, sulphur-containing amino acids, particularly L-cysteine, exhibit mechanistically distinct modes of toxicity owing to their unique redox and chemical reactivity. Unlike other amino acids, cysteine can directly influence intracellular redox balance, promote reactive oxygen species (ROS) generation, and interact with metal ions through its thiol group. However, exogenous cysteine if present in high concentration, can paradoxically have harmful effects on bacteria. Research on model organisms like *Salmonella enterica* and *Escherichia coli* has shown that cysteine toxicity involves several mechanisms, such as the production of hydrogen sulphide (H_2S) through enzymatic degradation, the generation of reactive oxygen species (ROS), and the acceleration of central metabolism and respiration [9-11]. The bactericidal potential of cysteine has been reported in several bacterial species, for eg, in *Xanthomonas citri* pv. *glycines*, cysteine at millimolar concentrations resulted in growth inhibition (2.5 mM) or complete growth arrest (10 mM) in M9 minimal medium [12]. The toxic effects were attributed to H_2S generation and promotion of the Fenton reaction, leading to hydroxyl radical formation and oxidative damage to cellular macromolecules [12].

Despite growing interest in metabolic targeting, a comprehensive comparative evaluation across all ESKAPE pathogens under metabolically constrained conditions remains lacking. Most prior studies have focused on individual model organisms or have been conducted in nutrient-rich media, potentially masking intrinsic metabolic liabilities. Minimal media such as M9 impose defined nutritional limitations that restrict metabolic plasticity and compel bacteria to rely on core biosynthetic, sulphur assimilation, and redox-balancing networks without access to exogenous

nutrient buffering. Such conditions more closely approximate nutrient deprivation encountered during host-associated persistence and dormancy. Within this framework, L-cysteine was selected as a metabolically active sulphur amino acid with dual redox potential, and its effects were systematically evaluated across five ESKAPE pathogens in M9 minimal medium. Strikingly, a clear organism-dependent bifurcation emerged: cysteine supplementation enhanced growth in certain pathogens, consistent with improved sulphur assimilation and redox support, while exerting pronounced growth-inhibitory effects in others under the same nutritionally constrained conditions. These findings demonstrate that minimal medium culture unmasks species-specific differences in cysteine utilisation, redox buffering capacity, and detoxification mechanisms. Collectively, this work identifies sulfur homeostasis as a context-dependent determinant of bacterial survival and exposes previously unrecognised metabolic vulnerabilities that may be exploitable for therapeutic intervention against multidrug-resistant ESKAPE pathogens.

2. Materials and Methods

2.1 Bacterial strain and culture conditions

Table 1 lists all of the strains utilised in this investigation, along with the conditions (as per MTCC guidelines) under which they were cultured. A multidrug-resistant (MDR) strain of *Klebsiella pneumoniae* (JIIT/NS72/I4) previously isolated in our lab from wastewater in Noida, India, was used in the present study for antibiotic susceptibility (13). After streaking the bacterial strains on Luria-Bertani (LB) agar (HiMedia) plates, they were incubated for the entire night at 37 °C. After being inoculated into LB broth, a single colony was cultivated for the entire night at 37 °C while being shaken at 150 RPM. For all subsequent experimental investigations, aliquots of these overnight cultures were kept at -80 °C.

Table1: Different bacterial strains used in this study and their growth conditions

S.No	Bacterial Strain	Incubation period	Optimum Temperature
1	*Enterococcus Faecium MTCC9728*	24 Hours	37 °C
2	*Staphylococcus Aureus MTCC737*	12 Hours	37 °C
3	*Klebsiella pneumoniae MTCC 4030*	24 Hours	37 °C
4	*Acinetobacter Baumannii MTCC9829*	48 Hours	30 °C
5	*Pseudomonas aeruginosa MTCC424*	24 Hours	37 °C
6	*Enterobacter spp MTCC 509*	24 Hours	30 °C
7	JIIT/NS72/I4	24 Hours	37 °C

2.2 Preparation of L-Cysteine Solutions

L-cysteine hydrochloride monohydrate (Calbiochem, Cat. No. 2430-100GM) was dissolved in sterile distilled water immediately before use to prepare stock solutions and filter-sterilised through a 0.22 μm membrane.

2.3 Determination of the effect of cysteine on the growth of ESKAPE pathogens

The experiment starts with streaking bacterial glycerol stocks stored at -80 ºCover Luria-Bertani (LB) agar and incubating for 18 to 24 hours at 37 °C. To create starter cultures, single colonies were inoculated into LB broth and cultivated overnight at 37 °C while being shaken at 200 rpm. Before the experimental setup, overnight cultures were standardised based on optical density (OD_{600}) to ensure uniform starting cell density across all conditions. The cultures were then diluted 1:100 in 0.5X M9 medium for experimental tests. A half-strength (0.5X) M9 medium was used to impose stress conditions compared to standard 1X M9, thereby minimising nutrient effects and enabling clearer

detection of cysteine-induced metabolic responses. Standard procedures were followed to generate a 5X M9 minimum medium stock, which contained (per litre): 64 g Na_2HPO_4, 15 g KH_2PO_4, 2.5 g NaCl, 5 g NH_4Cl, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.4% glucose as the only carbon source. Final doses of 2 mM, 5 mM, and 10 mM of cysteine were applied. Cultures were incubated at 37 °C with shaking at 150 rpm. Bacterial growth was monitored by measuring optical density (OD) at 595 nm at 2-hour intervals for up to 24 h. A positive control consisting of M9 medium with inoculum but without cysteine and a negative control containing uninoculated M9 medium were included. All experiments were performed in triplicate to ensure reproducibility, and data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA to evaluate the effect of cysteine concentration on bacterial growth. Analysis was performed at the 6-hour time point, corresponding to the mid-to-late exponential phase, where treatment effects were clearly distinguishable. A p-value < 0.05 was considered statistically significant.

2.4 Antibiotic susceptibility of JIIT/NS72/I4 against Meropenem in the presence of cysteine

The effect of the meropenem–cysteine combination was evaluated using the disk diffusion method. A single colony from a freshly streaked plate was used to prepare a bacterial suspension equivalent to a 0.5 McFarland standard in 0.8% saline solution. The standardised inoculum was uniformly swabbed onto the surface of both MHA and 0.5X M9 agar plates. Antibiotic discs containing varying concentrations of meropenem (10.24 µg/disc to 0.64 µg/disc) were placed on the agar surface. For test conditions, plates were supplemented with 10 mM cysteine, while control plates contained meropenem discs alone without the cysteine. The plates were incubated at 37 °C for 18–24 hours. Following incubation, zones of inhibition were measured to assess bacterial susceptibility and to evaluate the effect of cysteine on meropenem activity.

3 Results

3.1 Species-specific growth response to exogenous L-Cysteine in M9 Minimal Medium

All six species were cultivated in M9 medium supplemented with L-cysteine to assess the impact of exogenous L-cysteine on the viability of ESKAPE pathogens. Out of all, *Enterococcus faecium*, due to its strict dietary needs and reliance on exogenous amino acids and vitamins, was unable to grow in M9 medium under our experimental conditions. **Fig 1** exhibits species-specific growth response on exposure to exogenous Cysteine. When compared to the control (no cysteine; Orange), the addition of L-cysteine (2mM: Grey; 5mM: Yellow; 10 mM: Blue) significantly and dose-dependently reduced the growth of *Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter spp*. In comparison to the control, 10 mM cysteine resulted in growth inhibition of *Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter spp*. by 43.5%, 24.4%, and 13.1%, respectively. In contrast, the growth accelerated dramatically by 202.40% and 185.60% for *Staphylococcus aureus* and *Acinetobacter baumannii,* respectively, in response to exogenous cysteine (**Table2**).
Statistical analysis using one-way ANOVA at the 6-hour time point confirmed that these differences were significant. Significant growth inhibition was observed in *Pseudomonas aeruginosa* ($p < 0.001$), *Klebsiella pneumoniae* ($p < 0.001$), and *Enterobacter* spp. ($p < 0.01$), whereas *Acinetobacter baumannii* ($p < 0.01$) and *Staphylococcus aureus* ($p < 0.04$) exhibited significant growth promotion.

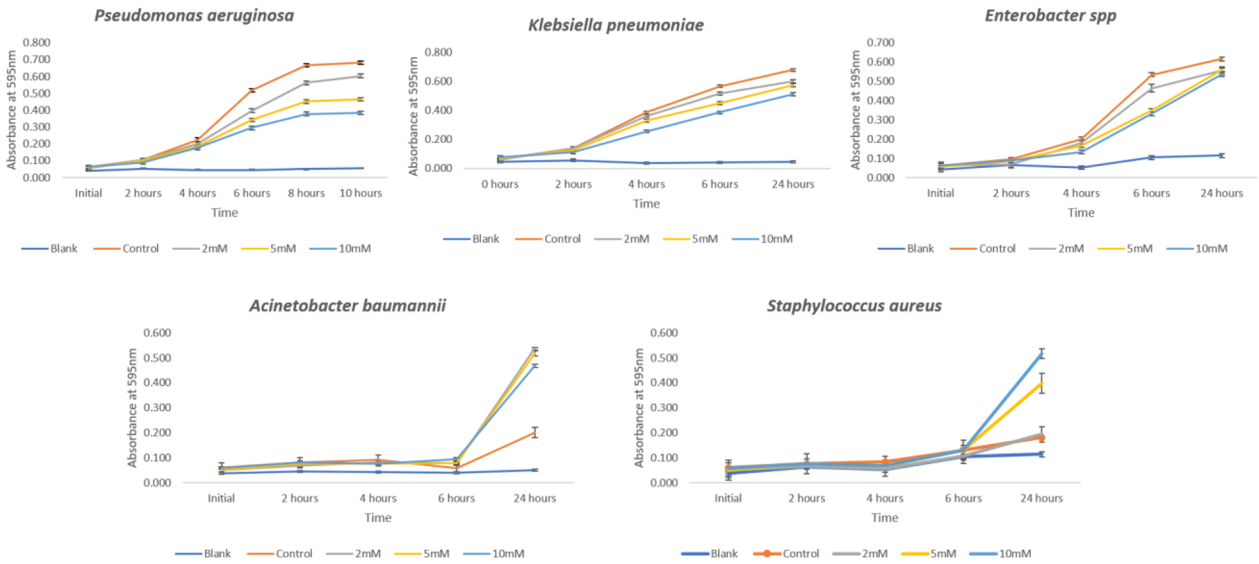

Fig 1: Divergent effect of L-Cysteine on the growth of ESKAPE pathogens showing $0D_{595}$ Vs Time graph

Table 2: Growth parameters of ESKAPE pathogens in response to cysteine treatment at 10 mM concentration

Bacterial Species	**Growth Response**	**Change in Growth Rate (%)**
Staphylococcus Aureus MTCC737	Promotion	+202.40%
Klebsiella pneumoniae MTCC 4030	Inhibition	−24.4%
Acinetobacter Baumannii MTCC9829	Promotion	+185.60%
Pseudomonas aeruginosa MTCC424	Inhibition	−43.5%
Enterobacter spp MTCC 509	Inhibition	−13.1%

3.2 Antibiotic susceptibility of JIIT/NS72/I4 against Meropenem in the presence of cysteine

The antimicrobial activity of meropenem was evaluated across six concentrations (0.64–10.24 µg/disc) in two growth media, Mueller-Hinton Agar (MHA) and M9 minimal medium, both in the absence and presence of 10 mM L-Cysteine supplementation. With meropenem discs alone, no zone of inhibition at any tested concentration in either MHA or 0.5X M9 medium was observed (**Fig 2A and 2B**), indicating that the bacterial isolate demonstrated resistance to meropenem under experimental conditions. In contrast, when meropenem discs were supplemented with 10 mM L-Cysteine, clear zones of inhibition were observed across all tested meropenem concentrations in both media, demonstrating sensitisation to the antibiotic. In MHA, the zones of inhibition ranged from 8 mm (at 0.64 µg/disc) to 12 mm (at 10.24 µg/disc) (**Table 3**), showing a modest but consistent increase in the zone of inhibition with rising meropenem concentration, providing proof of concept of cysteine's ability to act as an adjuvant to antibiotic for restoring their susceptibility. However, despite the appearance of a zone of inhibition in MHA media, the JIIT/NS72/I4 strain, as per FDA guidelines,

remains resistant to the antibiotic in combination with 10mM cysteine. Increasing the cysteine concentration may resensitize this strain, but since it may cause toxicity to human cells, no efforts were made to carry out experiments with increased cysteine concentration.

Intriguingly, in 0.5X M9 medium, substantially larger zones of inhibition were recorded (**Fig. 2C and 2D**), ranging from 20 mm (at 2.56 µg/disc) to 30 mm (at both 10.24 and 5.12 µg/disc) (**Table 3**), indicating a significant effect of cysteine in restoring meropenem sensitivity as opposed to MHA.

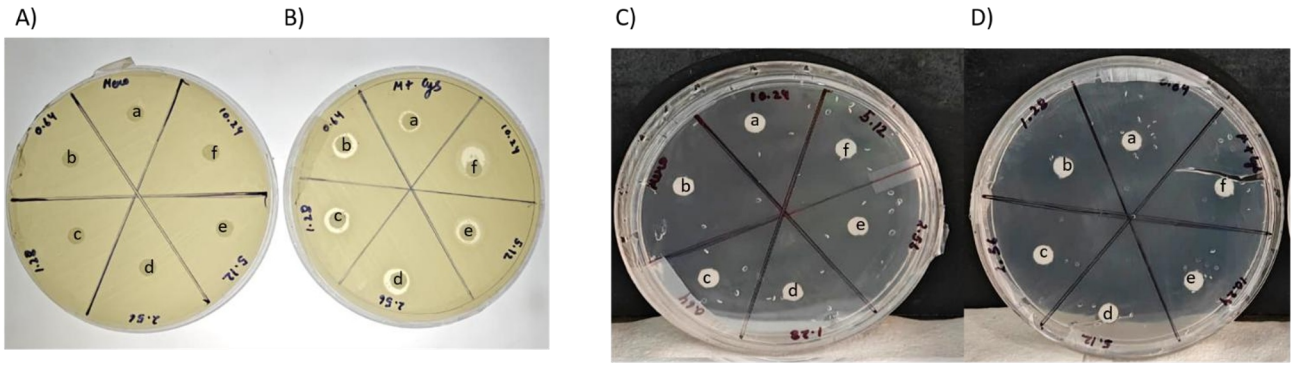

Fig 2: **Effect of exogenous L-Cysteine on the susceptibility of *S4* (*Klebsiella pneumoniae*) to meropenem under different nutrient conditions using the disc diffusion assay. (A)** Mueller–Hinton Agar (MHA; nutrient-rich medium) showing no zones of inhibition around meropenem discs at any tested concentration in the absence of L-Cysteine, indicative of meropenem resistance. **(B)** Mueller–Hinton Agar (MHA; nutrient-rich medium) showing visible zones of inhibition around meropenem discs supplemented with 10 mM L-Cysteine, demonstrating partial restoration of antimicrobial susceptibility under nutrient-rich conditions. **(C)** M9 agar (nutrient-limited medium) showing no zones of inhibition around meropenem discs at any tested concentration in the absence of L-Cysteine. **(D)** M9 agar (nutrient-limited medium) showing markedly enhanced zones of inhibition around meropenem discs supplemented with 10 mM L-Cysteine, reflecting greater sensitisation to meropenem under nutrient-restricted conditions. Disc concentrations: **a)** 0.1 µg/disc, **b)** 0.64 µg/disc, **c)** 1.28 µg/disc, **d)** 2.56 µg/disc, **e)** 5.12 µg/disc, **f)** 10.24 µg/disc.

Table 3: Zones of inhibition for various concentrations of meropenem discs supplemented with 10 mM of L-Cysteine

Concentration of Meropenem per disc (µg/disc)	**Zone of inhibition in mm supplemented with 10mM of L-Cysteine**	
	In MHA	In 0.5X M9 agar
10.24	12	30
5.12	10	30
2.56	10	20
1.28	10	27
0.64	8	23
0.1	5	21

4 Discussion:

The present study reinforces the species-specific growth response of ESKAPE pathogens to exogenous L- cysteine, were it promotes the growth of *Acinetobacter baumannii* and *Staphylococcus aureus* and inhibits the growth of *Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter spp*. The metabolic adaptability of ESKAPE pathogens in the nutrient-limited M9 minimum medium is highlighted by this divergence. The distinct metabolic wiring of each ESKAPE pathogen is shown by the sharp difference between the "toxic" response in *Pseudomonas aeruginosa* and the "beneficial" response in *Acinetobacter baumannii* and *Staphylococcus aureus*.
The mechanisms behind the inhibitory impact seen in *Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter spp*. are probably similar to those previously reported in other Gram-negative bacteria. Rapid conversion of cysteine to pyruvate can overstimulate the TCA cycle, which may result in an excessive increase in intracellular reactive oxygen species (ROS) and increased respiration [10]. The lack of complex organic components in minimum medium may lessen the ability of bacteria to withstand an oxidative burst, which leaves these pathogens more susceptible. Furthermore, exposure to millimolar amounts of cysteine can produce greater concentrations of H_2S, which can become hazardous even though it is frequently protective at low levels. If these particular pathways are responsible for the suppression seen in our growth curve trials, further research utilising respiration assays and ROS quantification will be required. On the other hand, the growth-promoting impact seen in *Acinetobacter baumannii* and *Staphylococcus aureus* marks a substantial divergence from cysteine's documented toxicity. Cysteine can be a useful source of sulphur and carbon/energy in M9 minimum media, where nutrients are limited. The extra nutritional supply promotes improved growth if an organism can effectively convert cysteine to pyruvate without causing a fatal oxidative imbalance. Extreme metabolic fitness and the capacity to use a variety of carbon sources in limited settings are characteristics of *Acinetobacter baumannii* [5][14]. Its ability to flourish on exogenous cysteine points to a higher capacity to disentangle oxidative stress caused by cysteine from cysteine-driven nutritional uptake. Similar to this, *Staphylococcus aureus* might have strong antioxidant and sulphur-fixation systems that enable it to efficiently counteract the harmful consequences of cysteine catabolism.

The analysis of cysteine biosynthesis pathways in each species may also provide some rationale for the above observations. There are two pathways for cysteine biosynthesis in the organisms that showed cysteine-induced growth inhibition: The Reverse transsulfuration (RTS) pathway, which produces cysteine from methionine-derived intermediates, and the *de novo* cysteine biosynthetic pathway (via L-serine) (**Table 4**). On the other hand, *Acinetobacter baumannii* and *Staphylococcus aureus*, the organisms that showed a growth-promoting response to cysteine, seem to rely mainly on the *de novo* synthesis pathway and do not have a functional RTS pathway. Under nutrient-limited 0.5X M9 medium conditions, the observed phenotypes can be plausibly explained by this metabolic difference. In metabolically adaptable Gram-negative organisms with both mechanisms, RTS can sustain endogenous cysteine synthesis even in the face of dietary stress. When exogenous cysteine is supplemented, it can cause an intracellular cysteine excess that might overload detoxifying mechanisms, disrupt redox equilibrium, and cause inhibitory consequences such as metabolic overactivation or ROS overproduction. In species without the RTS pathway, on the other hand, cysteine production is solely dependent on the *de novo* process, which may perform less well when nutrients are scarce because of decreased sulphur flux or limited precursor availability. Exogenous cysteine supplementation probably makes up for metabolic limitations in these species, promoting growth rather than causing toxicity. It is important to note, however, that the correlation observed between RTS pathway status and cysteine response across ESKAPE pathogens remains correlative, and causality cannot be established from the present data alone. Confounding factors such as differences in outer membrane composition, efflux pump activity, or broader metabolic architecture across species cannot be excluded. A definitive mechanistic link between RTS pathway possession and cysteine-induced growth inhibition would require targeted approaches such as gene expression

profiling, RTS pathway knockouts, or isogenic mutant studies, which are proposed as important directions for future investigation.

Table 4: Distribution of Cysteine Biosynthetic Pathways Among Tested ESKAPE Pathogens [15]

Organism	De Novo Cysteine Biosynthesis	Reverse Transsulfuration (RTS) Pathway	Observed Effect of Exogenous Cysteine in M9
Pseudomonas aeruginosa	Present	Present	Growth inhibition (dose-dependent)
Klebsiella pneumoniae	Present	Present	Growth inhibition (dose-dependent)
***Enterobacter* spp.**	Present	Present	Growth inhibition (dose-dependent)
Acinetobacter baumannii	Present	Absent (no functional RTS)	Growth promotion
Staphylococcus aureus	Present	Absent (no functional RTS)	Growth promotion

Importantly, the current work also shows that cysteine greatly influences antibiotic sensitivity, especially when meropenem resistance is present. The absence or minimal zone of inhibition in MHA and M9 medium without cysteine indicated that the tested isolate was resistant to meropenem under standard conditions. On the other hand, cysteine supplementation led to a significant increase in susceptibility and a significant expansion of the zone of inhibition at all tested dosages. This suggests that cysteine can function as an antibiotic adjuvant under stress conditions.

Overall, these findings demonstrate that exogenous L-cysteine exerts concentration-dependent and species-specific effects on bacterial growth in minimal media. While cysteine displays inhibitory potential against certain Gram-negative ESKAPE pathogens, it may paradoxically enhance growth in others. Therefore, one of the primary metabolic factors controlling cysteine responsiveness in all ESKAPE pathogens may be the presence or absence of the reverse transsulfuration pathway. This finding implies that whether cysteine acts as a metabolic stressor or a nutrient under minimum conditions is strongly influenced by intrinsic variations in the design of the sulphur absorption network. These observations warrant further mechanistic investigations, including assessment of branched-chain amino acid biosynthesis, ROS generation, and sulphur assimilation pathways, to elucidate the molecular determinants governing cysteine responsiveness across clinically significant pathogens.

5 Conclusion

This study demonstrates that exogenous L-cysteine exerts pronounced, species-specific, and dose-dependent effects on the growth of ESKAPE pathogens under nutrient-limited conditions. The observed bifurcation — growth inhibition in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterobacter* spp., and growth promotion in *Acinetobacter baumannii* and *Staphylococcus aureus* — underscores the heterogeneity of sulphur metabolism and redox buffering capacity within this clinically critical pathogen group. The presence of the reverse transsulfuration pathway in inhibition-susceptible species, and its absence in growth-promoted species, provides a plausible framework for understanding these divergent responses. Crucially, the study extends beyond growth physiology to

demonstrate a therapeutically relevant finding: L-Cysteine can function as an effective antibiotic adjuvant, capable of restoring meropenem susceptibility in a carbapenem-resistant *Klebsiella pneumoniae* isolate. The significantly enhanced zones of inhibition observed under nutrient-limited M9 conditions compared to nutrient-rich MHA further suggest that metabolic stress potentiates cysteine's adjuvant activity, pointing toward a context-dependent mechanism of antibiotic resensitisation. Taken together, these findings identify cysteine-induced redox imbalance as a tractable and species-selective metabolic vulnerability within the ESKAPE group. While mechanistic causality remains to be established through future studies involving ROS quantification, respiration assays, and pathway knockout models, the present work lays a compelling foundation for the development of cysteine-based adjuvant strategies as a novel approach to combating multidrug-resistant pathogens.

Acknowledgment:

The authors sincerely acknowledge Prof. R.K. Gupta and Prof. Prerna Diwan at the Department of Microbiology, Ram Lal Anand College, University of Delhi, South Campus, for providing the laboratory facilities and critical discussion necessary to carry out the growth curve analysis. Special thanks are extended to Mr Sushil Kumar for his constant technical support and assistance throughout the experimental work. Vibha Gupta wished to acknowledge the research grant from ICMR, Govt. of India, for financial assistance through grant id ICMR/2020/4780.

Reference:

1. Zalegh I, Bourhia M, Zerouali K, Katfy K, Nayme K, Khallouki F, et al. (2022). Molecular characterization of gene-mediated resistance and susceptibility of ESKAPE clinical isolates to *Cistus monspeliensis* L. and *Cistus salviifolius* L. extracts. *Evidence-Based Complementary and Alternative Medicine*, 2022, 7467279. https://doi.org/10.1155/2022/7467279
2. GBD 2021 Antimicrobial Resistance Collaborators. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. *The Lancet*, 404(10459), 1199–1226. https://doi.org/10.1016/S0140-6736(24)01867-1
3. Bhowmik A, Chakraborty S, Rohit A, Chauhan A. (2024). Transcriptomic responses of extensively drug-resistant *Klebsiella pneumoniae* to N-acetyl cysteine reveals suppression of major biogenesis pathways. *Journal of Applied Microbiology*, 135(6), lxae136. https://doi.org/10.1093/jambio/lxae136
4. Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E, et al. (2025). The WHO bacterial priority pathogens list 2024. *The Lancet Infectious Diseases*, 25(9), 1033–1043. https://doi.org/10.1016/S1473-3099(25)00118-5
5. Guccione E, Leon-Kempis MDR, Pearson BM, Hitchin E, Mulholland F, van Diemen PM, et al. (2008). Amino acid-dependent growth of *Campylobacter jejuni*. *Molecular Microbiology*, 69(1), 77–93. https://doi.org/10.1111/j.1365-2958.2008.06263.x
6. Shahjee HM, Banerjee K, Ahmad F. (2002). Comparative analysis of L-amino acid osmolytes in *Escherichia coli*. *Journal of Biosciences*, 27(5), 515–520. https://doi.org/10.1007/BF02705049
7. Cosloy SD, McFall E. (1973). Metabolism of D-serine in *Escherichia coli K-12*. *Journal of Bacteriology*, 114(2), 685–694. https://doi.org/10.1128/jb.114.2.685-694.1973
8. Kornelsen V, White D, Kumar A. (2025). Phenotypic and transcriptomic changes in *Acinetobacter baumannii*. *Canadian Journal of Microbiology*, 71, 1–8. https://doi.org/10.1139/cjm-2024-0175
9. Oguri T, Schneider B, Reitzer L. (2012). Cysteine catabolism in *Salmonella enterica*. *Journal of Bacteriology*, 194(16), 4366–4376. https://doi.org/10.1128/JB.00729-12

10. Liu Y, Yang K, Jia Y, Shi J, Tong Z, Wang Z. (2020). Cysteine potentiates bactericidal antibiotic activity. *Infection and Drug Resistance*, 13, 2593–2599. https://doi.org/10.2147/IDR.S263225
11. Nonaka G, Takumi K. (2017). Cysteine degradation gene *yhaM* in *Escherichia coli*. *AMB Express*, 7(1), 90. https://doi.org/10.1186/s13568-017-0389-y
12. Pluemjit O, Panthavee P, Pornsuriya S, Sunpapao S. (2020). Role of amino acid cysteine in suppression of *Xanthomonas citri* pv. *glycines*. *Chiang Mai University Journal of Natural Sciences*, 19(4). https://doi.org/10.12982/CMUJNS.2020.0052
13. Kundu A, Antil M, Rana S, Diwan P, Gupta RK, Gupta V. (2022). Surveillance of carbapenem-resistant ESKAPE bacteria. *International Journal of Health Sciences*, 6(S1), 14216–14225. https://doi.org/10.53730/ijhs.v6nS1.8615
14. Álvarez VE, Quiroga MP, Centrón D. (2023). Biomarker identification in *Acinetobacter baumannii*. *mSystems*, 8(3), e0073422. https://doi.org/10.1128/msystems.00734-22
15. Kies PJ, Hammer ND. (2022). Bacterial scavenging of host sulfur metabolism. *Infection and Immunity*, 90(5), e0057921. https://doi.org/10.1128/iai.00579-21