How nutrients shape antibiotic sensitivity of Pseudomonas aeruginosa: food for thought

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Food for thought

Chapter 2

Nutrient-driven metabolic modulation of antibiotic efficacy in *Pseudomonas aeruginosa*

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In submission

Abstract

Pseudomonas aeruginosa is a versatile pathogen that can adapt its metabolism to diverse nutritional environments. It is a frequent cause of chronic respiratory infections, particularly in people with cystic fibrosis (pwCF). In pwCF, the infectious microenvironment is characterized by a unique and patient-specific nutrient environment. The nutrient-rich yet hypoxic mucus suppresses aerobic metabolism and promotes alternative metabolic pathways such as denitrification and fermentation, as well as the establishment of a biofilm-associated lifestyle. These adaptations promote sustained bacterial survival in the CF respiratory tract and may impair the efficacy of antibiotic therapy. This review summarizes how physiologically relevant nutrient environments drive metabolic changes in *P. aeruginosa* and subsequently its responses to antibiotics. We also discuss how CF-related pathophysiology may contribute to nutrient heterogeneity, potentially altering antibiotic effects. In conclusion, the complex interplay between nutrient availability, bacterial metabolism, and antibiotic response may provide both explanations and opportunities for tailoring antibiotic therapies in patients with chronic *P. aeruginosa* infections.

2.1. Introduction

Cystic fibrosis (CF) is associated with the formation of a thick, dehydrated mucus layer, hindering both oxygen (O_2) diffusion and waste clearance (**Figure 1A**), which often results in the establishment of chronic respiratory tract infections. *Pseudomonas aeruginosa,* a versatile Gram-negative opportunistic pathogen, is among the most predominant causes of chronic bacterial respiratory tract infections in adult patients with CF (pwCF)[1,2,3].

Mucus in the respiratory tract of pwCF provides a complex environment with energy substrates that can be efficiently utilized by *P. aeruginosa*[4] (**Figure 1B**). Mucus composition varies substantially between individual patients[5,6] and is spatially distributed across the compartmentalized lung[7]. *P. aeruginosa* is capable of adapting to these varying local environments due to its versatile and well-regulated metabolism[8,9]. In pwCF, *P. aeruginosa* is typically present in a biofilm lifestyle, wherein the biofilm extracellular matrix serves as a protective shield against both host immunity and antibiotics[10]. Mature biofilm structures impose constraints on O_2 and nutrient penetration, leading to the segregation of aerobic and anaerobic metabolic subpopulations which can impact antibiotic treatment effects[11] (**Figure 1C-D**).

Understanding the intricate relationship between diverse nutrient microenvironments and antibiotic responses is key to improving antibiotic treatment of chronic *P. aeruginosa* respiratory tract infections in pwCF. The current review aims to provide an overview of: (i) *P. aeruginosa* metabolic adaptation within clinically relevant CF lung microenvironments; (ii) the influence of changing nutrient environments on biofilm formation and antibiotic sensitivity; and (iii) the role of patient heterogeneity in nutrient diversity and treatment response.

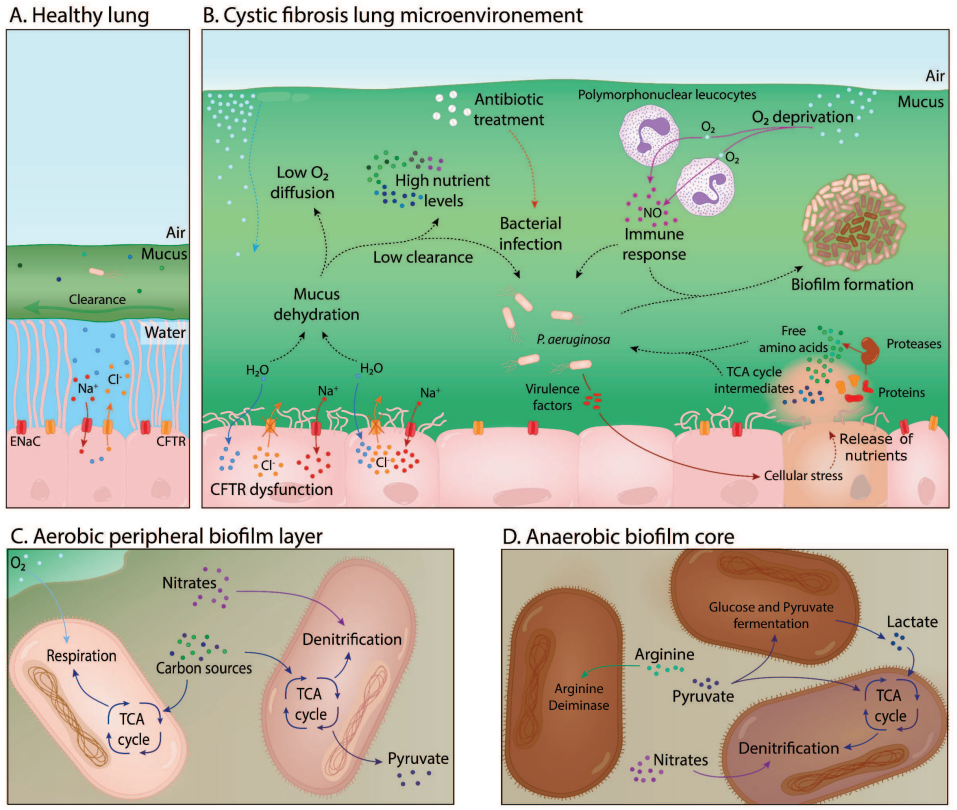

Figure 1. Overview of *P. aeruginosa* in the cystic fibrosis (CF) lung microenvironment. (**A**) demonstrates the efficient clearance of the mucus in healthy lungs with functional transmembrane proteins. (**B**) Accumulation of viscous dehydrated mucus and the microenvironmental influences in biofilm formation. Metabolic processes of cells in the (**C**) peripheral layer and (**D**) core of the biofilm.

2.2. Metabolic adaptation within the nutritional microenvironment in cystic fibrosis

The CF lung microenvironments are characterized by a large diversity in nutrients and O_2 levels. The following section describes *P. aeruginosa* metabolic pathways that are utilized or affected under these nutritional conditions.

Aerobic metabolism

Tricarboxylic acid (TCA) cycle

Amino acids and lactate are systematically increased in the CF lung and represent important nutrients for *P. aeruginosa* energy production through the TCA cycle[12,13,14]. These nutrients have different entry points into the TCA cycle, facilitating metabolic flux versatility (**Figure 2**). For instance, lactate is converted to pyruvate by lactate dehydrogenases (LldDE and LldA) to fuel the TCA cycle[15], while L-glutamate enters halfway in the cycle by glutamate dehydrogenases (GdhA and GdhB)[16]. The resulting electron carriers from the TCA cycle play a crucial role in oxidative phosphorylation (OXPHOS), supporting energy-demanding processes like extracellular matrix production during biofilm maturation[17,18]. Matrix-producing biofilm cells exhibit comparable TCA cycle activity to planktonic cells[19], underlining the high metabolic activity in the peripheral biofilm sub-population where nutrients and O_2 are still available. Finally, *P. aeruginosa* can also operate anaplerotic pathways in the TCA cycle, such as the pyruvate and glyoxylate shunt, if nutrients or O_2 become scarce. Shunting the TCA cycle reduces electron carrier production to maintain the redox balance during low energy demanding circumstances, such as the dormant biofilm core[19,20].

Glucose catabolism

Glucose levels are elevated in the CF respiratory fluid due to active stimulation of glucose leakage from lung epithelial cells and the induction of hemoptysis by *P. aeruginosa*[21–23]. Unlike many organisms, *P. aeruginosa* typically does not prefer glucose as primary carbon source in CF sputum due to the absence of glycolytic enzymes[5]. However, glucose catabolism remains crucial for the bacterial survival and pathogenicity, primarily through efficient production of pyruvate through the Entner-Doudoroff (ED) pathway[19,24]. *P. aeruginosa* employs a combination of enzymes from both the ED and Embden-Meyerhof-Parnas (EMP) pathway for a full carbohydrate degradation loop. The ED-EMP cycle is primarily used for anabolic functions, but also yields precursors for biofilm matrix and cell envelope production[19]. *P. aeruginosa* also actively secretes lipases and elastases to cleave macromolecules into metabolites suitable for the ED-EMP cycle[8,25]. For example, the peptidoglycan component N-acetylglucosamine present in CF sputum is processed within the ED-EMP system to be utilized intracellularly as a

carbon source[26,4]. The heightened levels of glycolytic substrates in CF sputum may contribute to the biofilm-aggregate structure observed in the CF lung. *P. aeruginosa* biofilms grown solely on glucose demonstrate reduced motility aggregate populations[27], suggesting an intricate interplay of glycolytic metabolism in shaping microbial community characteristics in the biofilm.

Amino acids and D-isoforms

Amino acids play a pivotal role in *P. aeruginosa* metabolism within the CF lung environment, serving as carbon or nitrogen sources and building blocks for proteins. Both *P. aeruginosa* and host immune cells contribute to the elevated amino acid concentrations in CF sputum through the excretion of peptidases[28,29]. The abundance of amino acids in CF sputum provides a favorable growth environment, whereby long-term evolution of pathogens in the CF lung can lead

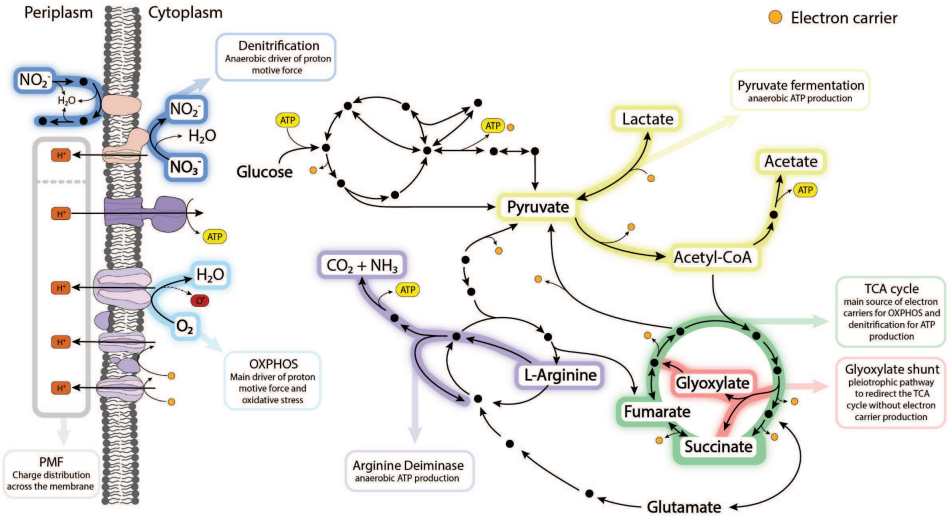

Figure 2. Central carbon metabolism of *P. aeruginosa* and the relation with antibiotic treatment. Metabolic map covering the EDEMP, TCA and urea cycle for electron carrier (orange) production for ATP (yellow) synthesis by oxidative phosphorylation (OXPHOS) and denitrification. Fermentation pathways are illustrated with colored arrows, glucose fermentation in blue, pyruvate fermentation in yellow and arginine fermentation in purple.

P. aeruginosa biofilm formation, with branched chain amino acids leucine, isoleucine and valine influencing *P. aeruginosa* growth rate and swarming motility[31,32]. Biofilm stimulation is isoform-dependent, as the D-isoform of leucine inhibits biofilm formation[32–36]. In contrast, other studies reported D-amino acid supplementation did not significantly improve survival outcomes in mice[35], and anti-biofilm effects of D-isoforms disappeared after several days of incubation[37]. These conflicting results highlight the relevance for further studies into the driving mechanisms of amino acids in *P. aeruginosa* infection in the CF lung.

Anaerobic metabolism

The dehydrated and thick mucus layer in the CF lung reduces O_2 diffusion. Levels of O_2 are further reduced by neighboring cells, such as lung epithelial cells and polynuclear monocytes (PMNs)[38,39]. This O_2 restriction contributes to the establishment of fully anaerobic microenvironments within the mature biofilm structure. In response to these O_2 constraints, *P. aeruginosa* employs adaptive strategies, utilizing two fermentation pathways and shifting from O_2 to nitrates as electron acceptors.

Glucose and pyruvate fermentation

The fermentation of glucose comprises two steps: the initial conversion of glucose to pyruvate, followed by the subsequent fermentation of pyruvate into lactate, acetate and succinate[40]. Glucose fermentation to pyruvate is typically influenced by redox constraints[41]. To overcome redox imbalances during anaerobic fermentation, *P. aeruginosa* actively produces radical-scavenging phenazines[41,42,43]. The NADH-dependent conversion of lactate to pyruvate conversion also preserves the cellular redox balance by limiting electron accumulation[41,42]. The emphasis on pyruvate metabolism in anaerobic conditions becomes evident by the increase in total biofilm biomass upon pyruvate supplementation and biofilm dispersion after pyruvate deficiency[44,45]. Efficient cross-feeding of pyruvate and lactate over the O_2 gradient demonstrates the cooperative metabolic activity between the biofilm sub-populations[15,46], and the role of carbon sources for maintaining matured biofilm structures.

Arginine deiminase

Arginine, an amino acid favored by *P. aeruginosa* as a carbon source, plays a crucial role in anaerobic metabolism and biofilm development. Arginine serves

as an energy source, undergoing fermentation through deiminase enzymes[32]. The transcription of the *arcCBAB* operon encoding for arginine deiminase enzymes is upregulated during biofilm formation, indicating its importance in this process[17]. The utilization of arginine deiminase enzymes is a relatively inefficient pathway for energy production, reducing motility and promoting the transition towards a static biofilm phenotype[47,48,32]. While fermentation maintains energy levels for cellular maintenance in anaerobic environments, it does not provide the efficiency to enable net growth[40,47], which might explain the presence of a dormant sub-population within the biofilm core. The role of arginine extends beyond serving as an energy source in anaerobic conditions. It also acts as a precursor of gene-modulating polyamines that contribute to the formation of the extracellular matrix of biofilms[49]. The central role of arginine in biofilm maturation is further supported by sensory domains, inducing *Pseudomonas Putida* biofilm formation in the presence of exogenous arginine[50].

Denitrification

P. aeruginosa capitalizes on denitrification for proliferation in anaerobic conditions. This process substitutes O_2 with nitrate (NO_3-) and nitrite (NO_2-) as electron acceptors (Figure 1D)[51,52]. This shift facilitated by the ample availability of these compounds in CF mucus, which also enables activation of the denitrification pathway despite the presence of O_2. The high abundance of these nitrates does not limit the utilization of the denitrification pathway within anaerobic environments[5,53]. *P. aeruginosa* also employs denitrification enzymes to neutralize nitric oxide (NO) produced by immune[14,54] and to mitigate ROS production by distributing the electron flow across the respiratory and denitrification pathways in O_2-rich conditions[8,40,55]. The functionality of denitrification is iron-dependent[51]. Reduced transcription of denitrification proteins hampers anaerobic metabolism under iron scarcity[56], whereas sufficient levels of iron stimulate anerobic metabolism and biofilm development[57–60]. This underscores the critical role of denitrification in the maturation of biofilm and adaptation of anaerobic sub-populations in the core, highlighting how metabolic processes are intricately linked to multiple nutrients in the surrounding microenvironment. This metabolic coordination is also regulated at the transcriptional level. The *Anr* transcriptional regulator, which controls denitrification enzymes, also has conserved regulatory effects in central carbon metabolic pathways[40,55]. For instance, under anaerobic yet nitrate-rich

conditions, arginine is no longer a preferred carbon source[5,47]. This metabolic heterogeneity is reflected in biofilm structures, where different metabolic pathways dominate at varying biofilm depths depending on nutrient availability[15,61]. Consequently, the metabolic adaptation of *P. aeruginosa* at the infection site is influenced by the overall nutritional composition, including O_2, metals, salts, and carbon sources. This highlights both the flexibility and diversity of *P. aeruginosa* metabolism within the CF lung, underscoring the importance of understanding the complex interplay between bacterial metabolism and the nutritional environment for effective therapeutic interventions.

2.3. Nutrient-driven effects of metabolic adaptation on antibiotic sensitivity

In this section, we discuss the role of nutrients in the metabolic activity of *P. aeruginosa*, and how this influences antibiotic sensitivity. Nutrient-limited environments typically induce low metabolic activity, which is generally linked to reduced activity of antimicrobials, since antibiotics often target energy-demanding cellular processes during cell division[62]. This includes processes such as DNA replication (e.g., fluoroquinolones), protein synthesis (e.g., aminoglycosides) and cell wall synthesis (e.g., β-lactams). In contrast, polymyxins are more effective in eradicating metabolically inactive cells[63]. An overview of specific nutrients present in the CF lung and their modulatory role on antibiotic efficacy is summarized in **Table 1**.

Fluoroquinolones

Fluoroquinolones require oxidative stress for effective bacterial killing in addition to their primary mode of action through inhibition of DNA gyrase and topoisomerase IV[82–84]. Oxidative stress primarily stems from aerobic metabolic activity, i.e., TCA cycle and OXPHOS, which spearhead ROS production. These processes can be suppressed in low oxygen environments, for example, in the O_2-and nutrient-deprived core of a biofilm, in addition to phenazine-mediated redox balancing mechanisms in anaerobic environments to reduce oxidative damage[61]. To illustrate, in *P. aeruginosa* biofilms, enhanced ciprofloxacin tolerance has been

Table 1. Nutrient supplements which change antibiotic sensitivity through metabolic changes

Antibiotic	Antimicrobial effect	Nutrient environment	Metabolic mechanism	Ref.
Ciprofloxacin	Potentiation	O_2	Electron transport chain	64
		Malic acid	TCA cycle activity	65,66
		Arginine	n.r.	67,68
	Reduction	n.r.	Denitrification	69–71
		Starvation	Dormancy	72
Tobramycin	Potentiation	O_2	n.r.	73
		Fumarate	Proton motive force, TCA cycle	74,75
		Glutamate and succinate	Proton motive force, TCA cycle	76
		bicarbonate	Alkaline pH	77
		Arginine	Alkaline pH	67,78,79
	Reduction	Glyoxylate	Proton motive force, TCA cycle	74
		n.r.	Denitrification	53
Meropenem	Reduction	Starvation	Oxygen radicals	80
Colistin	Potentiation	Nitrate	Anaerobic metabolism	63
	Reduction	Glucose	"Osmotic homeostasis"	81
		Formate	n.r.	81

n.r. = not reported

To illustrate, in *P. aeruginosa* biofilms, enhanced ciprofloxacin tolerance has been observed in metabolically inactive cells as compared to metabolically active cells[85]. Consequently, the supplementation of O_2, i.e., to promote aerobic respiration, improves ciprofloxacin efficacy against *in vitro* grown biofilms[64]. Similar ciprofloxacin potentiation was observed when supplementing with organic acids to increase TCA cycle activity[65,66]. By using metabolic shunts as a safeguard against oxidative stress while preserving anabolic flexibility[20], *P. aeruginosa* demonstrates a form of metabolic defense against fluoroquinolone action. This adaptability becomes particularly evident in the nitrogen rich CF lung environment, where *P. aeruginosa* shifts from OXPHOS to denitrification, thereby reducing oxidative stress and increase tolerance to fluoroquinolones while maintaining metabolic activity[69–71]. The reduced fluoroquinolone

susceptibility by the activation of anaerobic metabolism is not observed when anaerobic nutrients such as NO_3 and arginine are supplemented. In fact, the addition of arginine and NO_3 demonstrated enhanced ciprofloxacin activity in mature biofilm cultures, while no enhancement was observed in young or alginate-grown biofilms[67,68].

Aminoglycosides

Aminoglycosides penetrate bacterial cells through membrane pores and block protein synthesis by attaching to ribosomal proteins. Their entry relies on the electric potential across the cell membrane, driven by the proton motive force (PMF) during OXPHOS[86,87]. Stimulating the PMF by elevating OXPHOS activity through O_2 supplementation can enhance the effectiveness of tobramycin[73]. Similarly, the supplementation of fumarate increases the electron transport through the elevated TCA cycle, improving aminoglycoside action[74,75]. However, supplementing with glyoxylate activates the glyoxylate shunt, which reroutes the TCA cycle and shifts the balance between OXPHOS and denitrification. This reduces the PMF and consequently decreases susceptibility to tobramycin[20,8,53,74]. This metabolic adaptation is also observed in biofilms exposed to tobramycin, where cells in aerobic biofilm regions shift to denitrification upon exposure to tobramycin[53].

The transmembrane pH gradient is another component of the PMF that plays a crucial role in aminoglycoside activity. The acidic CF lung environment lowers both the net positive charge of tobramycin and the PMF of *P. aeruginosa*, thereby reducing aminoglycoside effectiveness[88]. This can be counteracted by increasing the pH through bicarbonate supplementation, which has shown to enhance tobramycin effect[77]. Adjusting the pH showed limited benefits for aminoglycoside treatment of biofilm, potentially due to the natural acidic pH-gradient in biofilm structures from accumulated extracellular DNA[77,78,88,89]. In contrast, arginine supplementation has shown promise in enhancing aminoglycoside efficiency in biofilms, due to metabolically induced pH increase[67,78,79]. Studies involving the use of 3D cultured lung cells have shown that a combination of altered pH, transmembrane potential, and carbon metabolism enhance aminoglycoside effect[76]. Alkalinizing the intracellular environment and increased TCA cycle activity through pyruvate metabolism increased the PMF-mediated aminoglycoside uptake. Furthermore, enriching the 3D culture media

with succinate and glutamate significantly improved aminoglycoside-mediated eradication of biofilms.

β-Lactam antibiotics

β-Lactam antibiotics exert their bactericidal effect by depleting cell wall building blocks through inhibition of cell envelope precursor synthesis. Primary mechanisms modulating β-lactam resistance typically have a genetic basis, including porin modifications, overexpression of efflux pumps, and inactivating β-lactamase enzymes[90]. Whereas the primary mechanisms of action and resistance to β-lactam antibiotics are generally stable across different environmental and metabolic conditions, secondary effects such as the induction of oxidative stress are closely linked to both[91–94]. The bactericidal activity of β-lactams can be potentiated by the interaction of ferrous ions with reactive oxygen species (ROS), which are generated as a result of the elevated metabolic activity associated with peptidoglycan recycling[95]. This oxidative mechanism aligns with observations that meropenem is more effective against *Pseudomonas aeruginosa* strains with compromised antioxidant defenses[80]. In contrast, activation of stress responses such as the stringent response can enhance antioxidant capacity prior to antibiotic exposure, thereby promoting antibiotic tolerance[80,96]. The stringent response also plays a key role during nutrient limitation in the biofilm core[97], contributing to biofilm physiology and potentially reducing susceptibility to β-lactams. Although the influence of the nutritional environment on the early bacterial response to β-lactams is not yet fully understood, it may be an important factor in shaping *P. aeruginosa* sensitivity.

Polymyxins

Polymyxins are polypeptide antibiotics that disrupt the bacterial cell envelope. Unlike many other antibiotics, polymyxins are particularly effective against dormant cell types that lack the high metabolic activity required for cell envelope remodeling[63]. These metabolic demands for lipopolysaccharide modifications are more readily supported in nutrient-rich environments, which can lead to a reduction in binding sites for colistin due to alterations of the lipid A component of lipopolysaccharide[85,98,99]. While such lipid A remodeling typically imposes a fitness cost in other Gram-negative bacteria, *P. aeruginosa* appears to tolerate these modifications without significant fitness penalties[99–101]. Nonetheless,

colistin susceptibility in *P. aeruginosa* can be influenced by the nutrient environment. For example, the carbon sources glucose and formate modulate antibiotic effects while not directly channeled into the central energy-generating pathways[81]. Glucose has been suggested to reduce susceptibility by alleviating osmotic stress, while formate induces a sensitizing effect through an as-yet undefined mechanism. These findings illustrate how *P. aeruginosa* leverages its metabolism to adapt to antibiotic exposure in ways that are uncoupled from core energy metabolism. However, such metabolic adaptations likely depend on nutrient-rich conditions that maintain energy homeostasis via alternative substrates.

2.4. Differences in the nutritional environments between and within patients

Differences in nutrients may impact the response of *P. aeruginosa* to antibiotics can occur at different biological scales, contributing to variability within and between patients. In the previous sections, we highlighted how nutritional diversity within the CF lung influences *P. aeruginosa* phenotype and antibiotic sensitivity at the cellular level, explaining heterogeneity at the cellular level (**Figure 3A**). Within the lung, accumulation of mucus and macronutrients can further contribute to this nutrient diversity. Nutrient conditions vary substantially across different lung regions due to varying host-pathogen interactions and oxygen availability[102] (**Figure 3B**). Patient-specific differences such as those related to disease severity, inflammation, comorbidities and microbial colonization significantly impact the nutrient microenvironments[104–109] (**Figure 3C**). In this section, we explore how nutrient heterogeneity at the tissue and patient level may further contribute to differences in antibiotic treatment response.

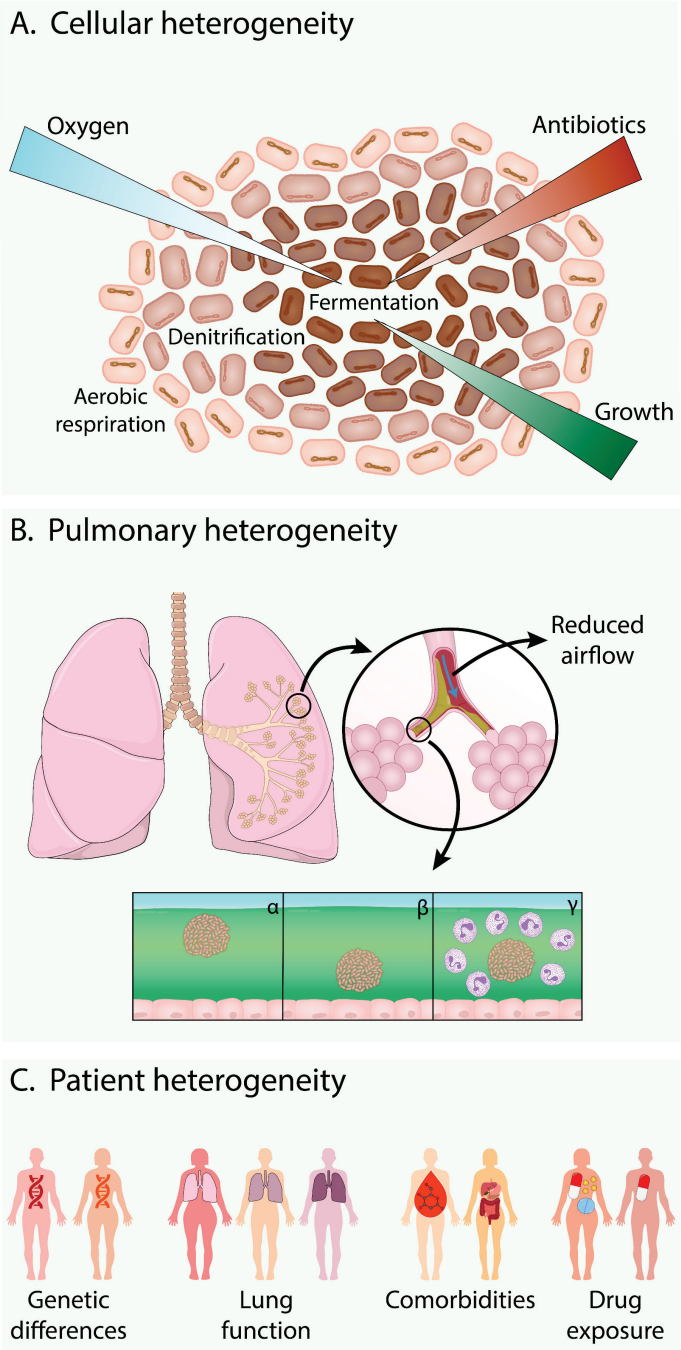

Figure 3. Multiscale heterogeneity contributing to variation in antibiotic treatment response in (**A**) patients, (**B**) site of infection, and (**C**) bacterial biofilm structure.

Pulmonary heterogeneity

The compartmentalization of the human lung creates a diverse microenvironment that significantly influences pathogen-host interactions[102]. One notable example is the respiratory zone, which is densely populated with PMNs and, as a result, experiences a pronounced depletion of O_2 from the typically aerobic zone[109]. It has been suggested that biofilm aggregates encased by PMNs no longer exhibit an oxygen gradient but instead experience uniform hypoxia[110]. Microbes originating from these hypoxic areas can adapt to become intolerant to O_2[111], a shift that will directly affect their metabolic processes and likely their response to antibiotics. For example, proposed strategies focusing on increasing aerobic respiration may have limited or even counterproductive effects on these strict anaerobic cells, especially when compared to *P. aeruginosa* lineages that have evolved increased aerobic respiration during long-term adaptation to the CF lung[25].

The O_2 depleted by PMNs is partly used for ROS production as a pathogen eradication mechanism, but also inducing oxidative stress in nearby host and microbial cells. This oxidative stress not only increases nutrient availability through cell lysis and epithelial cell nutrient leakage[112], but also primes *P. aeruginosa* by activating stress responses prior to antibiotic treatment. The pre-activation of these stress responses can undermine the secondary effects of antibiotics that depend on ROS production, such as fluoroquinolones and β-lactams, reducing antibiotic effectiveness[113].

These observations underscore the critical need to consider the role of the compartmentalized lung in nutrient availability, oxygen levels, and immune cell activity in *P. aeruginosa* treatment response. Understanding the intricate dynamics of the different CF lung microenvironments and microbial adaptation offers a pathway to more effective treatment approaches, potentially including the strategic manipulation of microbial metabolism to enhance antibiotic efficacy.

Patient heterogeneity

There are nearly 2000 possible mutations of the CF transmembrane regulator gene that cause CF, leading to a broad spectrum of disease severities[114]. These mutations result in diverse manifestations in CF pathophysiology which

eventually contribute to significant differences in lung function decline[115,116]. Progressive lung function decline caused by inflammation results in higher levels of free amino acids, nutrients that are preferred by *P. aeruginosa*[107]. The more severe lung damage in chronically infected pwCF is often accompanied by *P. aeruginosa* adaptations that further enhance its metabolic dominance in the inflamed environment[107,108,117]. These metabolic changes also include the shift from aerobic respiration toward denitrification[118], a transition that is associated with decreased antibiotic susceptibility. This suggests that changes in the nutrient environment and associated metabolic adaptations may be important considerations in treatment decision-making. Such factors may be particularly relevant in the treatment strategy during the substantial changes in lung function that occur at the transition from childhood to adulthood[119].

Diabetes mellitus is one example of an important and frequent comorbidity in pwCF[119], which further exacerbates the elevated glucose concentrations typically observed in the CF lung[120,121]. Elevated glucose levels in the CF lung have been repeatedly associated with an increased risk of developing respiratory infections[122,123,124]. In vitro studies have demonstrated that glucose induces metabolic shifts and increase biofilm formation in *P. aeruginosa*, which in turn reduces levofloxacin susceptibility[125]. Glucose also plays a key role in epithelial cells and PMNs, particularly in anaerobic conditions where it is fermented into lactate. The subsequent rise in lactate levels has been suggested as a biomarker for pulmonary exacerbations[13,23,126]. However, the lack of significant decrease in lactate levels following antibiotic treatment raises questions about its utility as a reliable biomarker[127]. Nonetheless, the sustained lactate levels indicate that *P. aeruginosa* continues to access lactate and glucose before, during, and after antibiotic treatment. This demonstrates that glucose serves as an important substrate for *P. aeruginosa*, despite not being one of its preferred carbon sources.

2.5. Considering nutrients in antimicrobial susceptibility testing

Current clinical decisions regarding antibiotic therapy are based on antimicrobial susceptibility testing, which typically uses standardized nutrient

and oxygen-rich conditions that do not reflect the *in vivo* nutrient environment shaped by CF-related pathophysiological factors. By integrating physiologically relevant growth conditions that closely mimic the CF-specific environments, the predictive accuracy of antibiotic effects may be improved[128]. Combining these nutrient-relevant conditions with recent advancements in the development 3D biofilm[25] and host-microbe[157] models promises a more accurate translation of laboratory findings into clinical outcomes.

2.6 Opportunities for antimicrobial drug development

The intricate relationship between metabolic activity and *P. aeruginosa* antibiotic sensitivity presents a promising avenue for developing a new class of antimicrobials that target bacterial metabolism[129]. These novel antimicrobials could aim to inhibit the specific metabolic pathways that pathogens exploit to evade or tolerate conventional antibiotics like aminoglycosides and fluoroquinolones. We have described how the metabolic shift of *P. aeruginosa* toward anaerobic energy-generating pathways reduces antibiotic uptake and decreases ROS production, which are essential to the activity of these antibiotics. By specifically targeting these anaerobic pathways in combination therapies, metabolism-targeting antimicrobials could effectively block bacterial escape routes from antibiotic treatments[130]. Unlike nutrient supplementation that relies on activation of aerobic metabolism, combination therapy with metabolism-targeting antimicrobials will be a consistent strategy to combat antibiotic resistance within the diverse oxygen gradients of the CF lung. The success of these therapeutics hinges on a deep understanding of pathogen metabolism within physiologically relevant microenvironments. Selecting metabolism-targeting candidates from drug libraries can only facilitate the development of successful candidates if the pathogens are studied in screening models that accurately represent the infection site[131,132]. The failure of many promising compounds during the development process often stems from a lack of consideration of physiological relevance of screening models[133], underscoring the importance of this strategy in the fight against antibiotic-resistant infections.

2.7. Conclusion

There is a complex interplay of nutrients in the CF lung environment, metabolic adaptations of *P. aeruginosa* and resulting consequences for antibiotic treatment efficacy. Various nutritional environments relevant to the CF lung influence antibiotic efficacy. These insights highlight the importance of further considering the CF lung microenvironment and its impact in order to refine susceptibility testing and treatment strategies, although characterization of the nutrient environment in patients remains challenging.

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