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Iron-dependent mechanisms in *Acinetobacter baumannii*: pathogenicity and resistance

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Acinetobacter baumannii is a Gram-negative opportunistic pathogen that poses a significant challenge in healthcare settings, particularly in ICUs, due to its MDR and high mortality rates, especially among critically ill coronavirus disease 2019 patients. Iron is crucial for the survival, growth and pathogenicity of *A. baumannii*, and the bacterium has developed multiple iron acquisition systems, including siderophore production, haem uptake and TonB-dependent transport mechanisms, to adapt to the iron-limited environment within the host. Although specific studies on *A. baumannii* are limited, mechanisms from other bacterial species suggest that similar iron acquisition strategies may play a key role in its virulence. Therapeutic approaches targeting these iron-dependent systems, such as the siderophore-conjugated cephalosporin cefiderocol, have shown potential in overcoming MDR *A. baumannii* infections. Additionally, strategies such as synthetic siderophores, TonB receptor inhibitors and iron chelators are under investigation to enhance treatment outcomes. Future research should prioritize validating these mechanisms in *A. baumannii*, advancing clinical trials for these therapies and exploring combination treatments to mitigate resistance and improve clinical outcomes in severely affected patients.

Introduction

Acinetobacter baumannii is an aerobic, Gram-negative opportunistic pathogen that has emerged as a major cause of healthcare-associated infections, particularly in ICUs worldwide. This pathogen can cause severe infections, including hospital-acquired pneumonia (HAP), ventilator-associated pneumonia (VAP), bacteraemia and wound infections, especially in immunocompromised patients and those undergoing invasive procedures.[1] It is remarkable environmental resilience, including the ability to persist on dry surfaces for months, combined with its MDR profile, makes *A. baumannii* a significant challenge in infection control. The rise of carbapenem-resistant *A. baumannii* (CRAB) strains has led to a surge in mortality rates, particularly in critically ill patients, such as those with coronavirus disease 2019 (COVID-19). Secondary infections with MDR *A. baumannii* have been increasingly documented in various regions, including Italy, where the incidence in hospitalized COVID-19 patients reached 1%; Wuhan, China, where studies reported rates of 1%–1.4%; and Egypt, where MDR strains accounted for 2.7% of cases, with high mortality observed.[2]

Iron plays a pivotal role in the growth and pathogenicity of bacteria, including *A. baumannii*. As an essential cofactor in several metabolic processes, such as DNA synthesis, cellular respiration and oxidative stress management, iron availability is critical for bacterial survival.[3–5] However, due to the host's iron-limiting defence mechanisms (termed 'nutritional immunity'), *A. baumannii* has evolved sophisticated iron acquisition systems to overcome these barriers.[5] These systems include siderophore-mediated iron uptake, haem acquisition and TonB-dependent transport mechanisms, which enable the bacterium to thrive in the iron-limited environments of the human host.[6]

Despite the significance of these iron acquisition mechanisms, research specific to *A. baumannii* remains relatively limited. Much of the current understanding of iron-dependent pathogenic mechanisms has been inferred from studies of other Gram-negative bacteria, such as *Escherichia coli* and *Bacillus* species.[7,8] While these inferences provide valuable insights, they also highlight the need for further experimental validation in *A. baumannii*. For instance, while siderophore systems like acinetobactin have been identified in *A. baumannii*, the exact regulatory pathways and their interactions with other virulence factors remain underexplored. This gap in knowledge underscores the importance of

further investigation into the unique iron acquisition strategies employed by *A. baumannii* and how they contribute to its virulence and resistance to antibiotic treatment.[7]

Overview of *A. baumannii*

Basic characteristics

A. baumannii is primarily associated with hospital environments, where its ability to survive under harsh conditions contributes significantly to its role in hospital-acquired infections (HAIs).[1] While it can occasionally be found in natural environments such as soil and water, its clinical relevance is predominantly linked to healthcare settings. A distinctive feature of *A. baumannii* is its highly plastic genome, which allows for the acquisition of multiple antibiotic resistance genes via horizontal gene transfer.[9] This adaptability, combined with its intrinsic resistance mechanisms, makes *A. baumannii* particularly formidable in clinical settings, especially in ICUs.[9] The WHO 2024 report identifies CRAB as the number one priority pathogen globally, highlighting the critical need for innovative therapeutic strategies to combat its extensive drug resistance and associated high mortality rates.[10]

Infection and resistance overview

A. baumannii has emerged as one of the most significant nosocomial pathogens, especially in critically ill patients, due to its ability to cause a wide range of infections, including pneumonia (HAP/VAP), bloodstream infections and wound infections.[1] These infections are particularly difficult to treat due to the bacterium's extensive resistance mechanisms, which include not only acquired resistance genes but also intrinsic mechanisms that make it inherently tolerant to many antibiotics.

One of the primary mechanisms of resistance in *A. baumannii* is the production of β-lactamases, including oxacillinase (OXA)-type carbapenemases, named for their ability to hydrolyze oxacillin, which confer resistance to carbapenem antibiotics—a critical class of drugs used to treat severe infections (Evans BA, Amyes SG, 2014).[2,11] These enzymes hydrolyze the β-lactam ring of carbapenems, rendering the antibiotics ineffective. In addition, *A. baumannii* expresses efflux pumps that actively expel a wide variety of antibiotics, further reducing their efficacy.[2] Other resistance mechanisms include modifications to outer membrane proteins, such as porins, which decrease membrane permeability and prevent antibiotics from entering the bacterial cell.[12]

Evans, B. A., & Amyes, S. G. (2014). OXA β-lactamases. *Clinical microbiology reviews*, *27*(2), 241–263. https://doi.org/10.1128/CMR.00117-13

The increasing prevalence of XDR and pan-drug-resistant (PDR) strains of *A. baumannii* has made treatment even more challenging. XDR strains are resistant to nearly all antibiotics, including last-resort drugs such as polymyxins and tigecycline, while PDR strains are resistant to all available antimicrobial agents[13] These resistant strains have been particularly problematic during the COVID-19 pandemic, as critically ill patients have been more susceptible to secondary infections with *A. baumannii*, leading to increased mortality rates.[14] Studies have shown that co-infection with MDR *A. baumannii* in ICU patients, particularly those on mechanical ventilation, significantly worsens patient outcomes and extends hospital stays.[14,15]

In addition to its resistance to antibiotics, *A. baumannii* also possesses various virulence factors that enhance its ability to persist in the host and evade the immune system. These include the ability to form biofilms on medical devices, such as catheters and ventilators, which protect the bacteria from both the immune response and antibiotic treatment.[16] Biofilm formation is facilitated by outer membrane proteins such as outer membrane protein A (OmpA), which play a key role in adherence to host tissues and biofilm stabilization.[17] The ability to survive under iron-limited conditions, a common environment within the host, is another critical factor in *A. baumannii*'s pathogenesis. The bacterium utilizes a variety of iron acquisition systems, including siderophores and haem uptake mechanisms, to obtain iron from the host and sustain its growth and virulence.[6]

Given these formidable resistance mechanisms and virulence factors, *A. baumannii* presents a serious threat to public health, particularly in healthcare settings. The urgent need for novel therapeutic strategies targeting these mechanisms, especially iron acquisition systems, is evident in considering the rising number of MDR and XDR infections.

Iron acquisition mechanisms in *A. baumannii*

A. baumannii exhibits major transcriptional changes under iron-limiting conditions, with up-regulation of siderophore biosynthesis gene clusters. These changes are regulated by the ferric uptake regulator (Fur), which controls the expression of iron-regulated outer membrane proteins essential for iron acquisition.[6,18] Among these, the FhuE receptor has been identified as a therapeutic target, as its inhibition disrupts siderophore-mediated iron uptake and compromises *A. baumannii* survival.[19]

Siderophore-mediated iron uptake

Siderophores are high-affinity iron-chelating molecules secreted by bacteria to sequester iron under limiting conditions. In *A. baumannii*, siderophores play a crucial role in overcoming iron limitation imposed by host nutritional immunity.[7] Receptors such as BauA and BauE facilitate the transport of iron–siderophore complexes across the bacterial outer membrane.[20,21]

Recent studies have highlighted the complementary roles of the acinetobactin isomers, Oxa and Isox. Oxa, recognized by the BauA receptor, forms a stable Fe (III) –Oxa complex through its specific and high-affinity interaction with BauA, ensuring efficient iron uptake. Isox supports Oxa by acting as an auxiliary iron collector under competitive or restricted iron conditions. This synergistic mechanism enables *A. baumannii* to adapt to varying iron availability during host infection, maintaining its growth and virulence.[20,21]

These receptors are crucial for recognizing and binding ferric siderophores (Fe^{3+}–siderophore complexes) in the extracellular environment and transporting these complexes into the periplasmic space (Figure 1). This transport system, mediated by BauA and BauE, plays a pivotal role in enabling *A. baumannii* to acquire iron, a critical nutrient for its survival and virulence. The ability to switch between Oxa and Isox ensures that *A. baumannii* can effectively adapt to environmental challenges, particularly during host infection where iron is tightly regulated by the host immune system.[20,21]

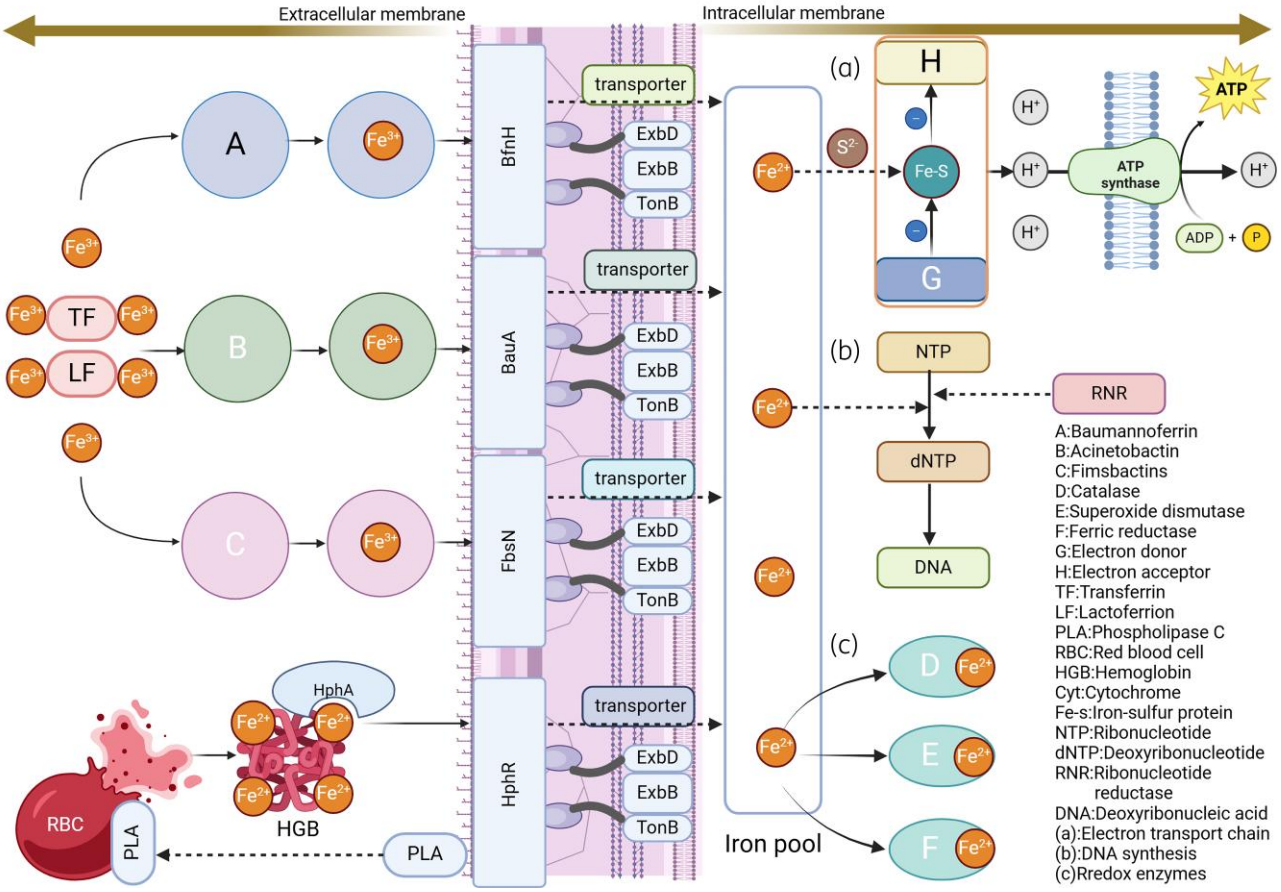

Figure 1. Mechanisms of iron acquisition and utilization in *A. baumannii*. Iron ions (Fe^{3+} and Fe^{2+}) are essential for bacterial growth, metabolism and pathogenicity. The bacterium acquires iron from various sources such as transferrin (TF), lactoferrin (LF) and haemoglobin (HGB) and specific siderophores like baumannoferrin (a), acinetobactin (b) and fimsbactins (c). Iron is transported across the extracellular membrane via siderophore-receptor complexes (BfnH, BauA and FbsN) with the help of the TonB–ExbB–ExbD complex, which provides energy for transport. Haem iron is released from haemoglobin by the haem-binding protein (HphA) and transported through the HphR receptor. Inside the cell, iron plays a crucial role in the electron transport chain (a), facilitating ATP synthesis via iron–sulfur proteins (Fe–S) and cytochromes (Cyt). It is also vital for DNA synthesis (b) as a cofactor for ribonucleotide reductase (RNR), converting ribonucleotides to deoxyribonucleotides (dNTP). Additionally, iron acts as an active centre in various redox enzymes (c) like catalase (d), superoxide dismutase (e) and ferric reductase (f), aiding in oxidative stress response and maintaining cellular redox balance. This comprehensive depiction underscores the importance of iron in *A. baumannii* survival and pathogenicity in iron-limited environments.

The BauB and BauC genes, part of the acinetobactin operon, encode enzymes essential for siderophore biosynthesis, which chelate iron and facilitate its upake into bacterial cells in *A. baumannii*.[20] These genes encode enzymes essential for forming siderophores, which chelate iron and facilitate its transport into the bacterial cell.[22] The Fur protein acts as a global regulator of iron homeostasis, repressing siderophore biosynthesis and transport genes in iron-replete conditions, thereby maintaining a balance between iron acquisition and storage.[23] Fur acts as a repressor in the presence of iron, maintaining iron homeostasis by down-regulating siderophore production when iron is abundant.[24]

Therapeutic strategies targeting siderophore systems focus on disrupting iron acquisition. For example, modifying siderophores and creating synthetic analogues with higher iron-binding affinities can outcompete natural siderophores, depriving *A. baumannii* of this essential nutrient. Additionally, inhibitors targeting siderophore biosynthesis reduce bacterial survival by limiting their ability to extract iron from the host environment.[11]

TonB-dependent systems and therapeutic implications

To thrive in iron-limited environments, *A. baumannii* has evolved efficient iron acquisition mechanisms, with siderophore systems and haem uptake systems being the most prominent.[8] These systems fulfil the bacterium's iron requirements and play crucial roles in its virulence and antibiotic resistance.

The TonB–ExbB–ExbD complex is a critical cytoplasmic membrane system that transduces energy from the proton motive force to outer membrane receptors, facilitating the active transport of ferric siderophores and other essential molecules into the bacterial cell.[25,26] In *A. baumannii*, the TonB system has been extensively studied and is crucial for the functionality of siderophore receptors like BauA, which specifically transports acinetobactin-bound iron across the outer membrane. Studies have demonstrated that mutations disrupting the TonB system led to significant growth defects under iron-limited conditions, highlighting the system's essential role in nutrient acquisition and virulence in *A. baumannii*.[22]

However, while TonB systems share functional similarities across different bacterial species, the interaction between TonB and outer membrane receptors like FhuA has been predominantly studied in *E. coli*. In *A. baumannii*, specific interactions between TonB and an FhuA-like receptor have not been conclusively demonstrated to the same extent, with most studies focusing on TonB's interaction with siderophore receptors like BauA.[27] Therefore, while the TonB system's energy transduction mechanism is well established in *A. baumannii*, the detailed interactions with receptors like FhuA remain less explored in this species.

Therapeutic strategies targeting TonB-dependent systems hold promise in impairing *A. baumannii*'s iron acquisition, reducing its virulence and enhancing susceptibility to antibiotics. One approach involves siderophore-drug conjugates, where compounds such as BAL30072 and MC-1 exploit TonB-dependent receptors like Ab-PiuA to transport antibiotics into bacterial cells, enhancing efficacy against MDR strains.[28] Another approach focuses on synthetic siderophore mimics, which compete with natural siderophores, disrupting iron acquisition pathways. Ga (III)-complexed siderophore mimics have shown potent inhibitory effects in iron-limited conditions.[11] Last, TonB receptor blockers that inhibit energy transduction required for iron uptake have been identified through high-throughput screening, demonstrating potential in weakening the bacterium by preventing iron acquisition.[29]

These therapeutic strategies are at various stages of research, with many showing promising results in both *in vitro* and *in vivo* models, indicating their potential in overcoming MDR in *A. baumannii*.

Acidic environment and iron acquisition

The importance of an acidic environment in bacterial infections is particularly evident in critically ill patients. In such cases, tissue perfusion is often compromised, leading to ischaemia and hypoxia, as seen in conditions like septic shock. These conditions result in a localized acidic microenvironment, which exacerbates the overall condition of the patient and increases the difficulty of infection control. Understanding the relationship between acidic environments and iron acquisition is crucial, as it provides insights into the challenges faced in treating *A. baumannii* infections under these conditions.[30–32]

To thrive in such acidic environments, *A. baumannii* adjusts its iron acquisition mechanisms in response to pH changes, particularly under conditions where iron solubility increases. The solubility of Fe (III) rises in acidic environments, enhancing the availability of iron for bacterial uptake. This affects siderophore biosynthesis and function, particularly acinetobactin and its isomer pre-acinetobactin, which adapt to different pH levels. Pre-acinetobactin remains stable in acidic conditions ($pH < 6$), while acinetobactin is more effective at neutral pH, allowing the bacterium to efficiently scavenge iron across varied pH environments.[7,32,33] Shapiro and Wencewicz[32] demonstrated this pH-triggered isomerization process in their study, illustrating how *A. baumannii* adapts its iron-scavenging mechanisms to the pH conditions encountered during infection.

In acidic conditions, the up-regulation of iron-dependent genes, such as those involved in siderophore production, is a crucial adaptive response. These genes, including *BauA* and *BauB*, which are part of a gene cluster responsible for the biosynthesis and transport of acinetobactin, are significantly influenced by environmental factors such as pH.[4,7,32] Studies have shown that *A. baumannii* exhibits enhanced growth and siderophore activity under acidic, iron-limited conditions, highlighting its ability to adapt to its iron acquisition mechanisms to fluctuating pH levels.[7,32]

A key regulatory mechanism behind this adaptation involves the *Fur)*, which plays a central role in iron homeostasis.[34] Under normal iron-replete conditions, Fur represses the expression of siderophore biosynthesis genes by binding to iron and interacting with specific promoter regions known as Fur boxes. However, under acidic and iron-limited conditions, the availability of free Fe (III) decreases despite its increased solubility. This results in Fur becoming inactive, thereby derepressing the siderophore-related genes and allowing for their up-regulation.[6,23,34] This regulatory mechanism ensures that *A. baumannii* can produce and transport siderophores efficiently, optimizing iron acquisition and homeostasis in challenging environments.

In addition to Fur regulation, the pH-triggered isomerization of pre-acinetobactin to acinetobactin further enhances iron chelation under varying pH conditions. This non-enzymatic isomerization allows *A. baumannii* to maintain effective iron acquisition across both acidic and neutral environments, which enhances its survival and adaptability during infection.[20,32]

Moreover, Fur's activity is modulated by other iron-responsive regulators, such as small RNAs and additional sigma factors, which further enhance *A. baumannii*'s ability to fine-tune its response to changing environmental conditions. These adaptive mechanisms, combining Fur-mediated gene regulation and pH-responsive siderophore isomerization, allow *A. baumannii* to thrive in iron-limited and acidic environments[35]

Experimental data indicate that the efficiency of iron acquisition via acinetobactin increases under acidic conditions, largely due to the up-regulation of siderophore biosynthesis and transport genes.[32] This enhanced siderophore production helps prevent iron toxicity, caused by excess solubility, and iron deprivation, due to competition with host iron-binding proteins.[36] By optimizing its iron acquisition mechanisms, *A. baumannii* can maintain growth and virulence even in hostile acidic conditions.[32]

Studies have also demonstrated that acidic environments promote the expression of other virulence factors, including outer membrane proteins and adhesion factors. Proteins such as *OmpA* and biofilm-associated proteins like *BapAb* are up-regulated, enhancing bacterial adhesion, biofilm formation and motility, which further contributes to the bacterium's environmental adaptability and resistance to host defences.[37,38] Biofilm formation, in particular, improves *A. baumannii*'s ability to evade the immune system and resist antibiotic treatment. Additionally, acidic conditions may influence the expression of stress-related proteins, further boosting the bacterium's virulence and adaptability.[38,39]

These combined factors underscore the importance of acidic environments in shaping the pathogenic potential of *A. baumannii*, as demonstrated in Figure 2.

Iron ions and host immune system interactions

Nutritional immunity overview

Nutritional immunity is a vital host-defence mechanism that limits the availability of essential nutrients, such as iron, to inhibit

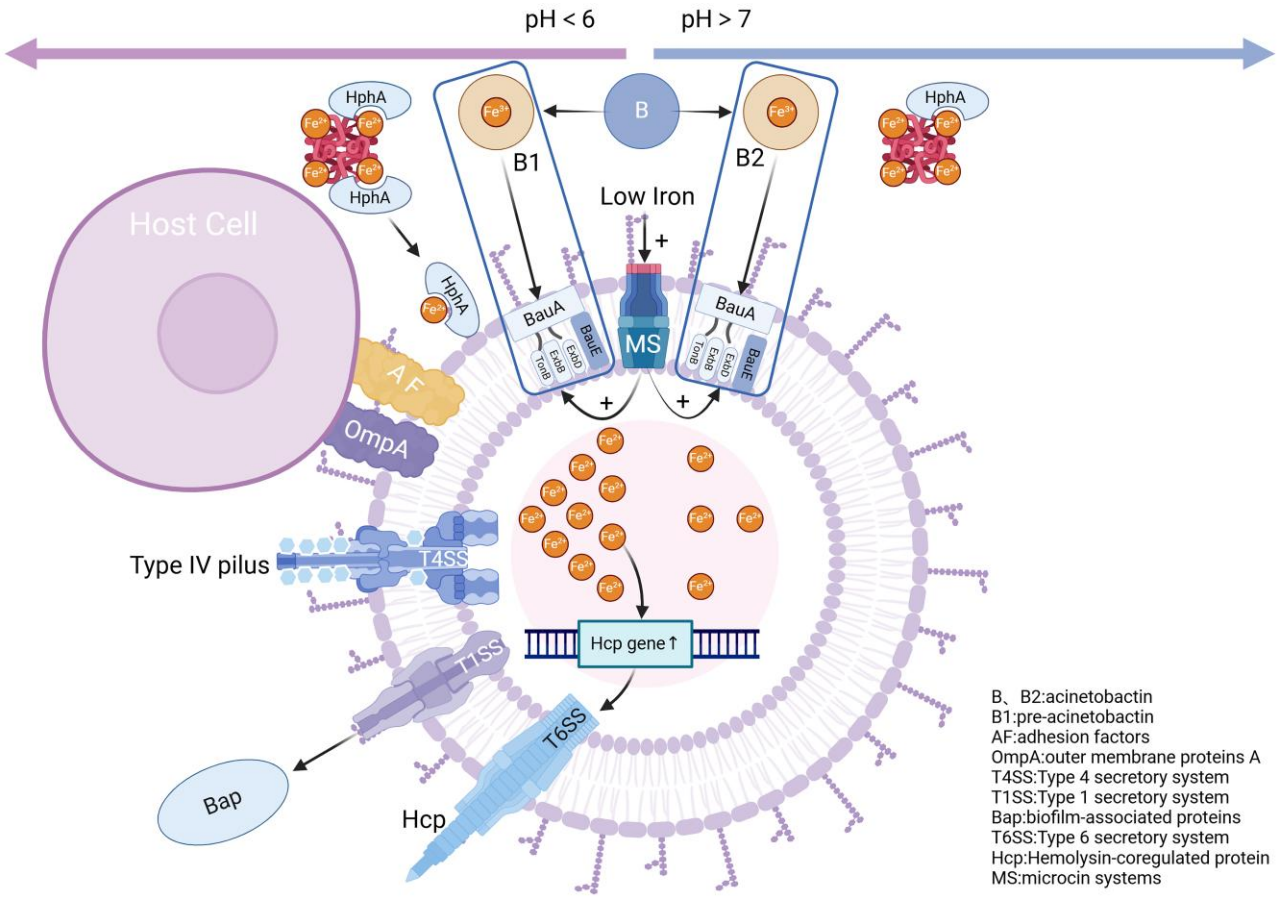

Figure 2. Iron acquisition and virulence mechanisms in *A. baumannii* under different pH conditions. This figure illustrates the mechanisms of iron acquisition and the regulation of virulence factors in *A. baumannii* under varying pH conditions. Under low pH (<6), pre-acinetobactin (B1) is the predominant form, while at higher pH (>7), acinetobactin (B2) is more active. Both forms of acinetobactin are transported into the bacterial cell via the BauA-BauE transport system, utilizing energy from the TonB–ExbB–ExbD complex. Additionally, haem iron is acquired from haemoglobin (HGB) via the haem-binding protein HphA and transported through the HphR receptor. Iron ions within the cell regulate the expression of the haemolysin-coregulated protein (Hcp) gene, which is part of the Type VI secretion system (T6SS), enhancing adhesion, motility and biofilm formation through structures such as Type IV pilus and biofilm-associated proteins (Bap). The outer membrane proteins (OmpA) and adhesion factors (AFs) further facilitate host–cell interactions. This comprehensive diagram highlights the adaptive strategies of *A. baumannii* in iron-limited environments, emphasizing the critical role of iron in bacterial pathogenicity and survival.

pathogen proliferation. Iron is critical for cellular processes like DNA replication and energy production, making it a key target during infections. To restrict iron access, the host employs a multi-faceted strategy that includes the production of iron-binding proteins, such as transferrin, lactoferrin and ferritin, and the redistribution of iron from the bloodstream to storage organs like the liver and spleen.[3–5]

Transferrin binds and transports iron in the bloodstream, creating an iron-depleted environment that limits pathogen access to this essential nutrient. Lactoferrin, found in mucosal secretions, restricts iron availability in areas like the respiratory tract, which is particularly relevant to *A. baumannii* infections. Ferritin stores intracellular iron in tissues, further depriving pathogens of this crucial resource. Additionally, during infections, the host redistributes iron from the blood to organs as part of its nutritional immunity response, thereby enhancing iron sequestration and reducing systemic iron levels to concentrations insufficient for bacterial growth.[3–5]

This stringent iron restriction presents significant challenges to pathogens like *A. baumannii*, which rely on advanced iron acquisition mechanisms to overcome these defences. One key strategy is the secretion of siderophores, such as acinetobactin, which exhibit a high affinity for iron bound to transferrin and lactoferrin. By hijacking the host's iron-binding systems, *A. baumannii* scavenges iron to sustain its metabolic needs.[7] This process is further facilitated by TonB-dependent receptors, such as BauA, which transport ferric siderophore complexes across the outer membrane, ensuring a steady iron supply under host-imposed iron-limiting conditions.[40]

Additionally, *A. baumannii* can exploit other metal uptake systems under iron-restricted conditions, allowing it to maintain metabolic flexibility. This adaptability is crucial during infections where iron availability fluctuates due to immune responses. Furthermore, its ability to form biofilms in iron-limited environments enhances resistance to immune defences and antibiotic treatment, complicating infection control.[38]

Although nutritional immunity serves as a robust first line of defence, *A. baumannii* has developed adaptive mechanisms to counteract these restrictions. A deeper understanding of these mechanisms is critical for designing new therapeutic strategies that inhibit bacterial iron acquisition and mitigate infections.

Calprotectin and iron deprivation response

Calprotectin, a host-defence protein primarily found in neutrophils, plays a crucial role in nutritional immunity by sequestering metal ions, though its role in iron sequestration is more indirect than direct. While calprotectin does not bind iron as efficiently as proteins like transferrin and lactoferrin, it indirectly contributes to the host's iron deprivation response by binding to other essential metals like zinc and manganese, depriving pathogens of these vital nutrients.[3,41] This depletion of critical metals places additional stress on pathogens like *A. baumannii*, forcing them to rely more heavily on iron-scavenging mechanisms.

During infection and inflammation, calprotectin is released to chelate zinc and manganese, which are required by *A. baumannii* to counter oxidative stress. Although calprotectin does not directly bind iron with high affinity, it creates an overall environment of metal deprivation that limits the ability of *A. baumannii* to acquire essential nutrients. This process starves the bacterium of both metals and iron indirectly, as the host's transferrin, lactoferrin and ferritin proteins further sequester available iron.[3,41] The combined effects of iron and other metal sequestration form a critical aspect of the host's nutritional immunity, aimed at inhibiting bacterial proliferation.[3]

In response, *A. baumannii* has evolved strategies to overcome these defences. It upregulates siderophore production, such as acinetobactin, to scavenge iron from host proteins. Additionally, the bacterium enhances the expression of metal transporters and enzymes like ZrlA, which help to maintain cell wall integrity and function under metal-limiting conditions.[42] These adaptive mechanisms enable *A. baumannii* to mitigate the nutritional stress imposed by calprotectin and other host-defence proteins, allowing it to persist and grow even in metal-deprived environments.

Role of iron ions in immune regulation

Iron ions are essential for the proper functioning and regulation of the host immune system. They play a critical role in the activity of immune cells such as macrophages and neutrophils, which utilize iron to generate reactive oxygen species and reactive nitrogen species during the process of pathogen killing after phagocytosis.[5] The presence of sufficient iron supports these immune cells' ability to mount an effective antimicrobial response, while iron deficiency can impair this function.

Beyond innate immunity, iron is crucial for the regulation of adaptive immune responses, particularly in T and B cells. Studies have demonstrated that iron is indispensable for T-cell proliferation and differentiation, as well as for the activation of B cells.[4] Through pathways such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase, iron ions modulate cytokine expression, which influences T-cell activity and immune signalling.[3] Therefore, iron deficiency not only limits the proliferation of T and B cells but also weakens the overall immune response, compromising the body's ability to fight infections effectively.[3,5]

In addition to promoting immune cell activity, iron ions are involved in regulating the host's anti-inflammatory responses through the hepcidin pathway. Hepcidin, a liver-produced hormone, controls systemic iron homeostasis by reducing intestinal iron absorption and promoting iron storage in ferritin. This regulation limits the amount of free iron available to pathogens, thus inhibiting their growth while also minimizing oxidative stress and tissue damage associated with excess free iron.[5,43]

Dysregulation of iron metabolism is closely associated with chronic inflammation and autoimmune diseases. In conditions such as chronic liver disease, cardiovascular disease and diabetes, iron overload exacerbates the inflammatory state, contributing to disease progression.[5,44] Therefore, maintaining proper iron balance is critical not only for immune function but also for managing chronic inflammatory conditions and preventing tissue damage.

In the context of *A. baumannii*, iron plays an additional role in biofilm formation, which significantly contributes to the pathogen's resistance to immune responses and antibiotics. Biofilms create a physical barrier that shields *A. baumannii* from immune attacks and therapeutic agents, making infections more difficult to eradicate.[38] Understanding the mechanisms behind biofilm formation is a key to developing more effective treatment strategies.

Moreover, *A. baumannii* employs a variety of immune evasion tactics to persist within the host. It produces CipA, a plasminogen-binding and complement-inhibitory protein, which interferes with all three complement activation pathways, reducing the effectiveness of the serum killing response.[45] Additionally, the bacterium's auto-transporter adhesin Ata binds to host glycans, facilitating adherence to host tissues and further aiding in immune evasion.[46] These factors, combined with its ability to form biofilms, allow *A. baumannii* to persist and thrive in hostile environments, complicating the treatment of infections.

Distinction between endogenous and exogenous iron in the host

The host carefully regulates the balance between endogenous and exogenous iron to maintain iron homeostasis and limit pathogen access to this essential nutrient. *Endogenous iron* refers to iron stored within body tissues and cells, primarily bound to proteins such as ferritin, transferrin and lactoferrin, which tightly control its availability. These proteins play a critical role in sequestering iron, effectively making it inaccessible to pathogens like *A. baumannii*, thus limiting bacterial growth.[3,5] By binding iron in intracellular and extracellular compartments, these proteins ensure that free iron concentrations remain low, which is crucial for preventing pathogens from exploiting host iron stores.

In contrast, *exogenous iron* originates from dietary sources, supplements or medical treatments and can significantly increase the pool of bioavailable iron in the bloodstream and tissues. Pathogens, including *A. baumannii*, have evolved sophisticated iron acquisition systems, such as siderophores and iron uptake proteins, which enable them to scavenge iron from exogenous sources. When dietary iron intake is elevated or iron supplements are introduced, these pathogens can exploit the increased iron availability to enhance their virulence and establish infections more effectively.[47] This makes the regulation of

exogenous iron equally important for maintaining host iron homeostasis and controlling pathogen proliferation.

The host's ability to regulate both endogenous and exogenous iron through pathways like hepcidin-mediated control of iron absorption is vital for preventing bacterial exploitation of these iron sources. Hepcidin, by regulating the expression of ferroportin, reduces iron absorption from the intestines and promotes iron sequestration in macrophages, effectively lowering the levels of free iron available to pathogens.[43] This dual regulation of iron from both internal stores and external intake is crucial for limiting pathogen growth and maintaining immune competence.[48]

A. baumannii's ability to thrive in iron-limited environments while capitalizing on increased exogenous iron availability underscores the importance of tightly controlling iron metabolism in the host. Infections are particularly challenging when there is an excess of exogenous iron, as pathogens can rapidly adapt to the increased nutrient supply, enhancing their virulence. Thus, understanding the distinction between endogenous and exogenous iron and how pathogens exploit these sources is a key to developing therapeutic strategies that target bacterial iron acquisition systems.

Impact of different host iron states on infection

Iron levels in the host have a profound influence on the progression and severity of bacterial infections. Both iron overload and iron deficiency can impact bacterial growth and modulate the host's immune response, creating distinct challenges in managing infections.

Iron overload

Iron overload conditions provide pathogens like *A. baumannii* with an abundance of iron, which enhances their growth and virulence. This excess iron supports bacterial metabolic processes, making infections more difficult to control.[49] Iron overload can result from genetic disorders such as haemochromatosis, excessive dietary iron intake or frequent blood transfusions. These conditions create an environment where iron is more readily accessible to pathogens, contributing to increased bacterial proliferation.

In addition to facilitating bacterial growth, iron overload suppresses the host's immune response by impairing macrophage function and reducing the production of antimicrobial peptides.[50,51] Macrophages, which play a key role in phagocytosing pathogens, become less effective when iron levels are high, diminishing their ability to control bacterial infections. Patients with iron overload are therefore at a heightened risk of severe infections, as the abundance of available iron not only supports bacterial virulence but also weakens immune defences.[52] Managing iron levels in these patients becomes a critical aspect of infection control and treatment, as excess iron directly contributes to increased pathogen virulence and immune suppression.

Iron deficiency

Conversely, iron deficiency can limit bacterial growth by depriving pathogens of the iron necessary for their metabolic activities. However, iron deficiency also negatively impacts the host's immune function. Iron is essential for the proliferation and activity of immune cells, including T cells and B cells, which are vital for mounting effective immune responses. When iron levels are insufficient, immune cell proliferation is compromised, resulting in a weakened immune system. This makes the host more susceptible to infections and can prolong recovery times.[49]

Iron deficiency can arise from various factors, such as inadequate dietary intake, chronic blood loss or conditions that impair iron absorption. While reduced iron levels may hinder bacterial growth, the host's immune system suffers, leading to an increased risk of infection. Thus, maintaining a balance in iron levels is essential for optimal immune function and infection control. Both excessive and deficient iron levels create vulnerabilities in the host, either by facilitating pathogen proliferation or by impairing immune defences.

A. baumannii has evolved sophisticated mechanisms to overcome host-imposed iron limitations and exploit available iron sources, whether in conditions of excess or deficiency. It employs multiple siderophores and iron-regulated proteins to acquire iron from host proteins like transferrin and lactoferrin, circumventing the iron restriction imposed by nutritional immunity.[19] In addition to siderophores, *A. baumannii* utilizes other metal transport systems, allowing it to adapt to different host iron states by acquiring not only iron but also essential metals like zinc and manganese.

The bacterium's ability to thrive under iron restriction is a key factor in its virulence. Host proteins such as calprotectin sequester metals, including iron, zinc and manganese, limiting *A. baumannii*'s access to these critical nutrients. This mechanism of nutritional immunity significantly impacts the bacterium's ability to grow and cause infection, underscoring the effectiveness of the host's defence strategies.[53] Understanding these iron-dependent interactions provides valuable insights into potential therapeutic targets aimed at disrupting *A. baumannii*'s ability to acquire iron, thereby limiting its capacity to cause infection.

Therapeutic strategies targeting iron acquisition systems

Iron acquisition is crucial for *A. baumannii* survival and pathogenicity, as the bacteria rely on siderophores and the TonB-dependent transport system to obtain iron from the host. Targeting this system has therefore become a key therapeutic strategy for treating *A. baumannii* infections. Cefiderocol, the most representative drug within this approach, mimics natural siderophores and exploits the bacterial iron uptake mechanism to penetrate the outer membrane via the TonB-dependent system, thereby bypassing conventional resistance mechanisms, making it a vital tool in combating MDR*A. baumannii*, including CRAB.

Mechanism of action of cefiderocol

Cefiderocol is a siderophore-cephalosporin antibiotic designed to overcome resistance barriers by mimicking bacterial siderophores, such as acinetobactin. By binding to iron ions and utilizing the TonB-dependent transport system, cefiderocol efficiently penetrates bacterial cell, bypassing traditional resistance mechanisms like reduced membrane permeability and efflux pumps.[54] Once inside, cefiderocol primarily targets penicillin-binding

proteins (PBPs), a critical enzyme in the divisome responsible for peptidoglycan synthesis. By inhibiting PBP3 activity, cefiderocol disrupts bacterial cell wall synthesis, impairs cell division and ultimately leads to bacterial death. This mechanism not only prevents bacteria from acquiring iron through their natural pathways but also directly compromises the integrity of the bacterial cell wall.[55]

In addition, cefiderocol inhibits *A. baumannii*'s ability to utilize haemoglobin as an iron source. Normally, *A. baumannii* degrades haemoglobin to extract iron, a process vital for its survival and growth in iron-limited host environments. Cefiderocol inhibits this pathway, further reducing bacterial access to iron and impairing its virulence.[52] This multi-layered approach enhances the drug's antimicrobial effects while also limiting bacterial pathogenicity within the host.

Research also suggests that inhibiting non-ribosomal peptide synthetases (NRPS), enzymes critical for siderophore production, could enhance xefiderocol's effectiveness. NRPS inhibitors have been shown to significantly reduce bacterial survival and improve antibiotic efficacy under iron-limited conditions, suggesting a potential synergistic approach for improving outcomes in infections caused by *A. baumannii*.

Resistance mechanisms of cefiderocol

Although xefiderocol demonstrates strong antibacterial activity against CRAB infections, the rapid emergence of bacterial resistance has led to several resistance mechanisms that compromise its efficacy. Theses mechanisms often target the processes critical for xefiderocol's iron-dependent entry and activity, significantly reducing its therapeutic potential.

One of the most common resistance mechanisms involves mutations in TonB-dependent receptors, which are essential for cefiderocol's transport into bacterial cells. The TonB–ExbB–ExbD complex facilitates energy transduction needed for transporting iron–siderophore complexes and cefiderocol across the bacterial outer membrane. Mutations in the TonB system or its associated receptors impair this process, leading to reduced cefiderocol uptake and increased MIC.[56] Mutations in TonB-dependent receptors, particularly PiuA and PirA, disrupt the uptake of iron-antibiotic complexes by altering the structure or function of these receptors, thereby reducing cefiderocol's antimicrobial efficacy. These mutations, such as frameshifts in *piuA* and transposon insertions in *pirA*, impair the siderophore-mediated transport mechanism essential for the drug's activity. Such genetic alterations are notably prevalent in CRAB strains, significantly complicating clinical management and contributing to resistance against cefiderocol[28,57]

In addition to receptor mutations, some resistant strains exhibit down-regulation of iron acquisition genes, such as *fiu*, *feoA*, and *feoB*. By decreasing their reliance on iron uptake systems, these bacteria limit cefiderocol's access to its target pathways, effectively evading its iron-dependent mechanism of action. This adaptive shift enables the bacteria to survive even under cefiderocol treatment by limiting their need for exogenous iron.[58]

In addition, the acquisition of β-lactamase genes, such as *blaPER-1*, through horizontal gene transfer represents a significant challenge to cefiderocol's efficacy. These genes encode enzymes that hydrolyze cefiderocol's β-lactam ring structure, compromising its antibacterial activity. Overexpression of these β-lactamases, rather than mutations, mediates resistance in MDR strains, particularly CRAB. This mechanism underscores the role of horizontal gene transfer in the dissemination of resistance genes among clinical isolates.[24,59]

Biofilm formation represents another significant barrier to cefiderocol's effectiveness. Within biofilms, *A. baumannii* exhibit distinct gene expression patterns, including alterations in genes related to iron acquisition and quorum sensing. Biofilms create a physical barrier that impedes antibiotic penetration, while simultaneously down-regulating iron transport genes. This dual effect limits cefiderocol's activity in biofilm-associated infections, further complicating the treatment of CRAB strains with robust biofilm formation.[59–61]

These resistance mechanisms, including TonB receptor mutation, β-lactamase overexpression, gene down-regulation and biofilm formation, highlight the complex interplay between bacterial iron acquisition system s and cefiderocol resistance. Table 1 summarizes the key pathways, associated genetic mutations and their role in resistance, providing a comprehensive overview of the intricate relationship between these factors.

Clinical application of cefiderocol

Despite the emergence of resistance mechanisms discussed previously, clinical trials have demonstrated cefiderocol's efficacy in combating MDR pathogens. Robust evidence from clinical trials supports its use in treating severe infections. The APEKS-cUTI trial demonstrated cefiderocol's non-inferiority to imipenem-cilastatin in treating complicated urinary tract infections, with a higher clinical and microbiological success rate (72.6% versus 54.6%).[62] The APEKS-NP trial established cefiderocol's non-inferiority to high-dose, extended-infusion meropenem for nosocomial pneumonia, showing similar 14-day all-cause mortality rates (12.4% versus 11.6%) and comparable safety profiles.[63] However, the CREDIBLE-CR trial highlighted a higher all-cause mortality in patients treated with cefiderocol compared with the best available therapy, particularly in severe cases involving *Acinetobacter spp.* infections.[64] These findings underscore cefiderocol's potential as a valuable therapeutic option while emphasizing the importance of cautious use and further research to optimize its application.[65]

Combination therapies to enhance efficacy

The available data suggest that there is an increased rate of failure when cefiderocol is used as a monotherapy; in cases where resistance arises, combination therapies have shown promise in improving cefiderocol's antibacterial activity and mitigating resistance risks, especially for infections caused by *A. baumannii*. Clinical improvement and microbial eradication have been achieved by enabling the cefiderocol combination regimen to treat more complex bacterial infections, such as cefiderocol combined with avibactam, colistin, gentamicin and so on.[66–70] Retrospective analyses suggest that while monotherapy and combination therapy yield comparable clinical outcomes in critically ill patients, combination therapies may be preferred in severe or high-risk cases, where they offer enhanced efficacy and potentially reduce resistance development.[71]

Table 1. Iron uptake pathways, gene mutations and their role in cefiderocol resistance

Iron uptake pathway/ system	Associated genes/ mutations	Functional role	Cefiderocol resistance mechanism
TonB-dependent receptors	*TonB, BauA, PiuA, PirA*	Facilitates transport of iron–siderophore complexes by binding and channelling through the outer membrane	Specific mutations (e.g. BauA V67E) reduce siderophore affinity, while PiuA/PirA structural changes impede transport; prevalent in IC2 CRAB strains
Siderophore-mediated iron uptake	*BauB, BauC*	Transports acinetobactin via TonB-dependent receptors	Mutations in siderophore biosynthesis reduce cefiderocol binding
PER-1 β-lactamase	*blaPER-1,*	Hydrolyzes β-lactam antibiotics	Acquisition through horizontal gene transfer leads to β-lactam ring degradation
Biofilm-associated mechanisms	Biofilm-associated genes	Enhances antibiotic resistance through physical barriers	Down-regulation of iron transport genes in biofilms limits cefiderocol efficacy

Iron uptake pathways: Highlight the role of TonB-dependent receptors and siderophore biosynthesis in cefiderocol transport and resistance. Genetic mutations: Provide specific examples of mutations contributing to resistance. Clinical implications: Emphasize how these mechanisms complicate the treatment of CRAB infections and necessitate further research into resistance mitigation strategies.
PER-1 β-lactamase, *Pseudomonas aeruginosa* extended-spectrum β-lactamase.

It is worth noting that, synthetic siderophores mimic natural bacterial siderophores and trick bacteria into absorbing non-functional iron compounds, thereby inhibiting bacterial growth.[11,66,72] Similarly, iron chelators, such as deferiprone, bind to free iron in the host environment, depriving bacteria of the iron necessary for survival.[65] Recent studies have further explored the combination targeting bacterial iron acquisition systems,[73] The combination of cefiderocol and iron chelators may be considered in the future to reduce resistance.

Traditional therapies as adjuncts to cefiderocol

In addition to iron-targeting strategies, traditional therapies remain valuable adjuncts to cefiderocol in treating CRAB infections. Colistin, a long-standing treatment for MDR infections, is used both as monotherapy and in combination with carbapenems, such as meropenem, to enhance treatment efficacy. Colistin disrupts the bacterial outer membrane, potentially improving carbapenem penetration. However, clinical outcomes vary based on infection severity. Some studies report improved survival with colistin–carbapenem combinations in patients with severe infections and high APACHE II scores (e.g. 25–29), while others find no significant difference in outcomes, including mortality, between monotherapy and combination therapy when adjusted for severity.[74,75] Colistin's nephrotoxicity remains a significant limitation, necessitating careful monitoring and further research to optimize its clinical use.[76] Notably, recent large-scale randomized controlled trials (AIDA and OVERCOME) provided robust evidence that for CRAB infections, colistin–meropenem combination therapy offers no significant clinical advantage over colistin monotherapy. These findings challenge previous reports and underscore the importance of reevaluating traditional combination therapy strategies. (Paul *et al*., 2020; Kaye *et al*., 2022)

Paul M, *et al*. Colistin plus meropenem for carbapenem-resistant Gram-negative infections: the AIDA randomized controlled trial. Clinical Microbiology and Infection. 2020;26(5):596–603.

Kaye KS, *et al*. Colistin Monotherapy versus Combination Therapy for Carbapenem-Resistant Organisms. NEJM Evidence. 2022;1(2).

Similarly, high-dose sulbactam combined with ampicillin remains a key option for treating CRAB infections, particularly in cases of pneumonia and complicated urinary tract infections. This combination therapy has demonstrated efficacy against MDR strains and serves as an important alternative when resistance limits other treatment options.[77] The ATTACK trial demonstrated that sulbactam-durlobactam is non-inferior to colistin in the treatment of CRAB infections, with potential advantages in terms of reduced mortality and lower toxicity. However, the high cost of this drug and its limited availability remain significant barriers to its widespread use. (Kaye KS, 2023)

Kaye, K. S., Shorr, A. F., Wunderink, R. G., Du, B., Poirier, G. E., Rana, K., Miller, A., Lewis, D., O'Donnell, J., Chen, L., Reinhart, H., Srinivasan, S., Isaacs, R., & Altarac, D. (2023). Efficacy and safety of sulbactam-durlobactam versus colistin for the treatment of patients with serious infections caused by Acinetobacter baumannii-calcoaceticus complex: a multicentre, randomised, active-controlled, phase 3, non-inferiority clinical trial (ATTACK). *The Lancet. Infectious diseases*, *23*(9), 1072–1084. https://doi.org/10.1016/S1473-3099(23)00184-6

Future directions for cefiderocol use

Cefiderocol's unique mechanism of targeting bacterial iron acquisition systems offers a novel approach to combating MDR Gram-negative infections. Combination therapies that target these pathways, such as those using iron chelators or TonB inhibitors, represent a critical opportunity to enhance cefiderocol's efficacy and mitigate resistance. Furthermore, optimizing the use of cefiderocol in clinical practice requires careful consideration of patient populations, particularly in high-risk cases or infections caused by pathogens like *Acinetobacter spp*., which pose significant treatment challenges.

In summary, cefiderocol plays a critical role in combating CRAB infections due to its unique mechanism of targeting the bacterial iron acquisition system. While its clinical efficacy has been widely recognized, the emergence of resistance underscores the continued relevance of traditional treatment options

such as colistin-based combinations and high-dose sulbactam. Future research should prioritize the development of effective combination therapies and strategies to address the evolving resistance challenges in MDR *A. baumannii* infections.

Review summary and future research directions

Summary of iron ions in A. baumannii *research and clinical prospects*

Iron acquisition is integral to the growth, metabolism and virulence of *A. baumannii*, enabling it to thrive in iron-limited environments such as the human body. The pathogen utilizes siderophores and haem uptake pathways to access host iron, which is crucial for its survival and pathogenicity. Recent advances in targeting these mechanisms, particularly through therapies like siderophore modification, synthetic siderophores, TonB-dependent receptor blockers and novel agents like cefiderocol, show promising potential for treating MDR *A. baumannii* infections.[4,5,7,20,50] Clinical studies have demonstrated that disrupting iron homeostasis reduces the virulence and resistance of *A. baumannii*, offering hope for more effective therapeutic strategies. Nevertheless, other approaches, including colistin-based therapies and high-dose sulbactam, also play important roles in managing these infections, underscoring the need for an integrated treatment strategy.[7]

Future research directions

Future research should prioritize targeted molecular and genetic studies to address the mechanisms of cefiderocol resistance in *A. baumannii*. Specifically, efforts should focus on understanding the role of TonB-dependent receptors, biofilm-related adaptations and iron-regulated gene expression under diverse environmental and clinical conditions.

Investigating TonB mutations and resistance mechanisms

TonB-dependent receptors are critical for cefiderocol's transport into bacterial cells, and mutations in these receptors represent a major resistance mechanism. Future studies should explore the structural and functional impact of specific mutations in TonB-dependent genes, such as *bauA, piuA* and *pirA*. Techniques such as site-directed mutagenesis, combined with functional assays (e.g. iron uptake efficiency and cefiderocol MIC evaluations), could provide valuable insights into how these mutations impair drug transport. Advanced structural biology methods, such as protein-ligand docking simulations or cryo-electron microscopy, may further elucidate how receptor-ligand interactions are altered. Expanding these studies to clinical isolates with naturally occurring mutations would also help bridge the gap between *in vitro* findings and clinical relevance.

Exploring the role of biofilms in resistance

Biofilm-associated resistance significantly reduces cefiderocol's efficacy by creating a protective environment and altering gene expression patterns. Future research should focus on high-throughput transcriptomic analyses, such as RNA-seq, to compare gene expression in biofilm-forming versus planktonic cells. Particular attention should be given to the differential regulation of genes involved in siderophore biosynthesis, TonB systems and efflux pumps. Experimental designs could include biofilm disruption assays combined with cefiderocol treatment to evaluate potential synergy with anti-biofilm agents.

Unravelling iron-regulated gene networks

The regulatory networks controlling iron acquisition in *A. baumannii* are complex and poorly understood. Employing CRISPR-Cas9 [clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9)]–based gene knockout or knockdown approaches could help dissect the roles of key iron-regulated genes, such as *fiu*, *feoA* and *feoB*, in siderophore production and iron transport. Coupling these genetic studies with *in vitro* and *in vivo* models of infection can clarify how these genes contribute to resistance and virulence under iron-limited conditions, such as those imposed by host nutritional immunity.

Developing experimental models for resistance monitoring

Robust experimental models that mimic clinical scenarios are essential for tracking the development of resistance. For instance, serial passage experiments under sub-inhibitory cefiderocol concentrations could be used to induce resistance, enabling the identification of adaptive mutations and resistance pathways. Whole-genome sequencing of resistant isolates from these models could provide insights into the genetic determinants of resistance, particularly in the context of biofilm formation and iron acquisition.

Optimizing iron-targeting therapies

In addition to genetic studies, optimizing the design of iron-targeting compounds remains critical. Structure–activity relationship studies and pharmacokinetic evaluations should focus on enhancing the stability, efficacy and specificity of these compounds. Siderophore-drug conjugates, which exploit bacterial iron acquisition systems for targeted antibiotic delivery, represent a promising avenue. Clinical trials evaluating these conjugates in the context of HAIs, particularly in ICU and COVID-19 patients, will be essential to validate their utility and address emerging resistance.

Personalized treatment strategies and biomarker development

To improve treatment outcomes, personalized approaches based on patient-specific iron metabolism and *A. baumannii* resistance profiles should be explored. Biomarker-driven strategies, such as monitoring the expression of iron-regulated genes or detecting specific mutations in TonB receptors, could guide the selection of tailored therapies. These approaches can maximize efficacy while minimizing side effects, particularly in critically ill populations.

Standardized monitoring and resistance management

Long-term monitoring of patients receiving cefiderocol or iron-targeting therapies is essential to detect resistance early. Standardized protocols for resistance surveillance, combined with adaptive treatment regimens, can ensure the sustained effectiveness of these therapies. Global surveillance programmes incorporating diverse patient populations will help track resistance trends and guide clinical decision-making.

By focusing on these targeted studies—TonB mutations, biofilm-related resistance, iron-regulated gene networks and compound optimization—future research can advance our understanding of cefiderocol resistance and refine therapeutic strategies for managing MDR *A. baumannii*. These efforts will be crucial to improving patient outcomes and addressing the growing threat of antimicrobial resistance in clinical settings.

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Author contributions

R.Z. and D.L. contributed equally to this work. They participated in the conception of the study, performed the literature review and were major contributors in writing the manuscript. H.F., Q.X. and H.T. assisted in the literature review and drafting of the manuscript. L.C. provided oversight and guidance throughout the project, critically revised the manuscript for important intellectual content and provided final approval for publication.

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