Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Resistance to Cefiderocol Involved Expression of PER-1 β-Lactamase and Downregulation of Iron Transporter System in Carbapenem-Resistant *Acinetobacter baumannii*

Yukun He[1,*], Yifan Wang[1,*], Xinqian Ma[1], Lili Zhao[1], Jie Guan[2], Jin Zhao[3], Wenyi Yu[1], Yanjun Li[4], Wentao Ni[1], Zhancheng Gao[1]

[1]Department of Pulmonary and Critical Care Medicine, Peking University People's Hospital, Beijing, People's Republic of China; [2]Clinical Laboratory, Peking University First Hospital, Beijing, People's Republic of China; [3]Department of Respiratory Diseases, Air Force Medical Center, Chinese People's Liberation Army, Beijing, People's Republic of China; [4]Clinical Laboratory, The Sixth Medical Center of PLA General Hospital, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Wentao Ni; Zhancheng Gao, Department of Pulmonary and Critical Care Medicine, Peking University People's Hospital, Beijing, People's Republic of China, Email wentao.qingdao@163.com; zcgao@bjmu.edu.cn

Background: Cefiderocol (CFDC) is a promising antimicrobial agent against multidrug resistant Gram-negative bacteria. However, CFDC resistance has emerged in carbapenem-resistant *Acinetobacter baumannii* (CR-AB) but the underlying mechanisms remain unclear.

Methods: Whole-genome sequencing and transcriptome sequencing were performed on CFDC-non-susceptible and CFDC-susceptible isolates. Two different recombinant plasmids was electro-transformed into the *E. coli* BL21 strain to determine the impact of bla_{PER} and the combined impact of bla_{PER-1} and bla_{OXA-23} on CFDC resistance.

Results: Fifty-five CR-AB isolates with minimum inhibitory concentrations (MICs) ranged from 0.06 mg/L to >256 mg/L were sequenced, including 47 CFDC-non-susceptible and eight CFDC-susceptible isolates. Two CFDC-non-susceptible isolates belonged to ST104 whereas the remaining isolates belonged to ST2, and bla_{PER-1} was present only in CFDC-non-susceptible isolates. Amino acid substitutions were noted in penicillin-binding proteins (PBPs) in four CFDC-susceptible isolates, with slightly elevated MICs. The MICs of recombinant *E. coli* BL21 carrying the bla_{PER-1} gene increased 64-fold and recombinant *E. coli* BL21 carrying both the bla_{PER-1} and bla_{OXA-23} genes increased 8-fold but both remained within the susceptibility range. Transcriptome sequencing of 17 CFDC-non-susceptible isolates and eight CFDC-susceptible isolates revealed that transcriptional levels of various iron transport proteins, such as *fiu, feoA*, and *feoB*, and the energy transduction system, TonB-ExbB-ExbD, were relatively downregulated in CFDC-non-susceptible isolates. GO enrichment analysis revealed that the upregulated genes in CFDC-non-susceptible isolates were mainly associated with redox homeostasis and stress response. Besides, the expression levels of the bla_{OXA-23} and exbD genes were negatively correlated with the MICs.

Conclusion: PER-1 production, iron transport system downregulation, and mutations in PBPs may synergistically impart high-level resistance to CFDC in CR-AB.

Keywords: carbapenem-resistant *Acinetobacter baumannii*, cefiderocol resistance, bla_{PER-1}, iron transporter systems

Introduction

Cefiderocol (CFDC) is a novel siderophore-cephalosporin that was approved successively by the United States Food and Drug Administration (FDA) and European Medicines Agency for treating complicated gram-negative infections in 2019 and 2020.[1] The catechol side chain of CFDC readily complexes with ferric iron, allowing it to utilize an iron-based

Received: 8 October 2022
Accepted: 2 December 2022
Published: 7 December 2022

transport system to penetrate the bacterial outer membrane. This "Trojan horse" strategy allows more drugs to enter the bacterial periplasmic space and bind to penicillin-binding proteins (PBPs), interfering with cell wall synthesis and ultimately causing bacterial death.[2] Susceptibility surveillance has revealed the potent activity of CFDC against a wide range of carbapenem-resistant gram-negative bacteria (CR-GNB), including isolates of *Acinetobacter baumannii, Enterobacterales*, and *Pseudomonas aeruginosa*.[3] The CREDIBLE-CR trial showed that the clinical and microbiological efficacy of CFDC was comparable to that of the best available therapeutic agents for various CR-GNB infections.[4]

However, resistance to CFDC has emerged among CR-GNB before its widespread use in clinical practice. For example, the SIDERO-CR-2014/2016 surveillance study identified 72 CFDC-non-susceptible isolates from 1873 carbapenem non-susceptible isolates, and the European test set of this study showed that only 82.8% of carbapenem-resistant *Enterobacterales* were susceptible to CFDC.[5] We previously evaluated in vitro antibacterial activity of CFDC against 126 carbapenem-resistant *A. baumannii* (CR-AB) isolates collected from four hospitals in Beijing, and reported high levels of CFDC resistance among these clinical isolates.[6] In addition, development of resistance during treatment has also been observed in some patients.[7]

We previous showed that bla_{PER-1} was only present in CFDC-non-susceptible CR-AB isolates, but not in susceptible isolates, suggesting the possible role of PER enzymes in CFDC resistance.[6] Other studies reported deficiency of the expressions of the iron transporters,[8–10] and/or alterations in penicillin-binding proteins (PBPs)[10] might also involve in CFDC resistance in gram-negative bacteria. However, PER enzymes could not fully explain the high-level of CFDC resistance in CR-AB in our study. The mechanisms associated with the reduced susceptibility to CFDC in CR-AB appear complicated and require further investigation. Considering that multi-omics can provide useful information regarding the biological activity and genetic characteristics of bacteria, in this study, we explored the differences in CFDC-non-susceptible and CFDC-susceptible CR-AB isolates via genomic and transcriptomic analysis to elucidate possible mechanisms leading to high-level CFDC resistance.

Materials and Methods

Bacterial Isolates and Antimicrobial Susceptibility Testing

A total of 126 non-duplicate CR-AB clinical isolates from different inpatients at four tertiary A-level hospitals in Beijing, China between 2012 and 2018 were described in our previous study.[6] All isolates were collected for parts of the routine laboratory test and identified using Vitek® matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *E. coli* ATCC® 25,922 was used as a quality control strain. Minimum inhibitory concentrations (MICs) of CFDC (purchased from Shionogi) were tested using the microdilution method in iron-depleted, cation-adjusted Mueller-Hinton broth (ID-CAMHB) according to the recommendations of the Clinical and Laboratory Standards Institute.[11] To explore comprehensive mechanisms of the large variation of resistance, we chose all 47 CFDC-non-susceptible isolates and eight CFDC- susceptible isolates were selected randomly as the control group in this study. The MICs of these isolates against CFDC and other antibiotics are listed in Table S1 in Supplementary Material.

Whole-Genome Sequencing and Analyses

Genomic DNA of 55 CR-AB isolates was extracted using a genomic DNA extraction kit (TIANGEN), according to the manufacturer's instructions. Genome sequencing was performed on the Illumina HiSeq×10 platform by generating paired-end libraries. High-quality sequencing data were generated by removing adaptors, low-quality reads, and short reads using fastp,[12] and filtered reads were assembled into contigs and scaffolds using SOAPdenovo2.[13] The sequencing and assembly quality are listed in the Table S2 In Supplementary Material. MLST analysis was conducted using MLST software.[14] Pairwise ANI values were converted to a Euclidean distance matrix and then used in the ggtree R package to view the unweighted pair group method with arithmetic mean (UPGMA) tree. Plasmids were identified and annotated based on the PlasFlow[15] and PLSDB databases.[16] The Comprehensive Antibiotic Resistance Database (CARD)[17] was used to identify antibiotic resistance genes (ARGs). Isolate variant analysis was carried out with Snippy 4.6.0 against the reference genome, *A. baumannii* ATCC 19606 (CFDC MIC = 0.125 mg/L, ST52), using default parameters. A phylogenetic tree based on cgSNPs was constructed using the PHYLIP package. PROVEAN was used to predict whether an amino acid substitution affected the biological function of a protein.[18]

Transcriptome Sequencing and Analyses

Due to the large variation in CFDC resistance among these 55 isolates and the differences found in the genome among the isolates, we selected representative isolates with different CFDC MICs for transcriptome sequencing. Total RNA was extracted from 25 CR-AB isolates (17 CFDC non-susceptible and eight CFDC susceptible isolates) using TRIzol® Reagent. The RNA-seq library was prepared using the TruSeq™ RNA sample preparation kit. Ribosomal RNA was removed using a Ribo-Zero Magnetic kit (Epicenter) and cDNA was synthesized using the SuperScript double-stranded cDNA synthesis kit (Invitrogen). Libraries were selected for cDNA target fragments of 200 bp and then sequenced on the Illumina HiSeq platform. The quality control and gene annotation methods were the same as those used for genomic analysis. For the two groups, differentially expressed genes (DEGs) were analyzed using DESeq2 (v.1.20.0) and identified based on the combined criteria of (FC) > 1.5, $P \leq 0.05$, and false discovery rate ≤0.01, after normalizing their expression. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations were performed for DEGs using the corresponding database as a reference. Weighted Gene Co-Expression Network Analysis (WGCNA) was used to explore co-expressed gene modules that have high biological significance and explore the relationship between gene networks and resistance to CFDC.[19] Gene significance (GS) was used to describe the relationship between gene and phenotypes. Module membership (MM) was calculated to evaluate the importance of a gene in the module. Genes with both |GS| > 0.3 and |MM| > 0.7 were defined as hub genes among the candidate gene modules.

Cloning Experiments

Two different recombinant plasmids were constructed to determine the impact of bla_{PER} and the combined impact of $bla_{PER\text{-}1}$ and $bla_{OXA\text{-}23}$ on CFDC resistance. The gene sequences were synthesized and cloned into the pET vector. The vector was linearized using restriction endonuclease BSAI-HFV2. Gibson Assembly® Master Mix was used for the Gibson reaction. The products of the Gibson reaction were transformed into competent UltraStable cells (VectorBuilder). The transformed cells were then inoculated onto Luria-Bertani (LB) agar containing 100 μg/mL ampicillin and incubated overnight at 37°C. Positive clones were selected to extract plasmid DNA, which was verified by sequencing. Since clinical isolates have a complex genetic background and diverse mechanisms leading to CFDC resistance, which might interfere with the validation of gene function, the recombinant plasmid was electro-transformed into the *E. coli* BL21 strain and the bacterial solution was inoculated onto LB agar containing 100 μg/mL of ampicillin following overnight incubation. PCR analysis of the positive clones was conducted to verify the success of the transformation. All transformants and parental isolates were subjected to test the CFDC MICs under induction using 0.5 mM isopropyl beta-d-1-thiogalactopyranoside (IPTG).

Results

Multi-Locus Sequence Typing (MLST) and Phylogenetic Analysis

In 55 CR-AB isolates MICs for CFDC ranged from 0.06 mg/L to >256 mg/L. MLST analysis showed that 53 isolates belonged to ST2, whereas the remaining two isolates, A43 and A45, belonged to ST104 (Table 1). Among the CR-AB sequences, all pairwise average nucleotide identity (ANI) values were >99% (Table S3 in supplementary Material). A UPGMA tree was constructed based on the ANI values (Figure 1A), and a maximum-likelihood tree was generated based on the core genome single nucleotide polymorphism (cgSNP) of all sequenced genomes (Figure 1B). Both phylogenetic trees showed that 41 resistant isolates and the susceptible strain A5 collected from the same hospital formed a clade (Figure 1A and B). The resistant isolates A43 and A45, A1 and A2, and A6, and A38 were closely grouped in pairs and were closer to the other seven sensitive isolates.

Genes Associated with Resistance to Cephalosporins

Diverse ARGs were identified using the CARD. Regarding the genes associated with resistance to cephalosporin-based agents, genes encoding β-lactamases were detected. Members of Ambler class C β-lactamases are encoded by four cephalosporinase bla_{ADC} variants. Among them, $bla_{ADC\text{-}25}$ was present in 45 CFDC-non-susceptible isolates and only one susceptible isolate, A5, whereas $bla_{ADC\text{-}79}$ was present only in two CFDC-non-susceptible isolates. Two ARGs belonging

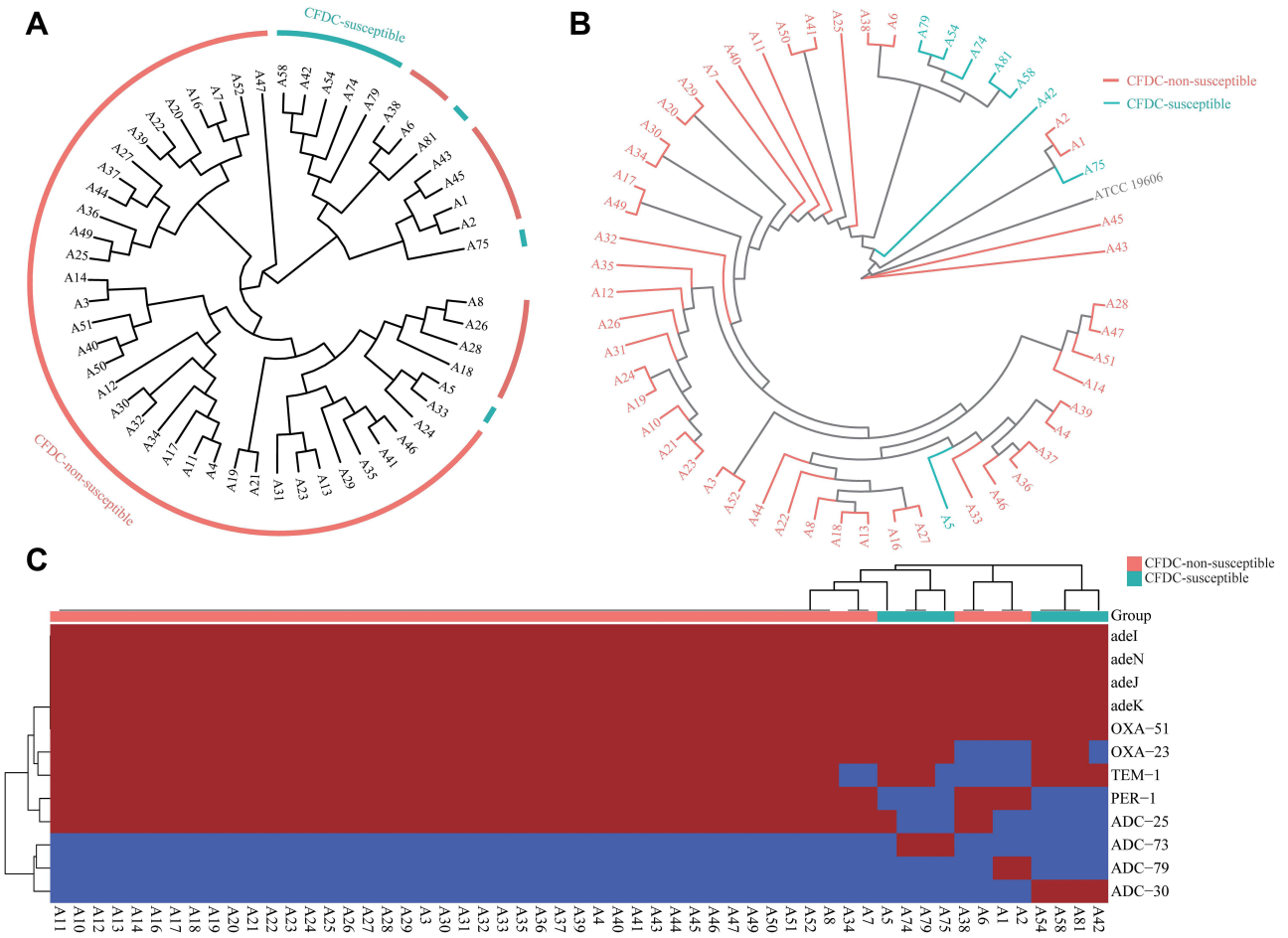

Figure 1 Genomic analysis of 55 carbapenem-resistant *Acinetobacter baumannii* isolates. (**A**) Evolutionary tree based on pairwise average nucleotide identity (ANI) among 47 cefiderocol-non-susceptible isolates and eight cefiderocol-susceptible isolates. (**B**) Phylogenetic tree based on core genome single nucleotide polymorphism (cgSNP) compared with ATCC 19606. (**C**) Distribution of cefiderocol resistance related genes in all 55 isolates.

to the Ambler class D β-lactamases were identified: bla_{OXA-51} was present in all 55 isolates, while bla_{OXA-23} was present in 43 resistant isolates and seven sensitive isolates (Figure 1C). Two ARGs belonging to Ambler class A β-lactamases (bla_{TEM-1} and bla_{PER-1}) were identified, of which, bla_{PER-1} was present only in CFDC-non-susceptible isolates. The bla_{PER-1} gene in nine resistant isolates was located on a pVPH1 plasmid, whereas bla_{PER-1} gene in 36 resistant isolates was located on unannotated plasmids based on current databases. No metallo-β-lactamases were identified in any of the isolates. In addition, four genes encoding the resistance-nodulation-division (RND)-type efflux pump associated with cephalosporin resistance (*adeN, adeI, adeJ*, and *adeK*) were detected in all the isolates.

Analysis of Genes Involved in CFDC Resistance

The number of SNPs among the 55 CR-AB isolates exceeded 39,000. Of these, 13% were non-synonymous mutations, compared to the strain ATCC 19606. Mutations in genes potentially conferring resistance to CFDC were examined, including *ftsI* (which encodes PBP3, the primary target of CFDC) and the TonB-ExbB-ExbD complex (which supplies energy to TonB siderophore receptors) (Table S4 in supplementary Material). Two amino acid substitutions (Ala515Val and Ala515Thr) were noted in *ftsI* encoded proteins in four susceptible isolates (A42, A74, A75, and A79), and both substitutions deleteriously affected protein function. These four isolates displayed slightly elevated MICs compared to the MICs of the other four susceptible isolates (Table 1). In terms of *exbD*, two resistant isolates (A6 and A38) and one susceptible isolate (A75) exhibited amino acid substitutions (Met25Thr and Thr37Arg), of which, Met25Thr substitution was predicted to exert a deleterious effect on protein function.

Table 1 Cefiderocol Minimum Inhibitory Concentrations (MICs) Distribution and MLST of 55 Carbapenem-Resistant *Acinetobacter baumannii* Isolates. The Isolates, A43, and A45 Were ST104 and the Remaining 53 Isolates Were ST2

MICs of Cefiderocol (mg/L)	Isolates
0.06	A5, A58
0.125	A54, A81
0.25	A42
0.5	A74, A79
2	A75
8	A7, A52
16	A19, A24, A43
32	A8, A14, A22, A26, A44, A45
64	A21, A23, A27, A30, A34, A38, A40, A41, A47, A50, A51
128	A1, A2, A10, A11, A16, A18, A25, A28, A29, A32, A33, A35, A39, A46, A49
≥256	A3, A4, A6, A12, A13, A17, A20, A31, A36, A37

Analysis of Differentially Expressed Genes

Twenty-five clinical isolates, including 17 non-susceptible and eight susceptible isolates, were selected for transcriptomic analyses to further explore the mechanisms underlying the development of CFDC resistance. In total, 3623 genes were identified. Among these, 86 genes were expressed only in the resistant group and 162 genes were expressed only in the sensitive group (Figure 2A). Principal component analysis revealed that samples in the two groups were distinct from each other, except A5 (Figure 2B), which was consistent with the results of genomic analyses. Comparison between the two groups revealed 785 DEGs (p.adj < 0.05, fold change [FC] > 1.5). These included 496 upregulated and 289 downregulated genes in the resistant group (Figure 2C). The bla_{PER-1} gene was significantly upregulated (Figure 2D) and there were no significant differences in the expression levels of other β-lactamase encoding genes between the groups. Additionally, the transcriptional levels of various iron transport protein encoding genes, such as *fiu, feoA* and *feoB*, were decreased in the non-susceptible isolates. Concomitantly, the expression of genes encoding typical virulent effectors, such as *fimC, fimD*, and *CsuA/B*, was considerably reduced in the non-susceptible group. The gene products of *CsuA/B* are essential for biofilm formation, community formation, and adhesion to solid substrates.[20] Moreover, the TonB-ExbB-ExbD energy transduction system was differentially expressed between the groups; the expression levels of TonB and ExbB were lower in the non-susceptible group than in the susceptible group.

Functional Analysis of DEGs

To further determine the molecular characteristics of the DEGs, functional categorization was performed using GO and KEGG annotations and GO enrichment analysis. GO annotation revealed a set of 519 DEGs (66%) with known annotations. According to the molecular function categories, the upregulated DEGs in the resistant group were related to "hydrolase activity", "ATP binding", and "oxidoreductase activity" (Figure 3A). The majority of the downregulated genes were linked to "DNA binding" and "structural constituent of ribosome" (Figure 3B). GO enrichment analysis revealed that the upregulated genes in non-susceptible isolates were mainly associated with redox homeostasis and stress response, which may be related to the disturbance of iron metabolism (Figure 3C). Related genes in "oxidoreductase activity" were listed in Table S5 in Supplementary Material. In contrast, the downregulated genes were associated with active protein synthesis (Figure 3D). According to the KEGG database, 189 DEGs were annotated and most of them were related to metabolic pathways. Pathways concerning carbohydrate metabolism, oxidative phosphorylation, nucleotide excision repair, and two-component system were more abundant in upregulated DEGs among the CFDC-resistant isolates, while 15 ribosome-related genes were observed in the downregulated DEGs among these isolates (Table S6 in Supplementary Material).

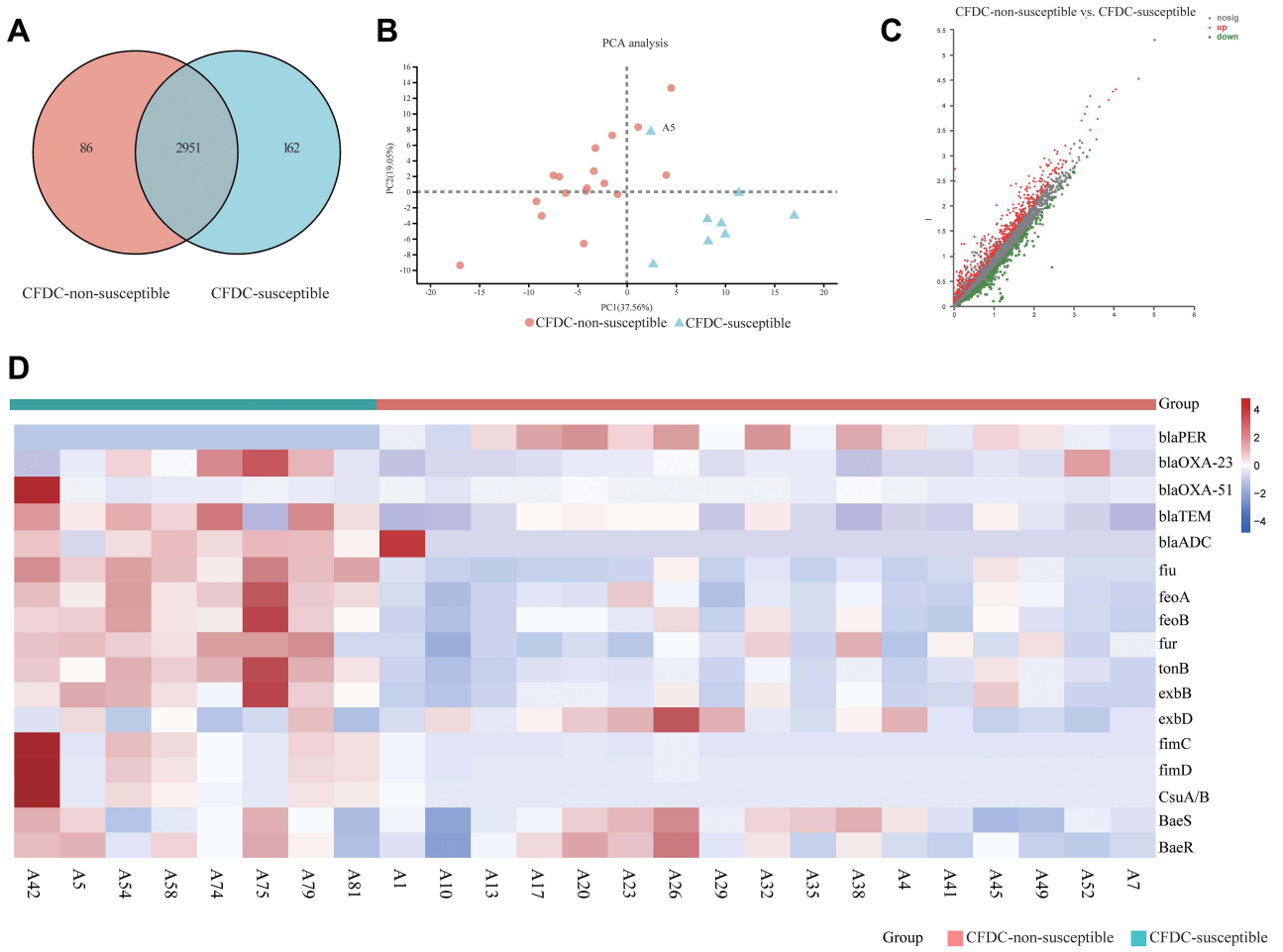

Figure 2 Transcriptome analysis of 25 carbapenem-resistant *Acinetobacter baumannii* isolates. (**A**) Venn plot of genes expressed in cefiderocol-non-susceptible isolates and eight cefiderocol-susceptible isolates. (**B**) Principal component analysis score plots of total transcriptome data. (**C**) Volcano plot of differentially expressed genes (DEGs) in each study group. DEGs were defined as genes with fold change > 1.5, P ≤ 0.05, and false discovery rate ≤0.01. (**D**) Expression levels of genes related to cefiderocol resistance.

Analysis of Weighted Gene Co-Expression Modules for Resistance to CFDC

To explore the core regulatory modules related to the high level of resistance to CFDC, we performed WGCNA using transcriptome data of 17 CFDC-non- susceptible isolates. The scale-free network was constructed by setting the optimal soft threshold power (β = 9) to the first set of power values when the scale-free topology index reached 0.9 (Figure 4A). Genes with the same or similar expression patterns were grouped into the same gene module using a "dynamic tree cutting" algorithm to form a hierarchical clustering tree (Figure 4B). Weighted hierarchical clustering analysis resulted in ten gene modules (Figure 4C). Obviously, the genes in the "red" module had negative correlation with MICs (Figure 4D, Table S7 In Supplementary Material), while the genes in the "green" module had the strongest positive correlation with MICs (Figure 4E, Table S8 in Supplementary Material). Among them, the genes bla_{OXA-23} and *exbD* were found in the hub gene set of the red module, indicating that the expression levels of them may be negatively correlated with the MICs. Two genes associated with iron-sulfur clusters were found in the hub gene set of the green module.

Effect of bla_{PER-1} on CFDC Susceptibility

To further clarify the contribution of bla_{PER-1} and bla_{OXA-23} to CFDC resistance, plasmids were constructed. MIC values showed that *E. coli* recombinant isolates producing bla_{PER-1} had 64-fold higher CFDC MIC values than the recipient strain; however, these values were still below the susceptibility breakpoints (Table 2). Besides, the effect of

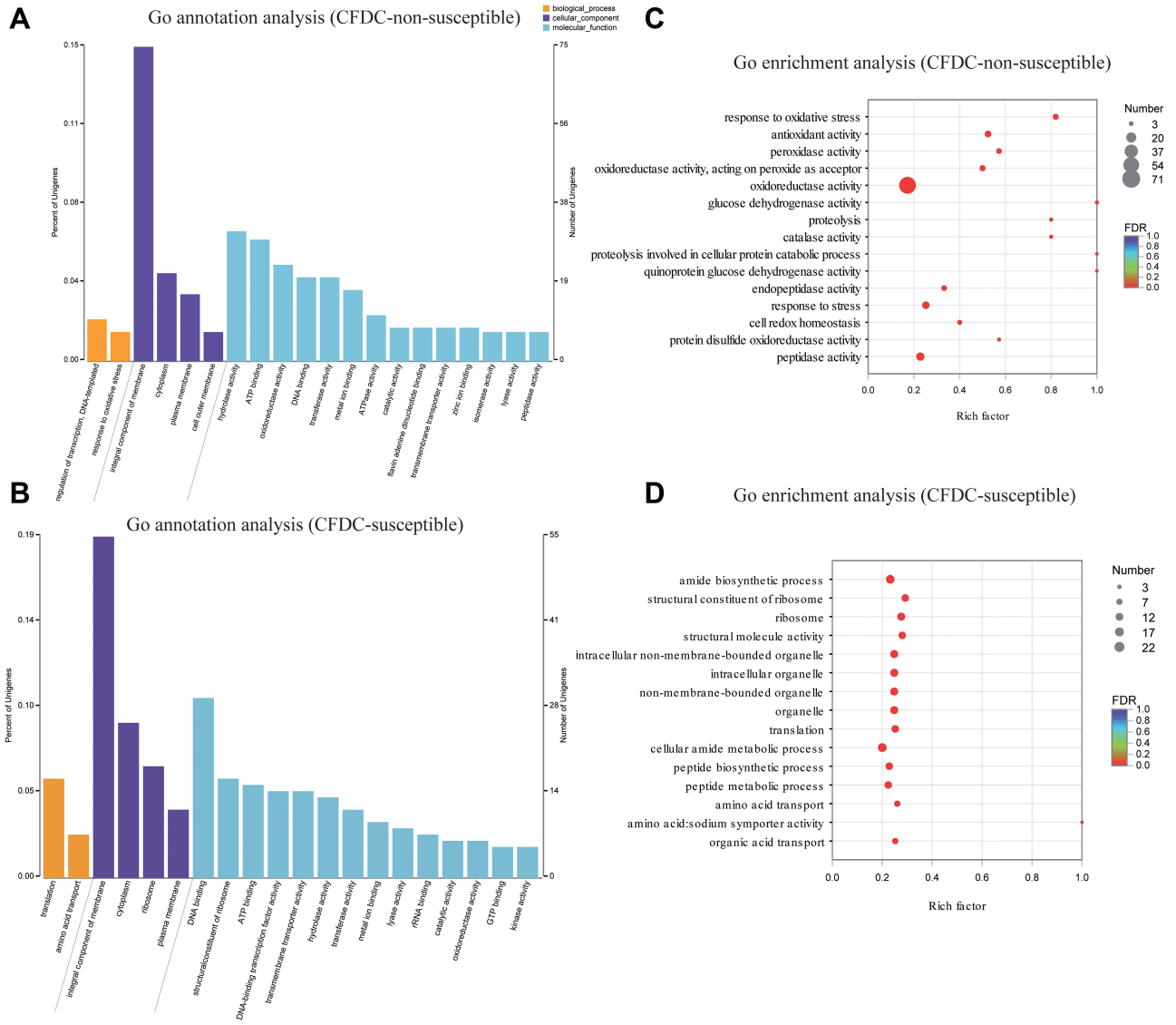

Figure 3 Gene Ontology (GO) analysis of all differentially expressed genes (DEGs). (**A** and **B**) GO annotation (**A**) and enrichment (**B**) analysis of DEGs upregulated in cefiderocol-non-susceptible isolates. (**C** and **D**) GO annotation (**C**) and enrichment (**D**) analysis of DEGs downregulated in cefiderocol-non-susceptible isolates.

co-expression of bla_{PER-1} and bla_{OXA-23} was explored. The *E. coli* clones producing both genes remained susceptible to CFDC, with 8-fold higher in the MICs, which is consistent with the result of WGCNA, that is, the expression level of bla_{OXA-23} may be negatively correlated with the resistance to CFDC.

Discussion

Infections caused by CR-AB are among the leading causes of nosocomial infections. CFDC is a novel cephalosporin-siderophore that was approved by the FDA in late 2019.[21] Several studies have highlighted the utility of CFDC for treating multidrug resistant gram-negative pathogens.[4,8] However, CFDC resistance has emerged in *A. baumannii*.[22,23] In this study, we characterized the genomic and transcriptomic profiles of CFDC-non-susceptible CR-AB isolates using next-generation sequencing. The data indicate that multiple factors contribute to high-level CFDC resistance in CR-AB.

The production of some β-lactamase enzymes, such as PER-1 and NDM, may be associated with CFDC resistance. Using the CARD database, we detected eight β-lactamase enzymes in the CFDC-resistant CR-AB. Similar to the SIDERO-WT-2014 study,[24] all CFDC-non-susceptible isolates harbored bla_{PER-1}, while none of the susceptible isolates harbored bla_{PER-1}. Other β-lactamases, such as bla_{OXA-23}, bla_{OXA-51}, bla_{ADC}, and bla_{-TEM}, were detected in both

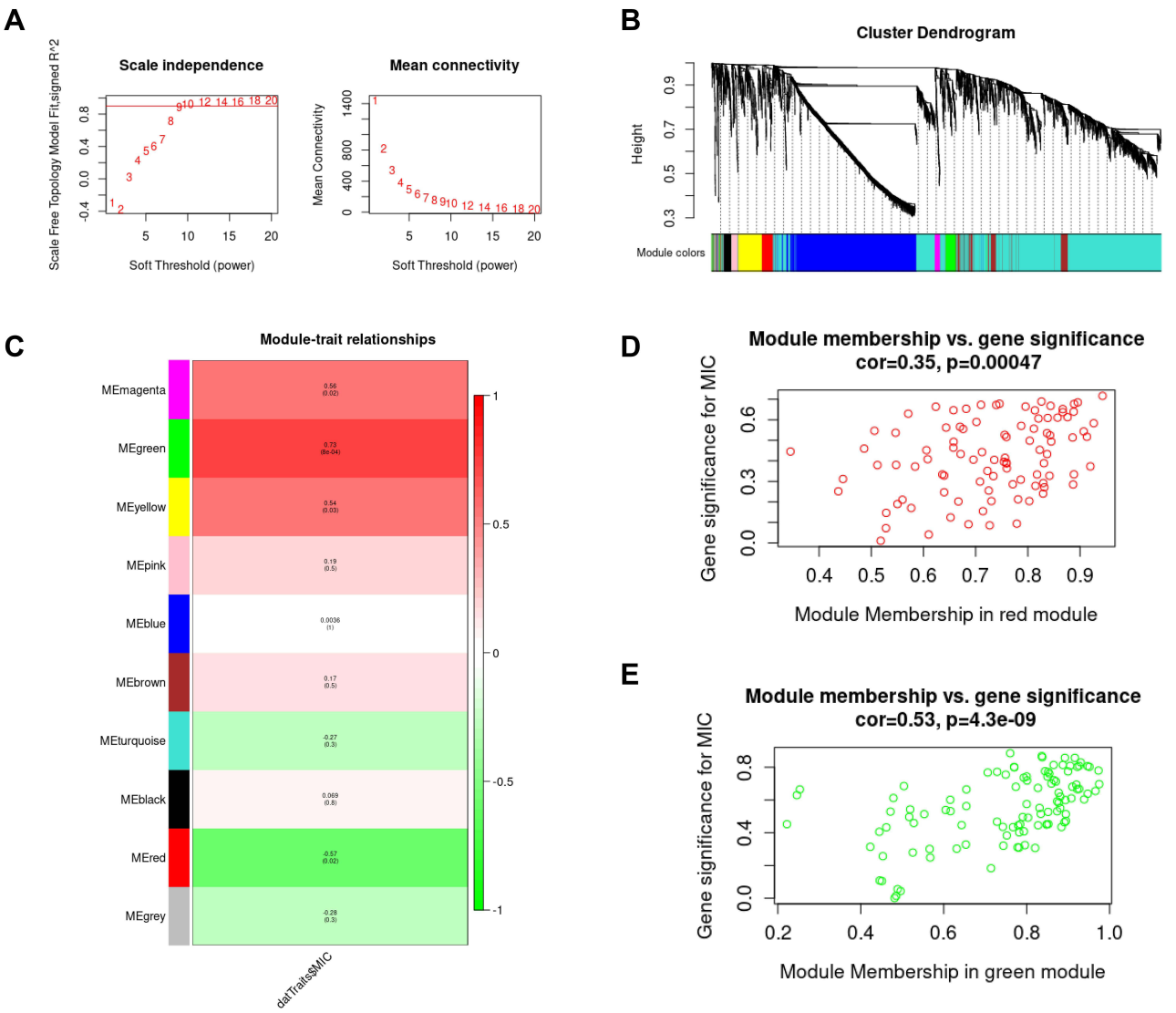

Figure 4 Weighted gene co-expression network analysis (WGCNA). (**A**) Selection of the best soft threshold. (**B**) Ten co-expression modules were obtained for genes with fusion distance less than 0.25. Among them, the gray modules contained genes that could not be clustered. (**C**) Correlation of modules with minimum inhibitory concentrations (MICs). (**D**) and (**E**) Module membership and gene significance in the red and green modules, respectively.

susceptible and non-susceptible isolates. *E. coli* BL21 strain was used to explore the effect of bla_{PER-1} and bla_{OXA-23} considering the complexity of genetic background of clinical isolates. The MIC of *E. coli* BL21 harboring bla_{PER-1} increased 64-fold compared to its original MIC, but remained within the susceptibility range. Meantime, the expression level of bla_{PER-1} showed no significant differences among different CFDC-non-susceptible isolates. Therefore, PER-1 production alone does not explain high-level CFDC resistance. Besides, although in vitro studies have demonstrated that CFDC is stable against bla_{OXA-23},[25] the MIC of the *E. coli* BL21 strain harboring both bla_{PER-1} and bla_{OXA-23} only

Table 2 Effect of bla_{PER-1} and Combined Effect of bla_{PER-1} and bla_{OXA-23} on CFDC Susceptibility in *E. coli* BL21

Strain	Description	MIC of CFDC (mg/L)
BL21	Wild type	0.03
BL21	Plasmid with bla_{PER-1}	2
BL21	Plasmid with bla_{PER-1} and bla_{OXA-23}	0.25

increased 8-fold and the WGCNA analysis indicated that the expression levels of bla_{OXA-23} them may be negatively correlated with the MICs to CFDC, which may be caused by more fitness costs to the host cell compared with the plasmid carrying only bla_{PER-1}, and needs further study.

In addition to PER-1 production and PBP3 mutations, changes in genes related to the iron transport system may also be responsible for imparting CFDC resistance. Ito et al implicated deletion of *fiu* with higher CFDC MICs in *E. coli*.[8] Yamano et al speculated that the deficient expression of *piuA* might cause CFDC resistance in *A. baumannii*.[26] However, two reported iron transport genes, *piuA* and *pirA*, were not detected in CFDC-susceptible or CFDC-non-susceptible isolates in our study. Therefore, we compared the transcriptomic profiles of CFDC-non-susceptible and CFDC-susceptible isolates. The expression of multiple iron transport-related genes (*fiu, feoA, feoB, tonB*, and *exbB*) was significantly decreased in the non-susceptible group, indicating that the reduced functional activity of various iron transport proteins potentially contributes to CFDC resistance in CR-AB. Therefore, the high-level resistance to CFDC in CR-AB is most likely caused by a combination of PER-1 production and downregulated iron transport systems. Wang et al reported that the MIC of a CFDC-susceptible *E. coli* harboring NDM-5 increased from 1 mg/L to 64 mg/L after deletion of *cirA* (which encodes a siderophore receptor). Furthermore, the MIC of an isolate harboring NDM-5 along with *cirA* deletion decreased from 64 mg/mL to 0.5 mg/mL following bla_{NDM-5} deletion. These results provide further evidence that the expression of β-lactamases and deficiency of iron transporters synergistically contribute to high CFDC resistance.[27]

We noticed that the CFDC MICs also varied significantly among the susceptible isolates. Mutations in genes involving targets for beta-lactam (PBPs) and iron transporters (TonB system) are also related to CFDC resistance.[6,8,10,27] Thus, we investigated other potential genetic determinants of resistance by comparing SNP variants among the isolates. Alterations in *ftsI* encoding PBP3 were found in four susceptible isolates (A42, A74, A75, and A79) with relatively higher CFDC MICs of 0.25–2 mg/L, suggesting that mutations in PBP3 might be responsible for the slightly elevated MICs. Malik et al also reported a CFDC-resistant *A. baumannii* isolate possessing mutations leading to Ile236Asn and His370Tyr alterations in *ftsI* encoded PBP3, which were predicted to moderately affect the its functionality.[10] Wang et al found that some CFDC-resistant *E. coli* isolates harbored mutations at amino acid residue 333 of PBP3, and an induced PBP3 mutation in *E. coli* DH5α could increase CFDC MIC by 4-fold (from 0.06 to 0.25 mg/L).[27]

In the present study, transcriptome analysis revealed that the expression of *CsuA/B* genes related to biofilm formation was significantly decreased in the CFDC non-susceptible isolates. Bao et al also found that genes involved in biofilm formation were downregulated and biofilm formation ability was reduced in CFDC-resistant *Klebsiella pneumoniae* due to the downregulation of siderophore transporter proteins.[28] Taken together, these results indicate a positive feedback between reduced biofilm formation and siderophore transporter protein expression in gram-negative bacteria.[28] Decreased iron content in bacteria may further reduce their biofilm formation ability. Moreover, GO enrichment analysis revealed that upregulated genes in non-susceptible isolates were mainly associated with redox homeostasis and stress response. This could be attributed to the tight relationship between iron metabolism and redox homeostasis, in other words, the activity of several major regulators depends on iron and the iron could also serve as an ion cofactor assembled in iron–sulfur clusters.[29]

Our study had several limitations. First, only eight CFDC-susceptible isolates and two STs (ST2 and ST104) were enrolled in our study. Studies including more isolates with various epidemiological characteristics from multiple regions need to be performed to confirm our findings. Second, the effect of other β-lactamases, such as bla_{ADC-25}, identified mutations in genes encoding the PBP3 protein and TonB-ExbB-ExbD complex are possibly related to CFDC resistance and need to be validated. Third, we observed downregulation of several genes belonging to the iron transport system in CFDC non-susceptible isolates. Whether there are key regulators involved in this process remains to be studied through further experiments.

In conclusion, our study provides evidence that high-level resistance to CFDC in CR-AB involves PER-1 production and iron transport system downregulation. Mutations in PBPs might also be involved in the reduced CFDC susceptibility of some isolates. Nevertheless, further studies aiming to understand the mechanisms underlying CFDC resistance in CR-AB are still needed, in order to identify effective strategies to prevent and tackle the resistance.

Data Sharing Statement

The datasets presented in this study can be found in online repositories, https://www.ncbi.nlm.nih.gov/, PRJNA848261.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Natural Science Foundation of China (81903672), and Peking University People's Hospital Research and Development Funds (RS2020-04, RDJP2022-43).

Disclosure

The authors declare no conflicts of interest in this work.

References

1. E.M.A. Fetcroja (cefiderocol); 2022. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/fetcroja. Accessed December 3, 2022.
2. Sato T, Yamawaki K. Cefiderocol: discovery, chemistry, and in vivo profiles of a novel siderophore cephalosporin. *Clin Infect*. 2019;69:S538–s543. doi:10.1093/cid/ciz826
3. Wang C, Yang D, Wang Y, Ni W. Cefiderocol for the treatment of multidrug-resistant Gram-negative bacteria: a systematic review of currently available evidence. *Front Pharmacol*. 2022;13:896971. doi:10.3389/fphar.2022.896971
4. Bassetti M, Echols R, Matsunaga Y, et al. Efficacy and safety of cefiderocol or best available therapy for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria (CREDIBLE-CR): a randomised, open-label, multicentre, pathogen-focused, descriptive, phase 3 trial. *Lancet Infect Dis*. 2021;21:226–240. doi:10.1016/S1473-3099(20)30796-9
5. Longshaw C, Manissero D, Tsuji M, Echols R, Yamano Y. In vitro activity of the siderophore cephalosporin, cefiderocol, against molecularly characterized, carbapenem-non-susceptible Gram-negative bacteria from Europe. *JAC Antimicrob Resist*. 2020;2:dlaa060. doi:10.1093/jacamr/dlaa060
6. Wang Y, Li Y, Zhao J, Guan J, Ni W, Gao Z. Susceptibility of cefiderocol and other antibiotics against carbapenem-resistant, Gram-negative bacteria. *Ann Transl Med*. 2022;10:261. doi:10.21037/atm-22-889
7. Karakonstantis S, Rousaki M, Kritsotakis EI. Cefiderocol: systematic review of mechanisms of resistance, heteroresistance and in vivo emergence of resistance. *Antibiotics*. 2022;11:723. doi:10.3390/antibiotics11060723
8. Ito A, Sato T, Ota M, et al. In vitro antibacterial properties of cefiderocol, a novel siderophore cephalosporin, against Gram-negative bacteria. *Antimicrob Agents Chemother*. 2018;62. doi:10.1128/aac.01454-17
9. Gupta A, Landman D, Quale J. Relationship of TonB-dependent receptors with susceptibility to cefiderocol in clinical isolates of Pseudomonas aeruginosa. *J Antimicrob Chemother*. 2022;77:1282–1285. doi:10.1093/jac/dkac022
10. Malik S, Kaminski M, Landman D, Quale J. Cefiderocol Resistance in Acinetobacter baumannii: roles of beta-Lactamases, Siderophore Receptors, and Penicillin Binding Protein 3. *Antimicrob Agents Chemother*. 2020;64. doi:10.1128/AAC.01221-20
11. Ito A, Nishikawa T, Matsumoto S, et al. Siderophore cephalosporin cefiderocol utilizes ferric iron transporter systems for antibacterial activity against Pseudomonas aeruginosa. *Antimicrob Agents Chemother*. 2016;60:7396–7401. doi:10.1128/aac.01405-16
12. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34:i884–i890. doi:10.1093/bioinformatics/bty560
13. Luo R, Liu B, Xie Y, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience*. 2012;1:18. doi:10.1186/2047-217X-1-18
14. Terreni M, Taccani M, Pregnolato M. New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. *Molecules*. 2021;26:2671. doi:10.3390/molecules26092671
15. Krawczyk PS, Lipinski L, Dziembowski A. PlasFlow: predicting plasmid sequences in metagenomic data using genome signatures. *Nucleic Acids Res*. 2018;46:e35. doi:10.1093/nar/gkx1321
16. Schmartz GP, Hartung A, Hirsch P, et al. PLSDB: advancing a comprehensive database of bacterial plasmids. *Nucleic Acids Res*. 2022;50:D273–D278. doi:10.1093/nar/gkab1111
17. Alcock BP, Raphenya AR, Lau TTY, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res*. 2020;48:D517–D525. doi:10.1093/nar/gkz935
18. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015;31:2745–2747. doi:10.1093/bioinformatics/btv195
19. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform*. 2008;9:559. doi:10.1186/1471-2105-9-559
20. McQueary CN, Actis LA. Acinetobacter baumannii biofilms: variations among strains and correlations with other cell properties. *J Microbiol*. 2011;49:243–250. doi:10.1007/s12275-011-0343-7
21. Price TK, Davar K, Contreras D, et al. Case report and genomic analysis of cefiderocol-resistant Escherichia coli clinical isolates. *Am J Clin Pathol*. 2022;157:257–265. doi:10.1093/ajcp/aqab115
22. Mushtaq S, Sadouki Z, Vickers A, Livermore DM, Woodford N. In vitro activity of cefiderocol, a siderophore cephalosporin, against multidrug-resistant Gram-negative bacteria. *Antimicrob Agents Chemother*. 2020;64. doi:10.1128/AAC.01582-20

23. Iregui A, Khan Z, Landman D, Quale J. Activity of cefiderocol against enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii endemic to medical centers in New York City. *Microb Drug Resist*. 2020;26:722–726. doi:10.1089/mdr.2019.0298
24. Kohira N, Hackel MA, Ishioka Y, et al. Reduced susceptibility mechanism to cefiderocol, a siderophore cephalosporin, among clinical isolates from a global surveillance programme (SIDERO-WT-2014). *J Glob Antimicrob Resist*. 2020;22:738–741. doi:10.1016/j.jgar.2020.07.009
25. Poirel L, Sadek M, Nordmann P. Contribution of PER-type and NDM-type beta-lactamases to cefiderocol resistance in Acinetobacter baumannii. *Antimicrob Agents Chemother*. 2021;65:e0087721. doi:10.1128/AAC.00877-21
26. Yamano Y, Ishibashi N, Kuroiwa M, Takemura M, Sheng WH, Hsueh PR. Characterisation of cefiderocol-non-susceptible Acinetobacter baumannii isolates from Taiwan. *J Glob Antimicrob Resist*. 2022;28:120–124. doi:10.1016/j.jgar.2021.12.017
27. Wang Q, Jin L, Sun S, et al. Occurrence of high levels of cefiderocol resistance in carbapenem-resistant Escherichia coli before its approval in china: a report from China CRE-network. *Microbiol Spectr*. 2022;10:e0267021. doi:10.1128/spectrum.02670-21
28. Bao J, Xie L, Ma Y, An R, Gu B, Wang C. Proteomic and transcriptomic analyses indicate reduced biofilm-forming abilities in cefiderocol-resistant Klebsiella pneumoniae. *Front Microbiol*. 2021;12:778190. doi:10.3389/fmicb.2021.778190
29. Sevilla E, Bes MT, Gonzalez A, Peleato ML, Fillat MF. Redox-based transcriptional regulation in prokaryotes: revisiting model mechanisms. *Antioxid Redox Signal*. 2019;30:1651–1696. doi:10.1089/ars.2017.7442