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Letter to the Editor

Zidebactam restores cefiderocol sensitivity in resistant bacteria

Dear editor,

We read with interest the article by Sarah Soueges et al. on the clinical use of cefiderocol (FDC) in immunocompromised patients[1]. The authors reported the notable efficacy of FDC in treating difficult-to-treat infections, particularly those caused by *S. maltophilia*. However, they also observed a concerning relapse rate and the acquisition of FDC resistance. Thus they recommended reconsidering the empirical use of FDC to prevent resistance and improve outcomes in vulnerable patient populations. Considering the scarcity of new antibiotics, the development of antibiotic adjuvants capable of restoring and enhancing the efficacy of FDC represents a promising approach.

In this study, we identified Zidebactam (Zid) as an FDC adjuvant that synergistically enhances the activity of FDC in eliminating FDC-resistant Gram-negative strains *in vitro*. Firstly, a total of 14 clinical FDC-resistant Gram-negative isolates from different species were collected, including *Ent. cloacae*, *K. pneumoniae*, *C. youngae*, *R. ornithinolytica*, *E. coli*, *Ser. marcescens*, *C. freundii*, *K. oxytoca* and *S. typhimurium* (MIC > 8 μg/ml), along with 2 FDC-sensitive *A. baumannii* isolates (Table 1). A checkerboard assay was then constructed to determine the synergistic bactericidal effect of FDC and Zid (Fig. 1A, Table 1). The results indicated that Zid could effectively restore and even enhance the antibacterial activity of FDC against both FDC-sensitive and FDC-resistant strains in a dose-dependent manner (FICI < 0.5). To further evaluate the antibacterial efficacy of the combination of Zid and FDC, a time-kill assay was conducted using the clinical strains mentioned above (Fig. 1B). Monotherapy with 2 μg/ml Zid or FDC failed to achieve complete eradication of the strains within 24 h. In contrast, the combination of FDC (0.5–2 μg/ml) and 2 μg/ml Zid effectively inhibited the growth of all tested strains within 24 h in a dose-dependent manner, except for NDM-1 producing *E. coli* E110 (Fig. 1B). This exception could be attributed to the overexpression of NDM-1, an enzyme that inactivates a broad range of beta-lactam antibiotics, including FDC.

Having demonstrated that Zid could restore and enhance the antibacterial effect of FDC *in vitro*, the morphological changes in *Ent. cloacae* P87-B following treatment with Zid and FDC were visualized using scanning electron microscopy (SEM). (Fig. 1C). No significant morphological changes were observed when the strain was treated with Zid (2 μg/ml) or FDC (2 μg/ml) as monotherapy. In contrast, treatment with the combination of Zid (2 μg/ml) and FDC (2 μg/ml) resulted in noticeable shrinkage of the bacterial cell membrane. This observation suggested that Zid and FDC synergistically led to leakage of cellular contents and ultimately bacterial cell death, possibly by increasing membrane permeability.

Following the visualization of the synergistic antibacterial effect of Zid and FDC, we next evaluated their bactericidal efficacy against intracellular bacteria using a cell infection model. The cytotoxicity of Zid and FDC on mammalian cells was assessed using MTT assays, which revealed no significant toxic effects on Vero cells (Fig. 1D). In the cell infection model, the viability of intracellular bacteria in Vero cells was quantified following treatment with Zid, FDC and their combination (Fig. 1E). Compared to the untreated group, treatment with 2 μg/ml Zid alone did not reduce the intracellular bacteria load, while 2 μg/ml FDC alone slightly suppressed bacterial growth. In contrast, the combined treatment of 2 μg/ml Zid and 2 μg/ml FDC resulted in a significant reduction in the number of viable intracellular bacteria compared to 2 μg/ml FDC monotreatment (p=0.0006).

To elucidate the molecular mechanisms underlying the synergistic antibacterial effects of Zid and FDC, transcriptomic analysis was performed on *K. pneumoniae* P22T treated with FDC alone and in combination with Zid to investigate changes in gene expression. A total of 519 upregulated and 1248 downregulated genes were identified in *K. pneumoniae* P22T treated with Zid-FDC combination, compared to treatment with FDC alone (Fig. 1F). Gene Ontology (GO) enrichment analysis revealed that the differentially expressed genes (DEGs) were highly associated with several pathways related to biological processes and molecular functions (Fig. 1G). Notably, as shown in Fig. 1H, pathways including oxidoreductase activity, the tricarboxylic acid cycle, quinone binding and NADH dehydrogenase were significantly enriched, with most DEGs in these pathways being downregulated. This suggested that the synergistic bactericidal effect of Zid and FDC may be related to the inhibition of key

Table 1
Strain Information and Synergistic Effect of Zid and FDC.

Species	Strain	MIC (μg/ml)				FICI
		Zid	FDC	FDC + 2 μg/ml Zid	FDC + 8 μg/ml Zid	
Ent. cloacae	P87-B	> 8	> 128	0.5	0.5	≦0.0332
Ser. marcescens	SM122	> 8	> 128	0.125	0.125	≦0.00830
E. coli	E110	> 8	128	0.125	0.125	≦0.00879
K. pneumoniae	P22-T	> 8	> 128	0.5	0.5	≦0.0332
C. youngae	Cit12	> 8	128	0.5	0.25	≦0.0176
R. ornithinolytica	Rao2	> 8	> 128	0.5	0.5	≦0.0332
S. typhimurium	STM1	> 8	8	0.0156	0.0156	≦0.00292
C. freundii	Cit3	> 8	> 128	1	1	≦0.0664
C. freundii	Cit9	> 8	> 128	2	1	≦0.0664
K. pneumoniae	P53	> 8	64	0.5	0.25	≦0.0195
K. oxytoca	P82	> 8	> 128	0.5	1	≦0.0664
Ent. cloacae	P86	> 8	> 128	1	0.5	≦0.0332
R. ornithinolytica	Rao3	> 8	> 128	1	1	≦0.0664
S. typhimurium	STM4	> 8	64	0.0156	0.0156	≦0.00122
A. baumannii	A26	> 8	4	1	1	≦0.312
A. baumannii	A28	> 8	4	1	1	≦0.312

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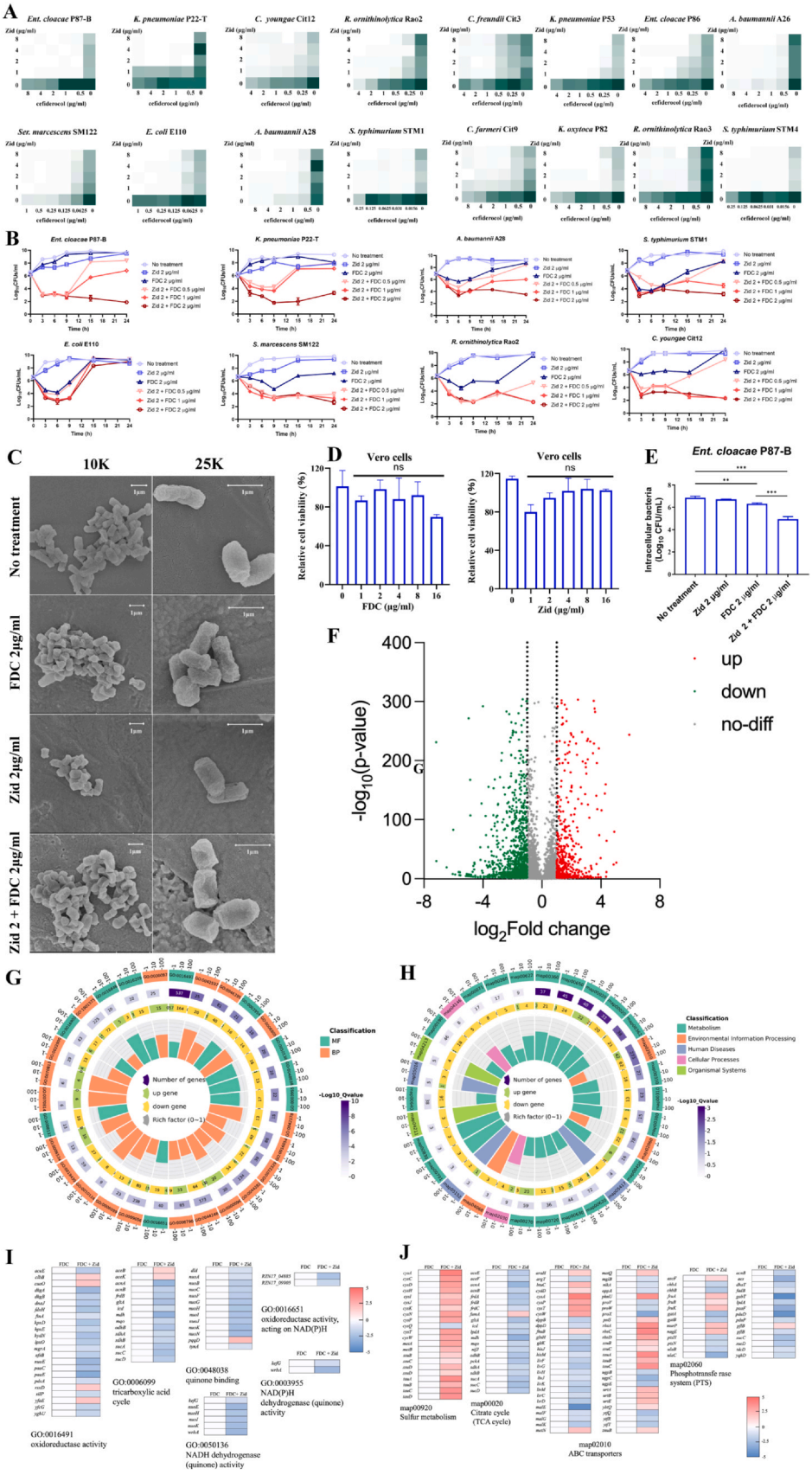

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Fig. 1. The synergistic antibacterial effect of Zid and FDC and the proposed mechanisms. (**A**) Checkerboard analysis of the synergistic antimicrobial effect of Zid and FDC on both FDC-resistant and FDC-susceptible Gram-negative bacteria. (**B**) Time-kill curve of FDC-resistant and FDC-sensitive strains in the treatment of Zid, FDC, and the combination of both drugs. (**C**) SEM imaging of *Ent. cloacae* P87-B incubated with Zid, FDC and the combination of both drugs. (**D**) Cytotoxicity determination of Zid and FDC on Vero cells using MTT assay. (**E**) Determination of the intracellular bacteria load of *Ent. cloacae* P87-B in Vero cells in the treatment of Zid, FDC, and their combination. (**F-J**) Transcriptomic analysis of *K. pneumoniae* P22T incubated with 0.5 μg/ml FDC and the combination of 1 μg/ml and 0.5 μg/ml FDC. (**F**) Volcano plot of the distribution of gene expression difference. (**G**) GO annotation analysis of the DEGs. (**H**) DEGs in the pathways which were significantly enriched using GO analysis. (**I**) KEGG annotation analysis of DEGs. (**J**) DEGs in the pathways which were significantly enriched using KEGG analysis.

respiratory chain enzymes, which lead to the aberrant bacterial respiration and cell death[2,3]. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis indicated that the DEGs were predominantly associated with metabolism pathways (Fig. 1I). In particular, most DEGs involved in the tricarboxylic acid cycle and propanoate metabolism were downregulated. This suggested that Zid restored the antibacterial effect of FDC by inhibiting the bacterial respiratory chain, which is consistent with the results of GO analysis. Additionally, the expression level of sulfate/thiosulfate ABC transporter gene, including *cysA*, *cysC*, *cysP*, *cysT*, *cysW*, and *cysB* were observed to be upregulated (Fig. 1J). We hypothesize that the induction of efflux mechanisms may represent a bacterial response to the synergistic stress exerted by Zid-FDC combination compared to FDC monotherapy[4].

In conclusion, we identifed Zid restored the antibacterial effects of FDC against FDC resistant Gram-negative strains *in vitro*. Transcriptome analysis indicated that the combinational use of Zid and FDC could inhibit the expression of the vital respiratory chain enzymes, which might lead to the aberrant bacterial respiration and cell death.

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Chen Xu: Conceptualization, Funding acquisition, Methodology, Investigation, Writing – original draft. **Xiaofan Li**: Methodology, Investigation, Writing – original draft. **Guangfen Zhang**: Investigation, Writing – original draft. **Chunli Li**: Investigation. **Jiayi You:** Methodology. **Chenjie Zhang:** Methodology. **Yuanyuan Li**: Investigation, Resources, Writing – review & editing. **Yunbing Li**: Investigation, Resources. **Xiangkun Zeng**: Investigation. **Lili Huang**: Resources. **Mi Zhou**: Resources. **Yi Yang**: Investigation. **Jiani Jiang**: Investigation. **Ruichao Li**: Supervision, Project administration, Writing – review & editing. **Shigui Yang**: Supervision, Project administration, Writing – review & editing. **Ning Dong**: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare no competing interests.

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