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**Abstract**
Fosfomycin exhibits broad-spectrum antibacterial activity, and is being re-evaluated for the treatment of extensively drug-resistant Gram-negative bacteria. However, the mechanism of fosfomycin resistance in these clinically relevant pathogens remains poorly understood. Using an integrating approach, we have discovered that wild-type K. pneumoniae and Escherichia coli mutants harboring a single nucleotide change in fosA exhibit fosfomycin resistance. These results show that fosA-mediated fosfomycin resistance in K. pneumoniae and E. coli is due to a single nucleotide change, and provide the opportunity to develop new strategies to inhibit FosA and potentiate fosfomycin activity.

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