Genomic and Transcriptomic Analyses Reveal Oxidative Stress Response Pathways as a Determinant of Cefiderocol Activity in *Klebsiella pneumoniae*

Lana Daoud*, Akela Ghazawi, Farah Al-Marzooq
Department of Medical Microbiology and Immunology, College of Medicine and Health Sciences, UAE University, Al Ain, UAE

Background: Cefiderocol (CFDC), a novel siderophore-cephalosporin, has potent activity against multidrug-resistant (MDR) Gram-negative bacteria. However, emerging resistance and the molecular mechanisms governing CFDC susceptibility remain poorly understood. The effects of iron entry with CFDC remain unexplored. We employed genomic and transcriptomic approaches to elucidate genetic basis and functional responses underlying CFDC resistance in *Klebsiella pneumoniae* (KPN).
Methods: We determined minimum inhibitory concentration (MIC) of CFDC in iron-depleted media. We conducted genomic analysis on 96 KPN clinical isolates and examined genes involved in oxidative stress (OS) response. Mutations were identified and correlated to the CFDC MIC. We profiled specific OS genes in 30 strains under treatment with CFDC. Gene expression was analyzed using Aligned Rank Transform nonparametric ANOVA.
Results: CFDC demonstrated 96.9% activity, with only eight non-susceptible isolates belonging to high-risk clones ST14/ST147. Resistant isolates showed enriched catecholate receptor mutations, particularly cirA frameshift mutation. Negative correlations between CFDC MICs and detoxification/repair mutations suggested that impaired OS responses enhance CFDC activity. KatE_D679V mutation was enriched in the susceptible group. Network analysis revealed iron regulation genes as central hubs in resistance. Expression of several OS genes was significantly increased under CFDC treatment such as, KatE; 8-fold, acnB; 82-fold, TrxA; 29-fold, sodB; 46.4-fold, and sodC; 20.4-fold ($p<0.001$). This indicates that the CFDC successfully penetrates susceptible strains and induces OS response, while CFDC-R strains fail to uptake the drug due to receptor defects; thus, CFDC shows minimal effects.
Conclusions: CFDC circumvents classical resistance mechanisms through siderophore-mediated entry. Iron uptake mutations (cirA) and OS pathway alterations predict resistance. Several OS genes are found exclusive to CFDC susceptible group indicating possible mechanism of action.

Keywords: Cefiderocol, oxidative stress, iron homeostasis, *Klebsiella pneumoniae*, high-risk clones