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Entitled

FACTORS AFFECTING THE ANTIBACTERIAL ACTIVITY OF CEFIDEROCOL ON KLEBSIELLA PNEUMONIAE WITH DIVERSE CHARACTERISTICS

by

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Abstract

Antimicrobial resistance (AMR) is a growing serious threat that poses a burden on the healthcare system. Spread of AMR bacteria limits treatment options; thus, new antimicrobial agents are required. Cefiderocol (CFDC) is a novel siderophore cephalosporin, approved by the FDA in 2019 for use in treating infections caused by multi-drug resistant (MDR) pathogens; but it is not yet in clinical use in the UAE. CFDC is equipped with stabilizers against β-lactamases as well as a siderophore side chain that allows it to penetrate the outer membrane of bacteria through the iron uptake pathways. The aim of this study is to determine the factors affecting CFDC activity on *Klebsiella pneumoniae*, with focus on β-lactamases, iron and its uptake pathways. It also aims to identify methods to improve CFDC activity.

A total of 103 *K. pneumoniae* strains were investigated. The strains were characterized to determine their susceptibility to CFDC and to detect β-lactamase and iron acquisition genes. The expression of these genes was related to CFDC susceptibility in iron depleted and iron enriched conditions.

CFDC had efficacy of 96.1%, inhibiting carbapenem and colistin resistant strains. CFDC was effective in killing strains with different β-lactamase genes (bla_{CTX-M}, bla_{DHA}, bla_{OXA-48}, bla_{NDM}, bla_{KPC}), even strains producing multiple β-lactamases. One strains, which was pan drug-resistant, exhibited extremely high minimum inhibitory concentration (MIC= 256 µg/ml). Whole genome analysis revealed presence of several resistance mechanisms such as production of multiple β-lactamases, mutation against colistin, efflux pumps, and mutations in the outer membrane porins.

CFDC MICs increased significantly in iron enriched media. When Fe^{3+} was highly available in the media, the bacteria reduced the expression of siderophore receptors and hence, lead to reduced uptake of CFDC. There was a negative correlation between enterobactin receptor (*fepA*) expression and the MIC of CFDC. In addition, we investigated for the first time the effect of iron transport genes, that were not studied before, on CFDC activity. There was a positive correlation between the expression of *fecA* and *kfu* and the MIC of CFDC; thus, both genes were associated with reduced susceptibility to CFDC.

Strains exhibiting increased MICs in iron enriched media were tested for synergistic effect when CFDC was combined with β-lactamase inhibitors (BLIs) and an outer membrane permeabilizer, namely polymyxin B nonapeptide (PMBN). Synergy was achieved with dual combination of CFDC and BLIs, especially avibactam (AVI), which caused a significant reduction in MICs in both iron depleted and iron enriched conditions. Treatment with PMBN did not have a significant effect when it was used alone, but it reduced the MIC in the triple combination (CFDC+AVI+PMBN) in both iron depleted and iron enriched conditions.

To conclude, CFDC was found to be highly potent against MDR *K. pneumoniae* even in strains exhibiting resistance to the last resort drugs such as colistin and carbapenems, including dual carbapenemase producers. CFDC lost its activity in iron enriched media, but the bacteria were re-sensitized through the combination with AVI. Synergistic combination of CFDC with β-lactamase inhibitors and outer membrane permeabilizers could be effective to treat infections in sites with increased iron levels such as the liver, but this should be tested *in vivo.* This study reports the emergence of CFDC resistance in *K. pneumoniae* from the UAE. We established that resistance to CFDC is caused by the interplay of many factors such as activity of resistance enzymes, membrane permeability defects and iron concentration in the environment. The emergence of highly resistant strains, even to the most recent antibiotics with novel mechanisms of action is alarming. This provides motivation for proactive surveillance and routine screening of strains for resistance.

Keywords: Antimicrobial resistance, cefiderocol, synergy, *Klebsiella pneumoniae*, β-lactamase inhibitors, polymyxin B nonapeptide, gene expression, cefiderocol resistance.