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## "Unraveling Antibiotic Resistance in Last Resort Therapies for Treatment of Multidrug Resistant *Enterobacteriaceae*"

by
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Thursday, October 13th, 2022
12:00 PM

Join Zoom Presentation:
https://hmhn.zoom.us/j/83631879157
Meeting ID: 83631879157

## ABSTRACT

Antibiotic resistance is a problem worldwide that continues to escalate, costing lives and creating a major financial burden to the healthcare system. Treatment of multidrug resistant Gram-negative pathogens specifically has become problematic after the rise of carbapenem resistance leaving classes of antibiotics available useless. This led to restoring the use of antibiotics previously introduced and removed from the market, combination therapies, and modifications to existing drugs for treatment of these infections as last resorts. Two such last resort therapies are colistin and cefiderocol.

Colistin is a drug that was first introduced in 1952 to treat Gram-negative infections but was quickly removed from the market in 1970 due to harsh neurological side effects and nephrotoxicity. Colistin was reintroduced in 2000 again to treat bacterial infections in response to carbapenem resistance. A rise in colistin resistance has been seen since its reintroduction.

Cefiderocol is a novel siderophore cephalosporin used to treat infections caused by multidrug resistant Gram-negative pathogens as well. Cefiderocol circumvents the periplasmic membrane barrier which challenges the entrance of most antibiotics into the bacterial cell by exploiting the bacteria's iron acquisition and uptake systems. As a recently developed drug that was licensed in 2019, limited research has been done to understand both susceptibility and resistance to the drug.

Transposon mutagenesis sequencing and CRISPR genomic editing are complementing and powerful tools for phenotypic screening and analysis of whole bacterial genomes. In this dissertation, I used these methodologies to investigate the response of a clinical KPC-3-producing *K. pneumoniae* strain to cefiderocol. A Himar1 transposon library was made in a KPC-3 positive ST258 *K. pneumoniae* isolate 30684. The library was treated with 2x and 8x the MIC of cefiderocol in both LB and Iron Depleted Mueller Hinton Broth (IDMHB), followed by transposon directed insertion sequencing. CRISPR gene editing and complementation were used to confirm the resistance in the targets identified. A total of 47 genes were identified in 2x (n=18) and 8x (n=42) the MIC of cefiderocol treated libraries in IDMHB. Among the 47 genes, 13 were found in common when tested at both cefiderocol concentrations, whereas, 5 were found only with 2x the MIC, and 29 were found only in treatment with 8x the MIC. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis categorized these genes into 8 main functional groups from most prevalent to least: protein export, peptidoglycan and lipopolysaccharide biosynthesis, β– lactam resistance, bacterial secretion system, two component systems, O-antigen nucleotide sugar biosynthesis, biosynthesis of secondary metabolites, and metabolism. Eleven individual knockouts were generated in representative genes *arnT, cirA, dedA, exbB, fepD, ftsE, KPC-3, lpoB, surA, tolR* and *tonB* using CRISPR–Cas9-mediated gene editing in the parent strain, and susceptibility testing confirmed the predicted phenotype.

In my dissertation research, I have added to our knowledge regarding genes involved in both susceptibility and resistance to cefiderocol in a clinical KPC-3-producing *K. pneumoniae* isolate while demonstrating the utility of using transposon mutagenesis sequencing and CRISPR genomic editing for investigating how bacteria adapt to antibiotic exposure.