Development of Cefiderocol Resistance in an *Enterobacter hormaechei* Strain Following Antibiotic Exposure

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Revised Abstract

Background: Cefiderocol (FDC) is a novel antimicrobial agent used for multi-drug resistant Gram-negative pathogens. To date, reports of mutations in β-lactamase and siderophore complex genes have been described and may contribute to FDC resistance. This case describes a New Dehli metallo-β-lactamase (NDM)-producing strain of *Enterobacter hormaechei* that developed FDC resistance following antibiotic exposure.

Methods: Serial respiratory and blood cultures were collected from a lung transplant candidate throughout 72 days of hospitalization. Confirmatory susceptibility and combination minimal inhibitory concentration (MIC) testing was performed using broth dilution and eTest assays. Short-read sequencing libraries were prepared using a seqWell plexWell 96 kit, and short-read whole-genome sequencing was performed using the Illumina NovaSeq platform. Reads from the sample genomes were aligned to the chromosome and three plasmid sequences of reference genome ENCL48880 for short-read sequencing. Long-read whole-genome sequencing using the Nanopore platform was performed on an FDC-sensitive isolate, and reads from the remaining sample genomes were aligned to the chromosome and four plasmid sequences of the sensitive isolate. RNA was extracted using a Qiagen Mini-Prep Kit followed by cDNA preparation and Quantitative polymerase chain reactions (QT-PCR).

Results: Four serial respiratory *E. hormaechei* isolates and one blood isolate were studied. Although initial isolates were susceptible to FDC (MICs 1-2 μg/mL), two respiratory isolates cultured after 41 days of FDC therapy had MICs of 128 μg/mL. The blood isolate remained FDC susceptible despite respiratory resistance. Whole-genome sequencing revealed no nonsynonymous single nucleotide variants (SNVs) within the chromosomes. Long-read sequencing revealed four plasmids in four of the isolates, and three plasmid in one sensitive isolate. A SHV-2 gene initially present in a low copy number plasmid was found to relocate to a high copy plasmid and the bacterial chromosome in resistant isolates. Finally, QT-PCR was notable for increased expression of the SHV-2 gene in resistant isolates.

Conclusion: This case documents emergence of FDC resistance in *E. hormaechei* isolates during a 41-day course of FDC therapy. Translocation of a SHV gene from a low-copy to a high-copy plasmid is the most likely cause of resistance, a novel mechanism in *Enterobacter* isolates. Other causes of resistance could include chromosomal and plasmid alleles deletions. Partnership between molecular testing and enhanced antimicrobial stewardship should be encouraged to optimize selection of regimens and durations to prevent resistance to FDC.

Background

- Cefiderocol is novel antimicrobial agent used for multi-drug resistant (MDR) Gram-negative pathogens
- β-lactamase and siderophore gene mutations may contribute to cefiderocol resistance
- This case report describes the development of cefiderocol resistance in a strain of New-Delhi metallo-β-lactamase (NDM)-producing *Enterobacter hormaechei*

Methods

- Institutional Review Board approval for specimen collection was obtained
- Serial respiratory and blood cultures were collected for clinical care during hospitalization were tested
- MIC testing was performed utilizing broth dilution in iron-depleted media
- Short-read sequencing libraries were performed utilizing a SeqWell plexWell 96 kit, and sequencing was performed using the Illumina NovaSeq platform
- Long-read sequencing was performed utilizing the Nanopore platform
- Short-read sequence reads were aligned to a long-read sequence of the chromosome and four plasmids of a cefiderocol sensitive isolate
- RNA extraction was performed using Qiagen Mini-Prep kit followed by cDNA preparation and quantitative PCR (QT-PCR)

Figure 1: Patient Timeline

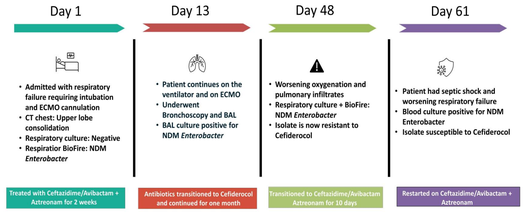

Table 1: Serial isolates demonstrated increasing cefiderocol MICs over time

Isolate Name	Day of Hospitalization	Cefiderocol MIC
Resp1	Day 13	2 μg/mL
Resp2	Day 40	1μg/mL
Resp3	Day 48	128 μg/mL
Resp4	Day 48	128 μg/mL
BCx	Day 61	2 μg/mL

Figure 2: SHV-2 Sequence Map demonstrates transition from low-copy to high-copy plasmid in cefiderocol resistant isolates

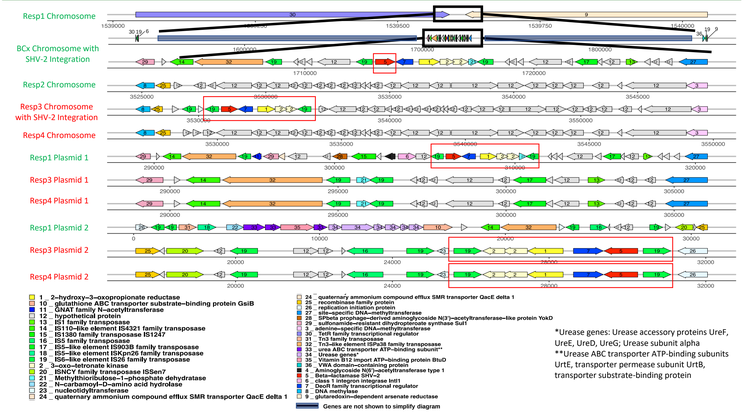

Figure 3: Graphical Representation of Isolate Chromosomes and Plasmids

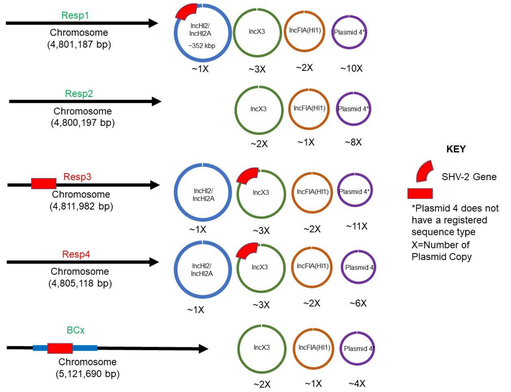

Table 2: QT-PCR notable for elevated SHV-2 expression

Fold Differences in Comparison to *gyrB* Housekeeping Gene		
Gene	Resp3	Resp4
SHV-2 Target 1	2.409	2.487
SHV-2 Target 2	1.537	0.481
NDM	1.314	0.961

Results

- Isolates resistant to cefiderocol were noted to have an MIC of 128 μg/mL
- Short-read whole-genome sequencing revealed no nonsynonymous single nucleotide variants within the chromosomes
- Long-read sequencing revealed transition of the SHV-2 gene from a low-copy plasmid to a high-copy plasmid along with chromosomal integration, and noted deletions of urease genes.
- One susceptible isolate was noted to have loss of SHV-2
- QT-PCR was notable for increased SHV-2 expression in a cefiderocol resistant isolate without increased NDM expression

Conclusions

- This case documents development of cefiderocol resistance in an *E. hormaechei* isolate after 41 days of therapy
- Movement of the SHV-2 gene from a low-copy plasmid to a high-copy plasmid, in addition to integration into the chromosome, are the likely mechanisms of cefiderocol resistance
- Increased expression of the SHV-2 gene in resistant isolates correlates with the genetic analyses

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