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Authors

Price, Travis K
Davar, Kusha
Contreras, Deisy
et al.

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Case Report and Genomic Analysis of Cefiderocol-Resistant *Escherichia coli* Clinical Isolates

Travis K. Price, PhD,[1] *Kusha Davar, MD, MBA, MS,*[2] *Deisy Contreras, PhD, D(DABMM),*[3] *Kevin W. Ward,*[1] *Omai B. Garner, PhD, D(DABMM),*[1] *Patricia J. Simner, PhD, D(DABMM),*[4] *Shangxin Yang, PhD, D(DABMM),*[1] *and Sukantha Chandrasekaran, PhD, D(DABMM)*[1]

From the [1]Department of Pathology and Laboratory Medicine and [2]Division of Infectious Disease, David Geffen School of Medicine, University of California, Los Angeles, CA, USA; [3]Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA; and [4]Division of Medical Microbiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

ABSTRACT

Objectives: Cefiderocol is a novel siderophore cephalosporin with in vitro activity against multidrug-resistant (MDR), gram-negative bacteria and intrinsic structural stability to all classes of carbapenemases. We sought to identify gene variants that could affect the mechanism of action (MOA) of cefiderocol.

Methods: We report a case of bacteremia in a liver transplant candidate with a strain of carbapenem-resistant *Escherichia coli* that was found to be resistant to cefiderocol despite no prior treatment with this antimicrobial agent. Using whole-genome sequencing, we characterized the genomic content of this *E coli* isolate and assessed for genetic variants between related strains that were found to be cefiderocol susceptible.

Results: We identified several variants in genes with the potential to affect the mechanism of action of cefiderocol.

Conclusions: The cefiderocol resistance in the *E coli* isolate identified in this study is likely due to mutations in the *cirA* gene, an iron transporter gene.

KEY POINTS

- Cefiderocol is a novel siderophore cephalosporin with in vitro activity against multidrug-resistant, gram-negative bacteria and intrinsic structural stability to all classes of carbapenemases.
- Resistance to cefiderocol is not common, and the mechanisms conferring resistance are unknown.
- Using closely related strains of *Escherichia coli* with varying susceptibility to cefiderocol, we identified genetic variants that may relate to resistance.

KEY WORDS

CP microbiology; AP infectious disease; Molecular diagnostics

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Corresponding author: Sukantha Chandrasekaran, PhD, D(ABMM); schandrasekaran@mednet.ucla.edu.

INTRODUCTION

Carbapenem-resistant Enterobacterales (CREs) are a significant public health concern. Carbapenem resistance in CREs is commonly mediated by the production of carbapenemases, enzymes that hydrolyze carbapenems and other β-lactams. Carbapenemases produced by CREs are grouped into 3 classes: class A (eg, *Klebsiella pneumoniae* carbapenemases [KPCs]), class B (eg, New Delhi metallo-β-lactamases [NDM]), and class D (eg, OXA-type carbapenemases).[1] Recently approved β-lactam/β-lactamase inhibitor combination drugs (eg, ceftazidime-avibactam, meropenem-vaborbactam) are not active against class B metallo-β-lactamases and may have limited activity against class D agents.[2-4] Other antimicrobial agents with the potential to treat CRE-associated infections (eg, aminoglycosides, polymyxins, tigecycline) are associated with toxicities; increasing resistance; and in some cases, intrinsic resistance.[5] Consequently, managing patients with serious infections caused by CREs presents a significant challenge.

The US Food and Drug Administration (FDA) approved cefiderocol for patients 18 years of age or older with complicated urinary tract infections, including pyelonephritis, as well as hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia

caused by susceptible gram-negative bacteria. Cefiderocol is a novel siderophore cephalosporin.[6] Siderophores are iron-chelating molecules that microorganisms produce to facilitate the transport of iron into the cell, which is required for many essential enzymatic reactions. In gram-negative bacteria, the siderophore binds to outer-membrane iron transporter proteins and is actively transported into the periplasm.[7] Attempts were made to "hijack" this system by conjugating the siderophore moiety to β-lactams, an approach commonly referred to as a Trojan horse strategy in the literature.[8-11] Cefiderocol, originally referred to as S-649266, is structurally similar to ceftazidime and cefepime, with an added chlorocatechol group on the end of the C3 side chain that confers siderophore activity.[6,12] Once in the periplasm, cefiderocol binds primarily to penicillin-binding protein 3 (PBP3) to exert its primary effect on cell wall synthesis, similar to other β-lactams.[13] Current data suggest that the in vitro activity of cefiderocol is not significantly affected by efflux pumps and porin channel mutations,[12] and the agent has intrinsic structural stability against all 3 classes of carbapenemases, including metallo-β-lactamases (MBLs).[14,15] Cefiderocol has potent antibacterial activity against multidrug-resistant (MDR), gram-negative bacteria, including CREs and carbapenemase-producing CRE strains.[6,13,16-18] International surveillance studies showed that the minimum inhibitory concentration (MIC) of cefiderocol inhibiting 90% of Enterobacterales clinical isolates (MIC_{90}), as determined using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method, was 0.5 µg/mL and 1 µg/mL for clinical isolates identified in North America and Europe, respectively.[5] The MIC_{90} was 4 µg/mL for carbapenem-nonsusceptible Enterobacterales strains.[19] NDM-positive strains had a higher MIC_{90} of 8 µg/mL.[20]

The utility of cefiderocol is promising, but currently, limited clinical data exist to support this hypothesis.[21] Case reports of the compassionate use of cefiderocol exist, with generally positive outcomes. For example, cefiderocol was used as an adjunctive therapy to successfully treat an MDR *Pseudomonas aeruginosa* intra-abdominal infection,[21] an extended-spectrum β-lactamase (ESBL)–producing strain of *P aeruginosa* in a patient with native aortic valve endocarditis,[22] and a co-infection of an MBL-producing *P aeruginosa* and an ESBL-producing *K pneumoniae* associated with chronic osteomyelitis.[23] Cefiderocol was successfully used as a monotherapy for a co-infection of an MDR *Acinetobacter baumannii* and KPC-producing *K pneumoniae* associated with ventilator-associated pneumonia and bloodstream infection.[24] Cefiderocol was also used as an adjunctive therapy to treat an intra-abdominal infection and secondary bacteremia caused by an MBL and OXA-48–like carbapenemase–producing *K pneumoniae*, but ultimately the patient died from other complications.[25]

Here, we report a case of cefiderocol-resistant, NDM-producing *Escherichia coli* isolated from a patient with no prior history of cefiderocol therapy. Using whole-genome sequencing (WGS), we characterized this isolate and assessed for genomic variations among related strains to identify potential novel mechanisms of resistance.

MATERIALS AND METHODS

Antimicrobial susceptibility testing was performed in accordance with CLSI standards. MIC testing was performed using broth microdilution on customized 96-well trays prepared in house. Preparation followed guidelines set forth by the CLSI. Results were interpreted manually according to CLSI M100-S30 Enterobacterales breakpoints.[26] Cefiderocol susceptibility was tested by both MIC and disk diffusion; results were interpreted according to the FDA Breakpoints (updated September 28, 2020).

WGS was performed using the Illumina MiSeq platform. The Qiagen EZ1 DNA Blood and DNA Tissue Kits and the EZ1 Advanced XL instrument were used to extract genomic DNA from pure isolates. Extracted DNA was quantified with the Qubit 1X dsDNA HS assay (Thermo Fisher Scientific) using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific). Acceptable quantities of DNA were 0.04 ng/µL or more. DNA was diluted in water to obtain concentrations within the range of 100 to 500 ng in 30 µL. Library preparation was performed using the Nextera DNA Flex Library Prep Kit (Illumina) according to the manufacturer's instructions. Tagmented DNA was amplified using a limited-cycle polymerase chain reaction set according to the input DNA concentrations. Library cleanup was performed using a 2-step bead purification procedure. Libraries were quantified, as described above, using Qubit; acceptable concentrations were 0.1 ng/µL or higher. Following quantification, the Agilent DNA 1000 Kit and Agilent 2100 Bioanalyzer instrument were used to analyze the average band size of the libraries, with an acceptable range of 300- to 900-base pair (bp) size. Acceptable libraries were normalized to 2 nmol/L or 4 nmol/L concentrations for the Illumina MiSeq v2 or v3 reagent kit, respectively; 5 µL of each sample were pooled, denatured, and diluted, and 600 µL were loaded into the reagent cartridge. The Illumina MiSeq System was used to produce 250-bp paired-end reads. Data were uploaded to the Illumina BaseSpace cloud and demultiplexed.

Strain typing, plasmid typing, and genetic determinants of antimicrobial resistance were identified using tools (KmerFinder v3.2, ResFinder v4.0, PlasmidFinder v2.1) available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org). We used the CLC Genomics Workbench, version 12.0.3 (Qiagen) to pair, trim, and map the sequence reads.

K-mer analyses were done using the Create K-mer Tree tool in CLC Genomics Workbench. K-mers of 16 nucleotide lengths were used; Jensen-Shannon divergence was used to construct the phylogenetic trees. Single-nucleotide variation (SNV—formerly single-nucleotide polymorphism [SNP]) analyses were done using the Basic Variant Detection tool in the CLC Genomics Workbench. This tool was used to create a variant track following read mapping to the reference genome. The variant tracks for each isolate were then compared using the Create SNP Tree tool. SNV trees were created using maximum-likelihood algorithms.

Sequence reads were also mapped to various other genes. Using the Extract Consensus Sequence tool, the consensus sequences were determined. Consensus sequences were then exported from

CLC Genomics Workbench and imported into Geneious Prime, version 2020.0.3, software (Biomatters) for further analysis. The consensus sequences and translated amino acid sequences were compared using the Geneious Alignment tool.

RESULTS

Case Report

A 55-year-old woman with medical history significant for cryptogenic cirrhosis thought to be the result of nonalcoholic steatohepatitis, complicated by ascites, hepatic encephalopathy, and hepatorenal syndrome, presented to a tertiary care medical center as a transfer from an outside hospital for higher level of care and liver transplant evaluation.

She was initially admitted to an outside facility for failure to thrive after recent hospitalization for an ESBL-producing *E coli* blood stream infection and upper gastrointestinal bleeding. While there, blood cultures were positive with *K pneumoniae,* and she was started on ertapenem therapy, with sensitivities pending at the time. Given her comorbidities and clinical condition, she was transferred directly to a tertiary care center for higher level of care and liver transplant evaluation. In terms of social history, she was born in Guatemala, with no recent travel outside the United States; she denied any alcohol, tobacco, or illicit drug use.

Upon transfer, she was hemodynamically stable and afebrile. She was evaluated by the infectious disease service, and after review of the *K pneumoniae* sensitivities before her transfer, she was switched to levofloxacin oral therapy to complete a 7-day course given a pansensitive isolate. Her course was complicated, however, by multiple seizures that required endotracheal intubation and resulted in subsequent septic shock, with respiratory and blood cultures revealing carbapenem-resistant *E coli* isolates.

TABLE 1 lists the susceptibility data and interpretations for the 2 *E coli* isolates. Isolate UCLA_315 was isolated from an endotracheal respiratory specimen collected on hospital day 9; isolate UCLA_314 was isolated from a blood specimen collected on hospital day 11. Both isolates were resistant to cephalosporins, carbapenems, aztreonam, ciprofloxacin and levofloxacin, tobramycin, trimethoprim-sulfamethoxazole, and all tested β-lactam/β-lactamase inhibitor combinations (ie, ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, and piperacillin-tazobactam). Both isolates were susceptible to amikacin, colistin, gentamicin, and tigecycline. UCLA_315 was susceptible to minocycline (2 µg/mL), while UCLA_314 had an intermediate MIC (8 µg/mL). Both isolates were resistant to cefiderocol by disk diffusion (zone diameter, 7 mm), and isolate UCLA_314 was resistant by MIC testing (>32 µg/mL). MIC testing was not performed for isolate UCLA_315.

Carbapenemase genotype testing by CARBA-R (Cepheid) indicated that both isolates were positive for bla_{NDM}. In vitro carbapenemase assays (ie, modified carbapenem inactivation method [CIM] and EDTA-modified CIM) confirmed the presence of MBLs. WGS analysis also confirmed the presence of bla_{NDM}.

The patient was initiated on ceftazidime/avibactam and aztreonam therapy while susceptibility testing was pending. Ultimately, she died on hospital day 14 because of ongoing sepsis, with a transition to palliative care based on her goals of care.

Genomic Characterization

E coli isolates UCLA_314 and UCLA_315 as well as 3 additional *E coli* isolates and 1 *K pneumoniae* isolate collected from the patient at the initial hospital (Olive View Medical Center [OVMC]), were sequenced. The 4 isolates from OVMC were susceptible to cefiderocol by disk diffusion (zone diameter ≥16 mm). K-mer analysis using KmerFinder[27,28] showed that UCLA_314 and UCLA_315 were most closely related to *E coli* strain AR_0435 (CP029115.1). Raw sequences of all *E coli* isolates were mapped to this reference strain, and further analyses revealed approximately 75,000 SNV differences between the 3 *E coli* isolates from OVMC, UCLA_314, and UCLA_315, showing that the 2 sets of isolates are genetically unrelated and likely represent a unique introduction event to the patient. UCLA_314 and UCLA_315 had only 3 SNV differences between them. For simplicity, UCLA_314, which was isolated from blood, was used for the remainder of the genomic analyses.

Several known antibiotic resistance genes were identified by ResFinder[29] **TABLE 2**. The presence of an MBL was confirmed (ie, bla_{NDM-5}), with 4 other β-lactamase genes: $bla_{CTX-M-15}$, bla_{OXA-1}, $bla_{TEM-176}$, and bla_{CMY-42}. Genes conferring resistance to aminoglycosides (ie, *aac(6')-Ib-cr*, *aadA2*, *aph(3')-Ia*), macrolides (ie, *mdf(A)*, *mph(A)*), fluoroquinolones (ie, *aac(6')-Ib-cr*, *qnrS1*), tetracyclines (ie, *tet(A)*), sulfonamides (ie, *sul1*), and trimethoprim (*dfrA12*, *dfrA14*) were identified. Gene alterations with known associations to resistance to nalidixic acid and ciprofloxacin in *parC* (p.S80I), *parE* (p.S458A), and *gyrA* (p.S83L and p.D87N) were also identified **TABLE 2**. PlasmidFinder[30] identified sequences matching 4 known plasmids: IncF1 (*E coli* K-12 plasmid F; AP001918.1), IncFII (*E coli* plasmid pC15-1a; AY458016.1), IncI(Gamma) (*Salmonella enterica* subsp enterica serovar Typhimurium plasmid R621a; AP011954.1), and IncX1 (*E coli* plasmid pOLA52; EU370913.1). Raw sequences from UCLA_314 were mapped to reference sequences for each plasmid: *E coli* plasmid F (59.6% coverage, 97.9% pairwise identity), pC15-1a (79.7% coverage, 95.9% pairwise identity), R621a (41.0% coverage, 98.7% pairwise identity), and pOLA52 (33.7% coverage, 94.5% pairwise identity).

Multilocus sequence typing analysis showed that UCLA_314 belonged to sequence type 167 (ST167). We performed a k-mer analysis using 18 ST167 genomes available through the National Center for Biotechnology Information (NCBI) GenBank (Supplemental Figure 1; all supplemental material can be found at *American Journal of Clinical Pathology* online). Consistent with the KmerFinder results, UCLA_314 was strongly related to *E coli* strain AR_0435 (CP029115.1); we were unable to find clinical information about this strain. UCLA_314 was also closely related to *E coli* strain WCHEC005237 (CP026580.3), a clinical isolate from a rectal swab culture obtained from a patient in China (Sichuan, Chengdu) in 2014.

Determination of Genes Involved in Cefiderocol Resistance

Mechanisms of resistance to cefiderocol are currently unclear. To investigate this phenomenon, we first tested cefiderocol susceptibility

TABLE 1 Antimicrobial Susceptibility Results for *Escherichia coli* Patient Isolates and Related ST167 Isolates[a]

	MIC (Interpretation) for *E coli* Isolate, µg/mL									
Antimicrobial	**UCLA_315**	**UCLA_314**	**EC_2016**	**AR_0435[b]**	**AR_0011[b]**	**AR_0014[b]**	**AR_0149[b]**	**AR_0150[b]**	**AR_0151[b]**	**AR_0162[b]**
Amikacin	≤4 (S)	≤4 (S)	—	>64 (R)	8 (S)	4 (S)	2 (S)	≤1 (S)	2 (S)	2 (S)
Aztreonam	>32 (R)	>32 (R)	—	>64 (R)	>64 (R)	64 (R)	≤2 (S)	32 (R)	>64 (R)	>64 (R)
Cefazolin	>32 (R)	>32 (R)	—	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)
Cefepime	>32 (R)	>32 (R)	—	>32 (R)	>32 (R)	>32 (R)	>32 (R)	>32 (R)	>32 (R)	>32 (R)
Cefiderocol	—	>32 (R)	—	>32 (R)	0.5 (S)	—	—	>32 (R)	—	>32 (R)
Ceftazidime	>32 (R)	>32 (R)	—	>128 (R)	128 (R)	32 (R)	>128 (R)	>128 (R)	>128 (R)	>128 (R)
Ceftazidime-avibactam	>32 (R)	>32 (R)	—	>16 (R)	≤0.5 (S)	≤0.5 (S)	>16 (R)	—	—	—
Ceftolozane-tazobactam	>32 (R)	>32 (R)	—	—	1 (S)	≤0.5 (S)	—	—	—	—
Ceftriaxone	>64 (R)	>64 (R)	—	>32 (R)	>32 (R)	>32 (R)	>32 (R)	>32 (R)	>32 (R)	>32 (R)
Ciprofloxacin	>4 (R)	>4 (R)	—	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)
Colistin	≤2 (S)	≤2 (S)	—	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)
Ertapenem	>4 (R)	>4 (R)	—	>8 (R)	0.25 (S)	≤0.12 (S)	8 (R)	>8 (R)	>8 (R)	>8 (R)
Gentamicin	≤1 (S)	≤1 (S)	—	>16 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	≤0.25 (S)	1 (S)
Imipenem	16 (R)	16 (R)	—	4 (R)	≤0.5 (S)	≤0.5 (S)	8 (R)	16 (R)	16 (R)	8 (R)
Levofloxacin	>8 (R)	>8 (R)	—	>8 (R)	>8 (R)	>8 (R)	8 (R)	>8 (R)	>8 (R)	>8 (R)
Meropenem	>16 (R)	>16 (R)	—	>8 (R)	≤0.12 (S)	≤0.12 (S)	8 (R)	>8 (R)	>8 (R)	>8 (R)
Meropenem-vaborbactam	>32 (R)	>32 (R)	—	—	≤0.5 (S)	≤0.5 (S)	—	—	—	—
Minocycline	2 (S)	8 (I)	—	—	—	—	—	—	—	—
Piperacillin-tazobactam	>128 (R)	>128 (R)	—	>128 (R)	8 (S)	8 (S)	>128 (R)	>128 (R)	>128 (R)	>128 (R)
Tigecycline	0.5 (S)	0.5 (S)	—	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)
Tobramycin	16 (R)	16 (R)	—	>16 (R)	>16 (R)	>16 (R)	1 (S)	1 (S)	16 (R)	≤0.5 (S)
Trimethoprim-sulfamethoxazole	>4/80 (R)	>4/80 (R)	—	>8 (R)	>8 (R)	≤0.5 (S)	≤0.25 (S)	>8 (R)	>8 (R)	≤0.5 (S)
	Zone Diameter (Interpretation) for *E coli* Isolate, mm									
Cefiderocol	7 (R)	7 (R)	9 (I)	NZ (R)	22 (S)	22 (S)	10 (I)	NZ (R)	NZ (R)	NZ (R)

CDC, Centers for Disease Control and Prevention; *E coli, Escherichia coli;* I, intermediate; MIC, minimum inhibitory concentration; NZ, no zone; R, resistant; S, susceptible.
[a]CLSI M100-S30 interpretive criteria were used. FDA Identified Breakpoints were used for cefiderocol interpretations (updated: September 28, 2020).
[b]The antimicrobial susceptibility data for these isolates were obtained from the CDC Antimicrobial Resistance Isolate Bank.

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TABLE 2 Presence of Genetic Determinants of Antimicrobial Resistance in Isolate UCLA_314[a]

Acquired AR Genes			
	Query Coverage, %	**Identity, %**	**Accession No.**
Aminoglycoside			
aac(6')-Ib-cr	100 (600/600)	100	DQ303918
aadA2	100 (792/792)	100	JQ364967
aph(3')-Ia	100 (816/816)	99.88	EF015636
β-lactam			
blaCMY-42	100 (1146/1146)	100	HM146927
blaCTX-M-15	100 (876/876)	100	AY044436
blaNDM-5	100 (813/813)	100	JN104597
blaOXA-1	100 (831/831)	100	HQ170510
blaTEM-176	100 (861/861)	100	GU550123
Quinolone			
aac(6')-Ib-cr	100 (600/600)	100	DQ303918
qnrS1	100 (657/657)	100	AB187515
Folate pathway antagonist			
sul1	100 (840/840)	100	U12338
dfrA12	100 (498/498)	100	AM040708
dfrA14	100 (474/474)	100	KF921535
Macrolide			
mdf(A)	100 (1233/1233)	99.92	Y08743
mph(A)	100 (906/906)	99.78	D16251
Phenicol			
floR	100 (1215/1215)	98.02	AF118107
Tetracycline			
tet(A)	100 (1275/1275)	100	AF534183
Chromosomal Variations Associated With AR			
	Nucleotide Change	**Protein Sequence Variant**	**PMID**
Nalidixic acid, ciprofloxacin			
parC	AGC → ATC	p.S80I	8851598
parE	TCG → GCG	p.S458A	28598203
gyrA	TCG → TTG	p.S83L	8891148
gyrA	GAC → AAC	p.D87N	12654733

AR, antimicrobial resistance; *E coli, Escherichia coli;* PMID, PubMed identifier.
[a]ResFinder 4.0 from the Center for Genomic Epidemiology was used to identify acquired AR genes and know chromosomal alterations resulting in AR from *E coli* isolate UCLA_314. Threshold for %ID was set to 90%, minimum coverage length was set to 60%. *Accession No.* refers to the GenBank accession number. *PMID* refers to the PubMed identifier of the paper showing association between the gene variation and AR.

for 7 other ST167 *E coli* isolates **TABLE 1**. Seven of these isolates were from the Centers for Disease Control and Prevention (CDC) Antimicrobial Resistance (AR) Isolate Bank: AR_0435, AR_0011, AR_0014, AR_0149, AR_0150, AR_0151, and AR_0162. One isolate (EC_2016) was obtained as a referral at the UCLA Clinical Microbiology Laboratory; WGS was performed on this isolate for an unrelated purpose, and it was found to be part of ST167. EC_2016 was isolated from a urine culture from a patient in 2016; no other clinical information was available for this patient. Six of these 8 isolates were found to be nonsusceptible to cefiderocol by disk diffusion; AR_0011 and AR_0014 were susceptible (22 mm) **TABLE 1**.

Genomes for all 7 CDC AR Isolate Bank isolates were downloaded from NCBI GenBank (AR_0149 [CP021532.1], AR_0150 [CP021736.1], AR_0151 [CP021691.1], AR_0011 [CP024855.1], AR_0014 [CP024859.1], AR_0162 [CP021683.1], and AR_0435 [CP029115.1]) and characterized using ResFinder.[29] Several known antibiotic resistance genes and point alterations were identified in the genomes (Supplemental Table 1). All isolates contained at least 1 known β-lactamase gene, and all except AR_0011 and AR_0014 contained an NDM β-lactamase gene (ie, $bla_{\text{NDM-1,}}$ $bla_{\text{NDM-5,}}$ $bla_{\text{NDM-7}}$) (Supplemental Table 1).

Using the genome sequences of the various ST167 isolates, we compared the 2 cefiderocol-susceptible isolates (ie, AR_0011 and AR_0014) to the nonsusceptible isolates (ie, UCLA_314, EC_2016, AR_0435, AR_0149, AR_0150, AR_0151, and AR_0162) to identify potential genetic determinants of resistance. We mapped raw sequences of each isolate to the *E coli* strain K-12 substr MG1655 (U00096.2) reference genome. A list of variants were identified

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between each isolate and the reference genome; these data were used to perform an SNV analysis FIGURE 1. A matrix of SNV differences among the various isolates is shown in Supplemental Table 2.

Using the list of variants, we identified genome positions where the nucleotide was identical among the nonsusceptible isolates but different from the susceptible isolates. This work applied to 2,881 variants from 271 unique genes. Considering the mechanism of action (MOA) of cefiderocol, we narrowed this list to genes involved in transport and peptidoglycan biosynthesis. This list applied to 704 variants from 58 unique genes (Supplemental Data 1). This list included several genes of interest: *ftsI* (encodes PBP3, the primary target of cefiderocol), *cirA* (encodes a ferric dihyroxybenzoylserine outer membrane transporter), and *fecB* (encodes a ferric citrate adenosine triphosphate–binding cassette transporter periplasmic binding protein). Consensus sequences for each of these genes were extracted and aligned to the K-12 reference gene to measure pairwise nucleotide identity TABLE 3. Consensus sequences were then translated to determine the effect on amino acid sequence. In FtsI, we identified a 4-amino-acid insertion ("YRIN") at position 334 that was present in all cefiderocol nonsusceptible isolates but absent in the 2 susceptible isolates TABLE 3. In *cirA*, all cefiderocol nonsusceptible isolates had a 2-nucleotide deletion present that resulted in a frameshift alteration after amino acid 89 that ultimately introduced a stop codon at amino acid position 105, significantly shortening the protein; this variation was absent in both cefiderocol-susceptible isolates TABLE 4. The variant in *fecB* did not result in different amino acid sequences between the cefiderocol-nonsusceptible and susceptible isolates TABLE 5.

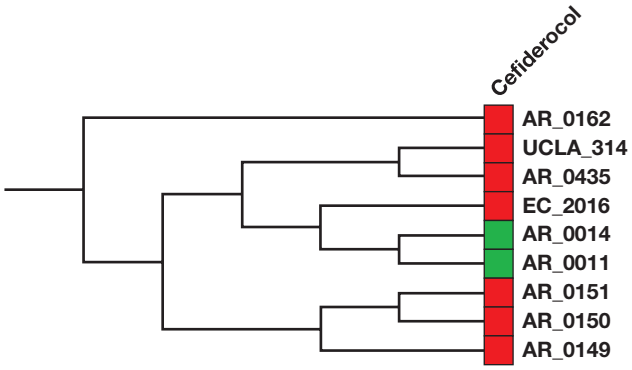

FIGURE 1 Single-nucleotide variant (SNV) analysis of ST167 *Escherichia coli* genomes. SNV analysis was performed in CLC Genomics Workbench (Qiagen) using variants obtained by mapping each genome to the *E coli* strain K-12 substr MG1655 (U00096.2) reference genome. Genome sequences from UCLA_314, EC_2016, and the following strains were used: AR_0149 (CP021532.1), AR_0150 (CP021736.1), AR_0151 (CP021691.1), AR_0011 (CP024855.1), AR_0014 (CP024859.1), AR_0162 (CP021683.1), and AR_0435 (CP029115.1). Strains in green were found to be susceptible to cefiderocol; strains in red were found to be nonsusceptible to cefiderocol.

Investigating the Role of NDM in Cefiderocol Resistance

All the isolates described above harbored an NDM β-lactamase gene except AR_0011 and AR_0014, which were also the 2 isolates that tested susceptible to cefiderocol (Supplemental Table 1). To investigate the role of NDM in cefiderocol resistance, we assessed the susceptibility of other *E coli* strains harboring NDM genes. We identified 5 CDC AR Isolate Bank isolates of *E coli* that harbored either $bla_{\text{NDM-1}}$ or $bla_{\text{NDM-5}}$ but tested susceptible to cefiderocol by disk diffusion and/or MIC testing (Supplemental Table 3). We then determined whether these strains harbored the nucleotide and amino acid variants seen in the cefiderocol-nonsusceptible strains TABLE 3. We found that although 2 strains (AR_0119 and AR_0128) had the 4-amino-acid insertion ("YRIN") at position 334 in FtsI, none of the strains had a frameshift variation in *cirA* TABLE 3.

DISCUSSION

Cefiderocol is a novel siderophore cephalosporin with intrinsic structural stability to both serine and MBL carbapenemases that

TABLE 3 Nucleotide and Amino Acid Sequence Variants of *ftsI*[a]

Cefiderocol Susceptibility	*E coli* Isolate	NDM Variant	Nucleotide Pairwise Identity, %	Protein Sequence Variant
Nonsusceptible	UCLA_314	NDM-5	98.90	p.P333_Y334insYRIN
	EC_2016			p.Q227H
	AR_0150			p.E353K
	AR_0151			p.I536L
	AR_0149	NDM-7		
	AR_0162			
	AR_0435	NDM-1		
Susceptible	AR_0011	None	99.90	p.Q227H
	AR_0014			
Susceptible	AR_0452	NDM-5	99.50	None
	AR_0055	NDM-1	98.50	p.A233T, p.I332V
	AR_0069		99.80	None
	AR_0119		98.40	p.P333_Y334insYRIN
	AR_0128			p.I536L

E coli, Escherichia coli; NDM, New Delhi metallo-β-lactamase.
[a]Nucleotide pairwise identity to *E coli* strain K-12 substr MG1655, and protein translation and variant analysis were performed using Geneious Prime (Biomatters).

TABLE 4 Nucleotide and Amino Acid Sequence Variants of *cirA*[a]

Cefiderocol Susceptibility	*E coli* Isolate	NDM Variant	Nucleotide Pairwise Identity, %	Protein Sequence Variant
Nonsusceptible	UCLA_314	NDM-5	97.70	p.S90Yfs*16[b]
	EC_2016			
	AR_0150			
	AR_0151			
	AR_0149	NDM-7		
	AR_0162			
	AR_0435	NDM-1		
Susceptible	AR_0011	None	100	None
	AR_0014			
Susceptible	AR_0452	NDM-5	99.40	None
	AR_0055	NDM-1	98.90	p.I174V
	AR_0069		99.00	p.I547F
	AR_0119		99.40	None
	AR_0128			

E coli, Escherichia coli; NDM, New Delhi metallo-β-lactamase.
[a]Nucleotide pairwise identity to *E coli* strain K-12 substr MG1655, and protein translation and variant analysis were performed using Geneious Prime (Biomatters).
[b]The *cirA* protein translation of the cefiderocol-nonsusceptible isolates contained other variants. These are not listed because they occur downstream of a stop codon introduced at amino acid position 105 resulting from a frameshift variation occurring after amino acid 89.

TABLE 5 Nucleotide and Amino Acid Sequence Variants of *fecB*[a]

Cefiderocol Susceptibility	*E coli* Isolate	NDM Variant	Nucleotide Pairwise Identity, %	Protein Sequence Variant
Nonsusceptible	UCLA_314	NDM-5	99.60	p.T23M
	EC_2016			p.I57S
	AR_0150			p.A214S
	AR_0151			
	AR_0149	NDM-7		
	AR_0162			
	AR_0435	NDM-1		
Susceptible	AR_0011	None	99.70	p.T23M
				p.I57S
	AR_0014			p.A214S

E coli, Escherichia coli; NDM, New Delhi metallo-β-lactamase.
[a]Nucleotide pairwise identity to *E coli* strain K-12 substr MG1655, and protein translation and variant analysis were performed using Geneious Prime (Biomatters).

the FDA approved for clinical use in late 2019. Several case reports[21-25] highlighted the clinical utility of cefiderocol for the treatment of difficult-to-treat MDR gram-negative infections. Although cefiderocol-resistant *E coli* isolates have been identified by surveillance studies, no case reports exist to our knowledge that describe the detection of a cefiderocol-resistant strain of *E coli* in a patient not previously treated with this drug. The detection of a cefiderocol-resistant, MBL-producing strain of *E coli* is considerably concerning.

The cefiderocol-resistant *E coli* isolates described here were identified as belonging to sequence type ST167. This sequence type is of particular public health concern because of its propensity for carrying plasmids that harbor MBL genes,[31-34] the presence of virulence factors (eg, type 2, 3, and 6 secretion systems; capsular synthesis genes),[35,36] and the recent discovery that it contains a previously uncharacterized O-antigen (O89b).[36] ST167 strains of *E coli* harboring bla_{NDM-5} have been described in Italy,[35,37] China,[31,38,39] and the United States.[40]

Mechanisms of resistance to cefiderocol are currently unclear. We sought to investigate this phenomenon by identifying differences among closely related strains of *E coli* that have differing susceptibility to cefiderocol. We found that all 7 nonsusceptible strains harbored an NDM gene, while the 2 susceptible strains did not. Recent literature, however, shows that NDM alone is not sufficient to confer resistance. Cefiderocol is stable against NDM-1 and other MBLs (ie, IMP-1, VIM-2, L1),[14] and although NDM-producing strains generally have elevated MICs,[20] the introduction of bla_{NDM-1} into an isogenic strain of *E coli* was not sufficient to confer resistance.[41] We were also able to provide evidence on this point by identifying 5 NDM-harboring *E coli* strains that tested susceptible to cefiderocol.

We investigated other potential genetic determinants of resistance by comparing genomic variants that were different between nonsusceptible and susceptible strains. We identified differences in several genes that relate to the MOA of cefiderocol. All nonsusceptible isolates had a 4-amino-acid insertion ("YRIN") at position 334 in FtsI. It was recently shown that an *E coli* isolate harboring this same insertion had significantly reduced susceptibility to cephalosporins.[42] The impact of an insertion in this region of FtsI on cefiderocol susceptibility is not known, but we did identify 2 cefiderocol-susceptible isolates that also harbored this insertion, showing that this insertion is not sufficient to confer resistance. More promising were the results of the *cirA* gene. The *cirA* gene of all resistant isolates was predicted to be significantly truncated because of a frameshift variation that was not present in all susceptible isolates tested. Ito et al[13] showed that strains of *E coli* harboring deletions in both *cirA* and *fiu* resulted in a 16-fold increase in the cefiderocol MIC. The *fiu* gene of all isolates described here had a 100% nucleotide pairwise identity to the K-12 reference sequence (data not shown).

Whether the cefiderocol resistance in these *E coli* isolates is the result of a combination of factors described here or a completely separate mechanism remains unclear. The impact of gene expression on resistance was not tested here, but recent data on *A baumannii* show that it may also be relevant to cefiderocol resistance.[43] Regardless, the discovery of a cefiderocol-resistant strain in a patient not previously treated with cefiderocol is clinically significant given the promising potential of this drug to treat infections with MBL-producing CREs.

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