Cefiderocol Activity Against Clinical *Pseudomonas aeruginosa* Isolates Exhibiting Ceftolozane-Tazobactam Resistance

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**Background.** Mutations in the AmpC-AmpR region are associated with treatment-emergent ceftolozane-tazobactam (TOL-TAZ) and ceftazidime-avibactam (CAZ-AVI) resistance. We sought to determine if these mutations impact susceptibility to the novel cephalosporin-siderophore compound cefiderocol.

**Methods.** Thirty-two paired isolates from 16 patients with index *P. aeruginosa* isolates susceptible to TOL-TAZ and subsequent *P. aeruginosa* isolates available after TOL-TAZ exposure from January 2019 to December 2020 were included. TOL-TAZ, CAZ-AVI, imipenem-relebactam (IMI-REL), and cefiderocol minimum inhibitory concentrations (MICs) were determined using broth microdilution. Whole-genome sequencing of paired isolates was used to identify mechanisms of resistance to cefiderocol that emerged, focusing on putative mechanisms of resistance to cefiderocol or earlier siderophore-antibiotic conjugates based on the previously published literature.

**Results.** Analyzing the 16 pairs of *P. aeruginosa* isolates, ≥4-fold increases in cefiderocol MICs occurred in 4 of 16 isolates. Cefiderocol nonsusceptibility criteria were met for only 1 of the 4 isolates, using Clinical and Laboratory Standards Institute criteria. Specific mechanisms identified included the following: AmpC E247K (2 isolates), MexR A66V and L57D (1 isolate each), and AmpD G116D (1 isolate) substitutions. For both isolates with AmpC E247K mutations, ≥4-fold MIC increases occurred for both TOL-TAZ and CAZ-AVI, while a ≥4-fold reduction in IMI-REL MICs was observed.

**Conclusions.** Our findings suggest that alterations in the target binding sites of *P. aeruginosa*–derived AmpC β-lactamas have the potential to reduce the activity of 3 of 4 novel β-lactams (ie, ceftolozane-tazobactam, ceftazidime-avibactam, and cefiderocol) and potentially increase susceptibility to imipenem-relebactam. These findings are in need of validation in a larger cohort.

**Keywords.** AmpC; antimicrobial resistance; ceftazidime-avibactam; omega loop.

*Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR; ie, *P. aeruginosa* resistant to all traditional β-lactams and fluoroquinolones) poses significant clinical challenges [1]. Several novel β-lactam agents have become Food and Drug Administration (FDA) approved with activity against DTR *P. aeruginosa*, including ceftolozane-tazobactam (TOL-TAZ), ceftazidime-avibactam (CAZ-AVI), imipenem-clastatin-relebactam (IMI-REL), and cefiderocol. Unreliable baseline susceptibility of DTR *P. aeruginosa* to the novel agents, as well as reports of resistance emerging during therapy, has tempered enthusiasm for several of these agents [2].

TOL-TAZ remains a preferred agent for the treatment of DTR *P. aeruginosa* infections [1]. We previously reported that in a cohort of 28 patients infected with DTR *P. aeruginosa* and paired clinical isolates before and after receipt of TOL-TAZ, half of patients had isolates that developed ≥4-fold increases in TOL-TAZ minimum inhibitory concentrations (MICs) after exposure to this agent [3].

Before the clinical use of cefiderocol, there was widespread belief that resistance would primarily result from mutations in TonB-dependent receptors (TBDs), a series of bacterial outer membrane proteins that mediate siderophore–iron complex transport [4-6]. While such mutations have been identified [7, 8], there have also been isolated reports of changes in the *ampC* region contributing to cefiderocol resistance among the Enterobacterales [9, 10]. This may occur after exposure to oxymenocephalosporins, such as CAZ-AVI or ceftazime, in the absence of exposure to cefiderocol. It is unknown what role exposure to TOL-TAZ, also an oxymenocephalosporin, has...
in contributing to cefiderocol inactivity against *P. aeruginosa*. A *P. aeruginosa* isolate infecting a 30-year-old liver transplant recipient developed a cefiderocol MIC increase from 2 to 8 mcg/mL after treatment with TOL-TAZ, in the absence of exposure to cefiderocol [11]. Mutations in the TBDR genes *piuD* and *pirR* were identified, in addition to a leucine-to-phenylalanine substitution at amino acid position 147 in the AmpC enzyme [11]. The relative role of mutations in the iron transport pathway and the role of the *ampC* gene in contributing to cefiderocol MIC increases in this case are unclear. Building on existing investigations, we sought to determine the frequency and putative mechanisms of cefiderocol resistance in a cohort of patients infected with DTR *P. aeruginosa* after TOL-TAZ exposure.

**METHODS**

**Study Population**

Sixteen unique patients from The Johns Hopkins Hospital with DTR *P. aeruginosa* isolates available both before and after at least 72 hours of TOL-TAZ (and up to 30 days after TOL-TAZ completion) between January 2018 and December 2019 had paired isolates available for additional testing. All initial DTR *P. aeruginosa* isolates were susceptible to TOL-TAZ. Patients contributing isolates were a median (range) of 55 (16–77) years, 44% had severe immunocompromise, and the most common sources of infection were pneumonia (69%) and bacteremia (31%) (Table 1). Patients received an average (range) of 12 (6–22) days of TOL-TAZ between the index and subsequent clinical *P. aeruginosa* isolate.

**Microbiological Testing**

Antimicrobial susceptibility testing (AST) for 32 DTR *P. aeruginosa* isolates from the 16 patients was determined using MDRGN2F lyophilized sensitizer broth microdilution (BMD) panels (Thermo Fisher Scientific, Waltham, MA, USA) [12]. Panels contain cefiderocol concentrations ranging from 0.03 to 64 mg/L and a proprietary chelator in the wells, removing the requirement for iron-depleted cation-adjusted Mueller Hinton broth. Isolates were tested in triplicate by BMD; modal MICs were used for analysis. Clinical Laboratory and Standards Institute (CLSI) interpretive criteria were applied to all agents to determine *P. aeruginosa* susceptibility [13]. Quality control organisms were performed each day of testing, including *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. The CLSI defines *P. aeruginosa* isolates with cefiderocol MICs ≤ 4 mcg/mL as susceptible to cefiderocol [13].

**Whole-Genome Sequencing**

Genomic DNA was extracted from the 32 isolates using the DNeasy Blood & Tissue Kit (QIAGEN, Inc., Valencia, CA, USA). Whole-genome sequencing (WGS) was conducted using Illumina MiSeq short-read sequencing (Illumina, San Diego, CA, USA). Sequenced isolates were evaluated using FASTQC, version 0.11.6, and MultiQC, version 1.6. Trimmomatic, version 0.39, removed adapters and trimmed low-quality paired-end reads. Trimmed and de-duplicated reads (FastUniq, version 1.1) were de novo assembled with SPAdes, version 3.12.0, and annotated with Prokka, version 1.13. Quast, version 4.6.3, confirmed assembly quality. Genomic distances for cluster analysis were calculated with SourMash 2.0.0a. MUMmer3, version 3.23, was used for pairwise differential genome analysis. Gene annotations were determined with nucleotide BLAST, version 2.9.0+, against the reference genome of *P. aeruginosa* PA01. Resistance genes were identified using ARESdb [14]. Intergenic and synonymous variants were removed. Isolate variant analysis was carried out with Snippy 4.6.0 against the reference genome for each species using default parameters.

More specifically, isolates with cefiderocol MICs >4 mcg/mL or those that developed a ≥4-fold increase in MICs when comparing index and subsequent isolates were compared with the PA01 reference genome and compared with their paired isolate using multiple sequence alignment to identify missense mutations resulting in changes to amino acid composition. Efforts focused on examining *P. aeruginosa* resistance targets described for earlier siderophore-antibiotic conjugates and/or cefiderocol. These include insertions, deletions, and frameshift mutations in *piuA, piuC, piuD, pirA, pirR, exbD3, tonB*; or mutations in the promotor region of *pvdS* or *fci* [4–7]—all components of the bacterial iron transport system (Table 2). Proteins associated with increased permeability were also assessed (OprD, mexoperon encoded proteins). Furthermore, based on reports of deletions, insertions, and amino acid substitutions in or proximal to the omega loop of AmpC contributing to cefiderocol resistance [9–11], this region was carefully examined. Bioinformatics analyses were conducted by Ares Genetics.

**RESULTS**

Phylogenetic trees were constructed to confirm relatedness between index and subsequent isolates, and sequence types were determined, also to ensure relatedness between isolates. Paired isolates for each patient met criteria for relatedness. Table 1 includes a brief description of the 16 patients, antibiotic exposures, antibiotic MIC data, and WGS results. For the 16 index isolates (ie, before TOL-TAZ exposure), susceptibility was as follows: TOL-TAZ 100%, CAZ-AVI 63%, IMI-REL 19%, and cefiderocol 100%. For the 16 subsequent isolates, susceptibility was as follows: TOL-TAZ 38%, CAZ-AVI 50%, IMI-REL 19%, and cefiderocol 94%. One pair of DTR *P. aeruginosa* isolates (isolates 9a-b) had cefiderocol MICs of 0.25 mcg/mL and 8 mcg/mL, respectively, transitioning from the susceptible category to the intermediate category. Isolates 8a-b, isolates 10a-b, and isolates 16a-b developed ≥4-fold increases in cefiderocol
| Isolate | Clinical Summary | TOL-TAZ MIC, mcg/mL | CAZ-AVI MIC, mcg/mL | IMI-REL MIC, mcg/mL | Cefiderocol MIC, mcg/mL | Potential Resistance Targets for Siderophore–Antibiotic Conjugates Identified in Subsequent Isolates but NOT Index Isolates |
|---------|-----------------|---------------------|---------------------|--------------------|------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| 1a-b    | 40 yo M, sickle cell disease and *P. aeruginosa* pneumonia. Received TOLTZ T3g q8h x 8d (No HD); other β-lactams: cefepime (4d), meropenem (8d). Alive at day: yes. | 0.5         | 256       | 2       | 1       | 1 1 1 1 -- -- -- -- OprD Stop mutation E426 |
| 2a-b    | 22 yo M, 56% body surface area burns with *P. aeruginosa* catheter-associated bacteremia. Catheter removed. Received TOLTZ 1.5g q8h x 19d (HD); other β-lactams: meropenem (17d). Alive at day: no. | 4         | 256       | 16      | 64      | 4 2 1 2 AmpC G183D -- -- -- -- PBP3 E466K |
| 3a-b    | 47 yo F, bilateral lung transplant with *P. aeruginosa* pneumonia. Received TOLTZ 3g q8h x 14d (HD); other β-lactams: meropenem (17d). Alive at day: yes. | 2         | 8         | 2       | 16      | 4 4 1 0.5 -- -- -- -- | AmpD G148A |
| 4a-b    | 59 yo M, pancreatic cancer with *P. aeruginosa* bacteremia secondary to an intra-abdominal abscess (subsequently drained). Received TOLTZ 1.5g q8h x 19d (no HD); other β-lactams: meropenem (10d). Alive at day: yes. | 4         | 256       | 64      | 64      | 8 8 4 4 -- -- -- -- PDC-3 |
| 5a-b    | 69 yo M, Hodgkin’s lymphoma with *P. aeruginosa* pneumonia. Received TOLTZ 3g q8h x 10d (HD); other β-lactams: meropenem (10d). Alive at day: 30: yes. | 1         | 16        | 4       | 256     | 8 64 0.25 0.12 -- -- -- -- | AmpR D135G |
| 6a-b    | 56 yo M, ventricular assist device with *P. aeruginosa* bacteremia and device-associated infection, device not removed. Received TOLTZ 3g q8h x 18d (no HD); other β-lactams: meropenem (2d). Alive at day: yes. | 4         | 8         | 2       | 8       | 2 2 0.5 0.5 -- -- -- -- | MexR A66V |
| 7a-b    | 66 yo M, pemphigus and immunocompromise with *P. aeruginosa* catheter-associated bacteremia. Catheter removed. Received TOLTZ 3g q8h x 8d (HD); other β-lactams: meropenem (2d). Alive at day: yes. | 1         | 1         | 4       | 4       | 4 4 0.5 0.5 -- -- -- -- | MexR L57D |
| 8a-b    | 55 yo F, myasthenia gravis and immunocompromise with *P. aeruginosa* pneumonia. Received TOLTZ 3g q8h x 8d (HD); other β-lactams: meropenem (2d). Alive at day: yes. | 1         | 4         | 2       | 8       | 4 4 0.5 2 -- -- -- -- | MexR A66V |
| 9a-b    | 77 yo M, acute myeloid leukemia with *P. aeruginosa* pneumonia. Received TOLTZ 3g q8h x 22d (No HD); other β-lactams: meropenem (19d). Alive at day: no. | 0.5       | 256       | 16      | 256     | 32 4 0.25 8 AmpC E247K |
| 10a-b   | 48 yo M, renal cell carcinoma with *P. aeruginosa* pneumonia. Received TOLTZ 3g q8h x 14d (No HD); other β-lactams: meropenem (17d). Alive at day: yes. | 1         | 2         | 2       | 2       | 0.5 4 0.25 2 -- -- -- -- | MexR L57D |
| 11a-b   | 30 yo M, quadriplegia with *P. aeruginosa* pneumonia. Received TOLTZ 3g q8h x 13d (No HD); other β-lactams: none. Alive at day: yes. | 2         | 1         | 4       | 2       | 4 4 0.5 0.25 -- -- -- -- | -- |
| Isolate  | Clinical Summary                                                                 | TOL-TAZ MIC, mcg/mL | CAZ-AVI MIC, mcg/mL | IMI-REL MIC, mcg/mL | Cefiderocol MIC, mcg/mL | Potential Resistance Targets for Siderophore–Antibiotic Conjugates Identified in Subsequent Isolates but NOT Index Isolates<sup>d</sup> |
|---------|----------------------------------------------------------------------------------|---------------------|---------------------|---------------------|-------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| 12a-b   | 16 yo M, ventilator-dependent with *P. aeruginosa* pneumonia. Received TOL-TAZ 3g q8h × 6d (no HD); other β-lactams: meropenem (7d). Alive at day 30: yes. | 4 2 32 4 8 8       | 0.25 0.25           | --                  | --                      | AmpC, AmpR, AmpD, MexR, OprD, TBDR, PBP3, PvdS, PDC-34                                                                 |
| 13a-b   | 53 yo M, 60% body surface area burns with *P. aeruginosa* pneumonia. Received TOL-TAZ 3g q8h × 6d (no HD); other β-lactams: meropenem (10d). Alive at day 30: no. | 1 0.5 16 4 4 4     | 0.5 0.5             | --                  | --                      | PDC-8                                                                                                                             |
| 14a-b   | 55 yo F, anoxic brain injury with *P. aeruginosa* pneumonia. Received TOL-TAZ 3g q8h × 7d (no HD); other β-lactams: meropenem (3d). Alive at day 30: yes. | 2 8 16 16 8 4      | 0.5 1               | --                  | --                      | --                                                                                                                                |
| 15a-b   | 74 yo M, ventilator-dependent with *P. aeruginosa* pneumonia. Received TOL-TAZ 3g q8h × 6d (HD); other β-lactams: none. Alive at day 30: yes. | 1 256 2 256 4 32   | 0.12 0.25           | --                  | AmpD                    | --                                                                                                                                |
| 16a-b   | 65 yo M, ventricular assist device with *P. aeruginosa* bacteremia and device-associated infection, device not removed. Received TOL-TAZ 3g q8h × 16d (HD); other β-lactams: meropenem (1d). Alive at day 30: yes. | 1 256 8 32 32 4    | 0.12 1              | --                  | AmpC, AmpD               | --                                                                                                                                |

Abbreviations: CAZ-AVI, ceftazidime-avibactam; HD, hemodialysis; IMI-REL, imipenem-relebactam; MIC, minimum inhibitory concentration; PDC, *Pseudomonas*-derived cephalosporinase; TBDR, TonB-dependent receptor; TOL-TAZ, ceftolozane-tazobactam.

<sup>a</sup>Green represents antibiotic MIC in susceptible range. Red represents antibiotic MIC not in susceptible range.

<sup>b</sup>Bold isolate numbers indicate ≥4-fold change in cefiderocol MIC against index to subsequent paired *P. aeruginosa* isolates.

<sup>c</sup>“Other β-lactams” includes β-lactam agents administered within 7 days before the index isolate was collected up to the time the subsequent isolate was collected. As all index isolates were resistant to ceftazidime, cefepime, piperacillin-tazobactam, and meropenem, there was limited use of “traditional” β-lactams.

<sup>d</sup>As only changes from index to subsequent isolates are included, mutations present in both index and subsequent isolates are not included. As an example, 9 of 13 index isolates not susceptible to imipenem-relebactam contained oprD mutants.
| Target | Organism(s) | Function | Description of Findings |
|--------|-------------|----------|-------------------------|
| piuA   | *P. aeruginosa*, *A. baumannii* | Encodes TonB-dependent receptor | Overexpression of *piuA* increased susceptibility to siderophore-conjugated antibiotics BAL30072 and MC-1 by 4- to 32-fold for *P. aeruginosa* [5]; transposon insertion in the iron transport receptor *piuA* increased cefiderocol MICs to *P. aeruginosa* but did not lead to frank resistance [6]; deletion of *piuA* in *A. baumannii* resulted in a 4- to 8-fold decrease in susceptibility to siderophore-conjugated antibiotics BAL30072 and MC-1 [5]; insertions, deletions, and frameshift mutations in the *piuA* gene in *P. aeruginosa* isolates led to increased MICs for the siderophore-conjugated antibiotic SMC-3176 [21]; *piuA* deleted mutants had a 8- to 32-fold reduction in cefiderocol MICs [4] |
| piuC   | *P. aeruginosa* | Encodes iron-dependent oxygenase and located adjacent to *piuA* | Frameshift mutation in *piuC* led to premature termination of translation of the PiuC protein and impacted the adjacent gene *piuA*, causing a reduction in expression of *PiuA* [22]; downregulation of the *piuC* gene increased MICs for siderophore-conjugated antibiotic BAL30072 8- to 16-fold [23]; insertions, deletions, and frameshift mutations in the *piuC* gene led to increased MICs for the siderophore-conjugated antibiotic SMC-3176 in *P. aeruginosa* [21] |
| piuD   | *P. aeruginosa* | Encodes TonB-dependent receptor | Deletion of *piuD* increased cefiderocol MICs by 32-fold [4]; clinical isolate with no prior exposure to cefiderocol demonstrated resistance potentially associated with mutation in *piuA* (deletion of an A nucleotide with premature stop codon at amino acid 89) [11] |
| pirA   | *P. aeruginosa*, *A. baumannii* | Encodes TonB-dependent receptor | Overexpression of *pirA* increased susceptibility to siderophore-conjugated antibiotics BAL30072 and MC-1 by 4- to 32-fold [5]; deletion of *pirA* in *A. baumannii* resulted in 4- to 8-fold decreased susceptibility to siderophore-conjugated antibiotics BAL30072 and MC-1 [5]; deletion of *pirA* led to a 2-fold increase in cefiderocol MICs [4]; *pirA* mutants had a 2-fold reduction in siderophore-conjugated antibiotics BAL30072 and MC-1 [4]; reduced expression of the siderophore receptor gene *pirA*, possibly in combination with *piuA*, was associated with cefiderocol resistance in *A. baumannii* isolates [31] |
| pirR   | *P. aeruginosa* | Encodes the response regulator of a 2-component regulatory system predicted to activate expression of *piuA* | Frameshift mutations in *pirR* increased MICs to SMC-3176, a siderophore-conjugated antibiotic [21]; clinical isolate with no prior exposure to cefiderocol demonstrated resistance potentially associated with mutation in *pirR* (insertion of a G nucleotide with premature stop codon at amino acid 201) [11] |
| pvdS   | *P. aeruginosa* | Required for pyoveridine production; mutations in *pvdS* lead to derepression of pyoveridine synthesis, which enhances production of the pyoveridine siderophore receptor FpvA | Mutation in promoter region of *pvdS* increased MICs for cefiderocol and the siderophore-conjugated antibiotic SMC-3176 [7, 8, 21] |
| fecI   | *P. aeruginosa* | Regulator of the synthesis of the iron transporter FecA, contributing to the transport of iron citrate | Single nucleotide change in *fecI* promoter increased MICs to siderophore-conjugated antibiotic BAL30072 8- to 16-fold [23]. Point mutations in the *fecI* promoter reduced activity of the siderophore-conjugated antibiotic SMC-3176 against *P. aeruginosa* [21]; mutations in the promoter region of *fecI* increased cefiderocol resistance [7] |
| exbD3  | *A. baumannii* | Component of inner membrane protein complex providing energy to TonB-dependent transporters | Frameshift mutations in *exbD3* increased the MICs of siderophore-conjugated antibiotics BAL30072 and MC-1 [5] |
| tonB   | *A. baumannii* | Component of inner membrane protein complex providing energy to TonB-dependent transporters | Frameshift mutations in *tonB* increased the MICs of siderophore-conjugated antibiotics BAL30072 and MC-1 [5] |
| ampC   | *P. aeruginosa* | Chromosomal β-lactamase gene | Substitution of leucine for phenylalanine at Ambler amino acid position 147 in the AmpC β-lactamase enzyme, potentially increased cefiderocol MICs [11] |
| PBP3   | *A. baumannii* | Target site of activity for cefiderocol | A isoleucine-to-asparagine substitution at position 236 and a histamine-to-tyrosine substitution at position 370 identified in a cefiderocol-resistant isolate. [31] |

Abbreviation: MIC, minimum inhibitory concentration.
MICs following TOL-TAZ exposure, with cefiderocol MICs increasing from 0.5 to 2 mcg/mL, 0.25 to 2 mcg/mL, and 0.12 to 1 mcg/mL, respectively, prompting further examination.

For isolates 8a-8b, a substitution in MexR A66V was identified. Additionally, a glycine-to-aspartic acid substitution at position 116 on AmpD was identified. *ampD* mutations have the potential to lead to AmpC overproduction, increasing β-lactam MICs in organisms with a chromosomal *ampC*, such as *P. aeruginosa* [15]. Similarly, for isolates 10a-b, a leucine-to-aspartic acid substitution in position 57 was noted in MexR. Mutations in *mexR* result in derepression of the mexAB-oprM multidrug efflux operon [16]. Despite these observations, cefiderocol MICs remained in the susceptible range for 8a-8b and 10a-b.

For isolates 9a-9b and 16a-16b, a glutamic acid-to-lysine substitution at position 247 in *ampC* was identified. This substitution has been previously identified as producing AmpC mutants exhibiting high-level resistance to TOL-TAZ and CAZ-AVI through reduced structural stability of the AmpC enzyme [17]. For isolates 9a-9b, TOL-TAZ MICs increased from 0.5 to 256 mcg/mL and CAZ-AVI MICs increased from 16 to 64 mcg/mL after 22 days of TOL-TAZ. Similarly, for isolates 16a-16b, TOL-TAZ MICs increased from 1 to 256 mcg/mL and CAZ-AVI MICs increased from 8 to 32 mcg/mL after 16 days of TOL-TAZ exposure. Interestingly, for both of these patients, IMI-REL MICs decreased from 32 to 4 mcg/mL, comparing index and subsequent isolates. Although remaining nonsusceptible to IMI-REL, this nonetheless represents a >4-fold decrease in IMI-REL MICs. All subsequent isolates remained resistant to all “traditional” β-lactams and fluoroquinolones.

**DISCUSSION**

In a cohort of 32 paired DTR *P. aeruginosa* isolates from 16 patients exposed to TOL-TAZ, 4 *P. aeruginosa* isolates developed >4-fold increases in cefiderocol MICs, although MICs remained in the susceptible range for 3 of the 4 isolates. The clinical significance of increased cefiderocol MICs in the absence of frank resistance is unknown. Additionally, as none of the included isolates were exposed to cefiderocol therapy, it is unknown if a furthering of MIC elevation would be anticipated after cefiderocol therapy. Antimicrobial resistance markers potentially contributing to cefiderocol MIC increases included mutations in *mexR* (2 isolates), *ampD* (1 isolate), and *ampC* (2 isolates). Of these, the E247K mutations identified in AmpC enzymes for 2 of the 4 isolates have been the most frequently described mechanism of resistance to TOL-TAZ and other cephalosporins [2, 17-19].

We did not identify mutations in TBDRs in the paired isolates contributing to cefiderocol nonsusceptibility in our cohort. However, identification of such mutations is likely more common in patients with previous exposure to cefiderocol. TBDRs are bacterial outer membrane proteins that enable uptake of specific siderophore–iron complexes across the bacterial membrane. They are dependent on 3 inner membrane proteins, TonB-ExbB-ExbD, for energy transduction [20]. TBDR expression is regulated by 2-component regulatory systems [21]. Mutations decreasing the function of components of this pathway may cause dramatic MIC increases for siderophore–antibiotic compounds. The deletion of the TBDRs PiuA and PirA in *Acinetobacter baumannii* decreased susceptibility to BAL30072 and MC-1, earlier siderophore-conjugated antibiotic prototypes, by 4-fold [5], while overexpression increased *P. aeruginosa* susceptibility to these agents by 4- to 32-fold [5, 22]. Frameshift mutations in *exbD3* or *tonB3* genes led to significant increases in BAL30072 and MC-1 MICs [5]. Elevations in cefiderocol MICs may also be associated with mutations in the upstream regions of *pvdS* (a regulator of pyoveridine synthesis) or the FecIRA operon (a regulator of iron transporter protein synthesis). Overexpression of these proteins can lead to ≥4-fold increases in cefiderocol MICs [7, 8, 21, 23].

Shields and colleagues demonstrated a 2-amino acid deletion in the R2 loop of the AmpC β-lactamase (ie, alanine and leucine at positions 292 and 293) in 2 *Enterobacter hormaaechei* isolates from distinct patients after exposure to ceftazidime [9]. These deletions appear to broadly impact cephalosporin antibiotics in that they confer resistance to ceftazidime, CAZ-AVI, and cefiderocol—in the absence of preceding exposure to CAZ-AVI or cefiderocol. The same group described a third patient with an *E. cloacae* clinical isolate with a cefiderocol MIC of >16 mcg/mL with an alanine–proline deletion at positions 294 and 295 and a leucine-to-valine substitution at position 296 in AmpC [10]. Conformation changes in the R2 loop of AmpC β-lactamases expand its substrate binding site, enabling entrapment of cephalosporins with bulkier R2 side chains, increasing their hydrolysis [24]. The omega loop borders the R1 and R2 regions of AmpC, and the R1 region contains position 247, where a substitution was identified in isolates 9b and 16b in our cohort, resulting in elevated cefiderocol, TOL-TAZ, and CAZ-AVI MICs.

Although our focus was on the emergence of resistance, we found that 69% of index isolates not susceptible to IMI-REL contained oprD mutants. Previous work has found that IMI-REL can remain effective against oprD mutants even when the pseudomonal AmpC is overexpressed, because of the potent activity of relebactam against AmpC enzymes [25, 26]. However, others have found that reduced expression of oprD can be sufficient to result in IMI-REL resistance, or at a minimum an increase in IMI-REL MICs compared with isolates with oprD mutants [27-29]. Our understanding of *P. aeruginosa* resistance to IMI-REL remains incomplete and will likely become clearer as it is used more frequently in clinical practice. An interesting observation in our cohort was the >4-fold reduction in IMI-REL...
MICs in both isolates 9b and 16b, which both contained E247K AmpC mutations. A similar finding was observed by Rubio and colleagues, who found that 81% of TOL-TAZ-resistant \textit{P. aeruginosa} isolates with \textit{ampC} mutations were susceptible to IMI-REL, including several isolates that developed reduced IMI-REL MICs in conjunction with an elevation in TOL-TAZ MICs \cite{29}. It is hypothesized that mutations resulting in AmpC structural modifications can enable carbapenems such as imipenem to rotate their bulky 6α-hydroxyethyl side chain to prevent hydrolysis \cite{30}.

We identified 2 different mutations in MexR, the negative regulator of the MexAB-OprM efflux pump, in isolates 8b and 10b with a 24-fold in cefiderocol MICs. The role of MexAM-OprM overexpression in reducing cefiderocol activity warrants further exploration as cefiderocol appears to be a substrate of this efflux pump, although other investigators did not find an association with MexAB-OprM overproduction and reduced cefiderocol activity \cite{6}. Similarly, the role of the AmpD G116 substitution (isolate 8b) remains unclear, as this mutation did not appear to impact TOL-TAZ or CAZ-AVI MICs, leading us to suspect that there was likely incomplete disruption of this gene. Mutations in AmpD G116D, MexR A66V, and MexR L57D were associated with modest increases in cefiderocol MICs to 2 mcg/mL, with cefiderocol MICs remaining in the susceptible range.

Our findings suggest that substitutions in the region of the AmpC omega loop contribute to increased cefiderocol MICs to \textit{P. aeruginosa}. This is particularly concerning as a single amino acid substitution has the potential to inactive 3 of the 4 novel antipseudomonal β-lactams (ie, TOL-TAZ, CAZ-AVI, and cefiderocol) while potentially increasing activity of the fourth (ie, IMI-REL). Our findings also bolster the hypothesis that resistance markers leading to \textit{P. aeruginosa} nonsusceptibility to cefiderocol are diverse \cite{32}. Our study is small and exploratory. Cloning and transformation studies are needed to confirm the significance of the mutations we identified as contributing to cefiderocol resistance.

\textbf{Acknowledgments}

\textit{Financial support.} This work was supported by an R21-AI153580 from the National Institutes of Health and an American Lung Association Research Grant, both awarded to P.T.D.

\textit{Potential conflicts of interest.} S.B. and A.E.P. are employees of Ares Genetics. Ares Genetics performed the bioinformatics analysis for this work. S.E.C. receives consulting fees from Novartis, Theravance, and Balslea outside the submitted work. P.J.S. has received grants and personal fees from Accelerate Diagnostics, OptGen Inc, and BD Diagnostics; grants from bioMerieux, Inc., Affinity Biosensors, and Hardy Diagnostics; and personal fees from Roche Diagnostics, GeneCapture, and Shinogen Inc. P.D.T. and Y.B. report no disclosures. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

\textit{Patient consent.} This work was approved by the Johns Hopkins University Institutional Review Board with a waiver of informed consent.

\textbf{References}

1. Tamma PD, Aitken SL, Bonomo RA, et al. Infectious Diseases Society of America Guidelines on the Treatment of Extended-Spectrum β-lactamase Producing Enterobacteriaceae (ESBL-E), Carbapenem-Resistant Enterobacteriaceae (CRE), and \textit{Pseudomonas aeruginosa} with Difficult-to-Treat Resistance (DTR-P aeruginosa) \textit{Clin Infect Dis} \textbf{2021}; 72:e169–e183.

2. Papp-Walace KM, Mack AR, Taracila MA, Bonomo RA. Resistance to novel β-lactam-β-lactamase inhibitor combinations: the “price of progress.” \textit{Infect Dis Clin North Am} \textbf{2020}; 34:773–819.

3. Tamma PD, Beisken S, Bergman Y, et al. Modifiable risk factors for the emergence of ceftolozane-tazobactam resistance. \textit{Clin Infect Dis} \textbf{2020}; ciaa1306.

4. Luscher A, Moynie I, Auguste P, et al. TonB-dependent receptor repertoire of \textit{Pseudomonas aeruginosa} for uptake of siderophore-drug conjugates. \textit{Antimicrob Agents Chemother} \textbf{2018}; 62:e00997–18.

5. Moynie L, Luscher A, Rolo D, et al. Structure and function of the PiuA and PirA siderophore-drug receptors from \textit{Pseudomonas aeruginosa} and \textit{Acinetobacter baumannii}. \textit{Antimicrob Agents Chemother} \textbf{2017}; 61:e02531–16.

6. Ito A, Sato T, Ota M, et al. In vitro antibacterial properties of cefiderocol, a novel siderophore cephalosporin, against gram-negative bacteria. \textit{Antimicrob Agents Chemother} \textbf{2017}; 62:e01454–17.

7. Ito A, Nishikawa T, Isihi R, et al. Mechanism of cefiderocol high MIC mutants obtained in non-clinical FoR studies. \textit{Poster presented at: ID Week 2018}; October 3–7, 2018; San Francisco, CA. \textit{Poster 598}.

8. Kohara N, Ito A, Ota M, et al. Frequency of resistance acquisition and resistance mechanisms to cefiderocol. \textit{Poster presented at: American Society of Microbiology Annual Meeting; June 6–11, 2018}; Atlanta, GA. \textit{Poster 619}.

9. Shields RK, Iovleva A, Kline EG, et al. Clinical evolution of AmpC-mediated ceftazidime-avibactam and cefiderocol resistance in Enterobacter cloacae complex following exposure to ceftolozane. \textit{Clin Infect Dis} \textbf{2020}; 71:2713–6.

10. Kawai A, McElheny CL, Iovleva A, et al. Structural basis of reduced susceptibility to ceftazidime-avibactam and cefiderocol in Enterobacter cloacae due to AmpC R2 loop deletion. \textit{Antimicrob Agents Chemother} \textbf{2020}; 64:e00198–20.

11. Sreling AP, Al Obaidi MM, Lainhart WD, et al. Evolution of cefiderocol nonsusceptibility in \textit{Pseudomonas aeruginosa} in a patient without previous exposure to the antibiotic. \textit{Clin Infect Dis} \textbf{2021}; ciaa1969.

12. Morris CP, Bergman Y, Tekle T, et al. Cefiderocol antimicrobial susceptibility testing against multidrug-resistant gram-negative bacilli: a comparison of disk diffusion to broth microdilution. \textit{J Clin Microbiol} \textbf{2020}; Dec 17:59(1):e01649–20. 

13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute. \textbf{2021}.

14. Ferreira I, Beisken S, Lueftinger L, et al. Species identification and antibiotic resistance prediction by analysis of whole-genome sequence data by use of ARESdb: an analysis of isolates from the unyvero lower respiratory tract infection trial. \textit{J Clin Microbiol} \textbf{2020}; 58:e00273–20.

15. Schmidtke AJ, Hanson ND. Model system to evaluate the effect of \textit{ampD} mutations on AmpC-mediated beta-lactam resistance. \textit{Antimicrob Agents Chemother} \textbf{2006}; 50:2030–7.

16. Adewoye I, Sutherland A, Srikrum R, Poole K. The mexR repressor of the MexAB-OprM efflux pump of \textit{Pseudomonas aeruginosa} inactivation of mutations compromising activity. \textit{J Bacteriol} \textbf{2002}; 184:1308–12.

17. Slater CL, Winogrzdzki I, Frade-Ribet PA, et al. Adding insult to injury: mecha-nistic basis for how AmpC mutations allow \textit{Pseudomonas aeruginosa} to accelerate cephalosporin hydrolysis and evade avibactam. \textit{Antimicrob Agents Chemother} \textbf{2020}; 64:e00894–20.

18. Haidar G, Philips NJ, Shields RK, et al. Ceftolozane-tazobactam for the treatment of multidrug-resistant \textit{Pseudomonas aeruginosa} infections: clinical effectiveness and evolution of resistance. \textit{Clin Infect Dis} \textbf{2017}; 65:110–20.

19. MacVane SH, Pandey R, Steed LL, et al. Emergence of ceftolozane-tazobactam-resistant \textit{Pseudomonas aeruginosa} during treatment is mediated by a single AmpC structural mutation. \textit{Antimicrob Agents Chemother} \textbf{2017}; 61:e01183-17.

20. Schalk JJ, Mislin GI, Brillet K. Structure, function and binding selectivity and stereoselectivity of siderophore-iron outer membrane transporters. \textit{Curr Top Membr} \textbf{2012}; 69:37–68.

21. Kim A, Kutschke A, Ehmann DE, et al. Pharmacodynamic profiling of a siderophore-conjugated monocarban in \textit{Pseudomonas aeruginosa}: assessing the risk for resistance and attenuated efficacy. \textit{Antimicrob Agents Chemother} \textbf{2015}; 59:7743–52.

22. McPherson CJ, Aschenbrenner LM, Lacey BM, et al. Clinically relevant gram-negative resistance mechanisms have no effect on the efficacy of MC-1, a novel siderophore-conjugated monocarban. \textit{Antimicrob Agents Chemother} \textbf{2012}; 56:6334–42.

23. van Delden C, Page MG, Köhler T. Involvement of Fe uptake systems and AmpC β-lactamase in susceptibility to the siderophore monosulfactan

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24. Berrazeg M, Jeannot K, Ntsogo Enguéné VY, et al. Mutations in β-lactamase AmpC increase resistance of *Pseudomonas aeruginosa* isolates to antipseudomonal cephalosporins. Antimicrob Agents Chemother 2015; 59:6248–55.

25. Fraile-Ribot PA, Zamorano L, Orellana R, et al. Activity of imipenem-relebactam against a large collection of *Pseudomonas aeruginosa* clinical isolates and isogenic beta-lactam-resistant mutants. Antimicrob Agents Chemother 2020; 64:e02165–19.

26. Barnes MD, Bethel CR, Alsop J, et al. Inactivation of the *Pseudomonas*-derived cephalosporinase-3 (PDC-3) by relebactam. Antimicrob Agents Chemother 2018; 62:e02406-17.

27. Livermore DM, Warner M, Mushtaq S. Activity of MK-7655 combined with imipenem against Enterobacteriaceae and *Pseudomonas aeruginosa*. J Antimicrob Chemother 2013; 68:2286–90.

28. Lapuebla A, Abdallah M, Olufisayo O, et al. Activity of imipenem with relebactam against gram-negative pathogens from New York City. Antimicrob Agents Chemother 2015; 59:5029–31.

29. Rubio AM, Kline EG, Jones CE, et al. In vitro susceptibility of multidrug-resistant *Pseudomonas aeruginosa* following treatment-emergent resistance to ceftriaxone-tazobactam. Antimicrob Agents Chemother 2021; 65:e00084-21.

30. Lahiri SD, Walkup GK, Whiteaker JD, et al. Selection and molecular characterization of ceftriaxone/tazobactam-resistant mutants in *Pseudomonas aeruginosa* strains containing derepressed AmpC. J Antimicrob Chemother 2015; 70:1650–8.

31. Malik S, Kaminski M, Landman D, Quale J. Cefiderocol resistance in *Acinetobacter baumannii*: roles of beta-lactamases, siderophore receptors, and penicillin binding protein 3. Antimicrob Agents Chemother 2020; 64:e01221-20.

32. McCreary EK, Heil EL, Tamma PD. New perspectives on antimicrobial agents: cefiderocol. Antimicrob Agents Chemother 2021; AAC.02171-20.