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MAJOR ARTICLE

An NDM-Producing *Escherichia coli* Clinical Isolate Exhibiting Resistance to Cefiderocol and the Combination of Ceftazidime-Avibactam and Aztreonam: Another Step Toward Pan-β-Lactam Resistance

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Background. Cefiderocol and ceftazidime-avibactam plus aztreonam (CZA-ATM) are preferred treatment regimens for New Delhi metallo-β-lactamase (NDM)-producing infections.

Methods. We report the case of a US patient who traveled to India to receive a renal transplant. He subsequently experienced pyelonephritis by an NDM-producing *Escherichia coli*. Broth microdilution and the broth disk elution method indicated resistance to all β-lactams, including cefiderocol and CZA-ATM. Whole-genome sequencing investigations were undertaken to identify resistance mechanisms.

Results. An *E. coli* isolate belonging to sequence type (ST) 167 containing a $bla_{\text{NDM-5}}$ gene was identified on a plasmid of the IncFIA/IncFIB/IncFIC replicon groups. When compared with the genome of another ST167 *E. coli* clinical isolate containing $bla_{\text{NDM-5}}$ and exhibiting susceptibility to cefiderocol and CZA-ATM, a 12–base pair insertion in *ftsI*, translating to a 4–amino acid duplication in PBP3, was identified. Moreover, a $bla_{\text{CMY-59}}$ gene was harbored on an IncI-γ replicon type, and frameshift mutations were identified in the *cirA* iron transport gene.

Conclusions. This is the first clinical case of a US patient harboring an NDM-producing isolate exhibiting resistance to all available β-lactam agents. The isolate's unexpected resistance to cefiderocol and CZA-ATM was likely due to a combination of (1) a modified PBP3 (increased MICs to both regimens), (2) truncated iron-binding protein (increased cefiderocol MIC), and (3) a bla_{CMY} gene (reduced CZA-ATM activity). *E. coli* ST167 clinical isolates harboring $bla_{\text{NDM-5}}$ genes are a recognized international high-risk clone. When coupled with the additional mechanisms identified in our patient's isolate, which is not uncommon for this high-risk clone, pan-β-lactam resistance may occur.

Keywords. PBP3; antimicrobial resistance; aztreonam; cefiderocol; ceftazidime-avibactam.

Enterobacterales isolates producing New Delhi metallo-β-lactamases (NDMs) were first described in a Swedish patient with a *Klebsiella pneumoniae* urinary tract infection hospitalized in India in late 2007 [1]. Within 3 years, bacteria harboring bla_{NDM} genes were identified in tap water and other environmental reservoirs in New Delhi, causing significant public health concerns [2]. They have since disseminated internationally and across bacterial genera [3, 4]. Of concern, NDM-producing infections are associated with mortality >30% [5–8].

Current NDM-active β-lactam treatment options are limited to cefiderocol or the combination of ceftazidime-avibactam plus aztreonam (CZA-ATM) [9]. Cefiderocol is a siderophore-conjugated cephalosporin with activity against ~80% of NDM-producing Enterobacterales [10–14].

CZA-ATM capitalizes on the combination's ability to evade the activity of NDM enzymes as well as several other β-lactamase enzymes commonly co-produced with NDMs (eg, extended-spectrum β-lactamases [ESBLs], AmpC β-lactamases, *Klebsiella pneumoniae* carbapenemases [KPCs], and OXA-48-like carbapenemases). More specifically, ATM is able to withstand hydrolysis from NDM enzymes. The β-lactamase inhibitor avibactam inactivates co-produced serine β-lactamases, enabling ATM to bypass hydrolysis from these enzymes and to safely reach its site of activity (ie, penicillin binding protein 3 [PBP3]) [15–17]. Susceptibility estimates of NDM-producing Enterobacterales to the combination of CZA-ATM are unclear given the heterogeneity of antimicrobial susceptibility testing (AST) methods to this combination of agents.

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PBP3, encoded by the *ftsI* gene, is also a site of action of cefiderocol [18]. It follows that PBP3 mutants have the ability to compromise the activity of cefiderocol and CZA-ATM through an incremental increase in their minimum inhibitory concentrations (MICs). Moreover, when PBP3 mutants are found in conjunction with specific serine or metallo-beta-lactamase genes or iron transport mutations, they can lead to frank resistance to these agents, resulting in no effective β-lactam treatment options against NDM-producing organisms [19–22].

Herein, we report the case of a patient from the United States who traveled to India to receive a renal transplant and 9 months later developed an NDM-5-producing *Escherichia coli* pyelonephritis exhibiting resistance to both cefiderocol and CZA-ATM. Using novel AST approaches and whole-genome sequencing (WGS), we describe the putative mechanisms that led to resistance to all available β-lactam agents. Furthermore, we review why this is likely not an isolated case and rather the harbinger for the next epidemic of resistance.

METHODS

Clinical Case Presentation

A 66-year-old male from the United States with diabetes and end-stage renal disease traveled to India in January 2022 to obtain a renal transplant from a living related donor. He was subsequently maintained on mycophenolate mofetil, tacrolimus, and prednisone. Between July and September 2022, he had 6 emergency department visits for cystitis. Urine cultures indicated the presence of *Escherichia coli* exhibiting resistance to all routinely tested β-lactam agents. For all of these encounters, his treatment consisted of CZA. Of note, neither carbapenemase testing nor CZA susceptibility testing was performed during these earlier encounters.

Within 48 hours of most recently discontinuing CZA (ie, his sixth treatment course with CZA), he presented to the Johns Hopkins Health System with a temperature of 101.2°F, rigors, and pain over his donor kidney site. He was initiated on CZA (2.5 g intravenously every 8 hours) in combination with ATM (2000 mg intravenously every 8 hours), both over 3 hours, given his previous health care exposures in India. Computed tomography imaging of his kidneys revealed perinephric stranding of his donor kidney suggestive of pyelonephritis. A urinalysis indicated a white blood cell count of 599 cells/mL, and a urine culture grew >100 000 CFU/mL of *E. coli*. AST results indicated that the isolate exhibited resistance to both cefiderocol and CZA-ATM. As no other viable options were available, he was continued on CZA-ATM in the event that in vitro resistance may not translate to clinical failure. Unfortunately, he experienced a relapse of his infection <3 weeks later.

The first *E. coli* isolate recovered from a urine culture after his renal transplant (isolate 1) and the most recent *E. coli* isolate that brought him to the Johns Hopkins Health System (isolate 7) were available for further microbiological analysis. Of note, the patient did not have an *E. coli* infection at any time before the collection of isolate 1. He had never received CZA, CZA-ATM, or cefiderocol before the time isolate 1 was collected.

Antimicrobial Susceptibility Testing

Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (Bruker Daltonics Inc., Billerica, MA, USA) was used for bacterial genus and species identification. Initial AST results were determined using the gram-negative Emerge panel on the BD Phoenix Automated System (Becton Dickinson Diagnostics, Sparks, MD, USA). AST was confirmed using lyophilized Sensititer broth microdilution (BMD) GN7F and MDRGNX2F panels (Thermo Fisher Scientific, Waltham, MA, USA). Additional antibiotic agents tested by manual methods included cefiderocol by the disk diffusion method (Hardy Diagnostics, Santa Maria, CA, USA) and colistin by the disk broth elution method [23]. Reference BMD was performed to determine the activity of 3 investigational antibiotics that are not currently available clinically (ie, cefepime-taniborbactam, cefepime-zidebactam, and meropenem-xeroborbactam). These 3 agents were specifically tested as preclinical studies indicate that they have a relatively high likelihood of activity against NDM-producing bacterial isolates [24]. For all AST studies, quality control organisms were prepared weekly or each day of testing, as appropriate. Clinical and Laboratory Standards Institute (CLSI) interpretive criteria were applied to determine susceptibility for most agents [25]; Food and Drug Administration (FDA) criteria were used for tigecycline as CLSI criteria are not available [26].

Disk Approximation Method for Ceftazidime-Avibactam and Aztreonam Activity

To determine if in vitro activity was present between CZA and ATM, a McFarland 0.5 standard was prepared from fresh cultured growth of the recovered *E. coli* and inoculated to form a lawn on cation-adjusted Mueller-Hinton agar plates (Becton Dickinson Diagnostics). Disks containing CZA (Hardy Diagnostics) and ATM (Becton Dickinson Diagnostics) were placed 20 mm apart from disk centers and incubated overnight at 37°C [27]. After overnight incubation, zones of clearance between the disks were assessed for enhanced activity with the combination of agents (expansion of the ATM zone diameter toward the CZA disk) or no enhanced activity (no expansion of the ATM zone) compared with the zones on the outer sides of disks. Of note, there is no standardized approach to performing the disk approximation method recognized by the CLSI.

Combination Ceftazidime-Avibactam and Aztreonam Broth Disk Elution Method

Since recognition of the role of CAZ-ATM in treating metallo-beta-lactamase-producing Enterobacterales infections, there has been no standardized approach to identify in vitro

Table 1. Antimicrobial Susceptibility Testing Results of an *Escherichia coli* Sequence Type 167 Isolate Producing NDM-5 With a 4–Amino Acid Insertion in PBP3, a Bla_{CMY-59} Gene, and a Truncated CirA Protein[a,b,c]

AMK	ATM	ATM-AVI	CAZ	CEF-TAN	CEF-ZID	CIP	CZA	CRO	CST	ERV	FDC	FEP	GEN
>32	64	16/4	>64	16/4	≤0.25/0.25	>2	>128/4	>64	≤0.5	≤0.5	>32	>64	>8
IMI	I-R	LVX	MER	MVB	MER-XER	MIN	OMC	PLZ	SXT	TGC	TOB	TZP	
>16	8/4	>8	>32	>16	≤0.25/8	2	16	>8	>2/28	≤1	>8	>64/4	

Abbreviations: AMK, amikacin; ATM, aztreonam; ATM-AVI, aztreonam-avibactam; CAZ, ceftazidime; CEF-TAN, cefepime-taniborbactam; CEF-ZID, cefepime-zidebactam; CIP; ciprofloxacin; CZA, ceftazidime-avibactam; CRO, ceftriaxone; CST, colistin; ERV, eravacycline; FDC, cefiderocol; FEP, cefepime; GEN, gentamicin; IMI, imipenem; I-R, imipenem-relebactam; LVX, levofloxacin; MER, meropenem; MVB, meropenem-vaborbactam; MER-XER, meropenem-xeruborbactam; MIN, minocycline; OMC, omadacycline; PLZ, plazomicin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline; TOB, tobramycin; TZP, piperacillin-tazobactam.

[a]All antibiotic minimum inhibitory concentrations (MICs) for isolate 1 and isolate 7 were within 1 doubling dilution.

[b]MICs for isolate 7 displayed in table.

[c]All values reported as μg/mL.

activity of this combination. However, a CLSI-led multicenter study revealed the CZA and ATM broth disk elution method to be a practical, reproducible, and accurate method to determine the in vitro activity of the combination [28]. This approach was approved in January 2023 by the CLSI as an acceptable approach to test susceptibility to CZA-ATM [29].

To summarize how this approach was used to test susceptibility to our patient's isolates, no disks, a 30-μg ATM disk (Becton Dickinson), a 30/20-μg CZA disk (Hardy Diagnostics), and both disks were added to each of four 5-mL tubes and incubated at 30–60 minutes at room temperature to allow elution of the antibiotics from the disks. The final antibiotic concentrations in the tubes were 6/4 μg/mL CAZ-AVI and 6 μg/mL ATM, individually or in combination. A 0.5 McFarland standard was prepared from a subculture of the *E. coli* clinical isolate, and 25 μL of the inoculum standard was added to each tube, vortexed vigorously to ensure adequate distribution, then incubated at 35°C ± 2°C under ambient air for 16–20 hours. After overnight incubation, tubes were assessed for turbidity (indicating not susceptible [ie, intermediate or resistant] to the tested agents). Quality control strains included *E. coli* ATCC 25922 (susceptible to all agents), *Klebsiella pneumoniae* BAA-1705 (not susceptible to ATM), *K. pneumoniae* BAA2146 (NDM producer not susceptible to ceftazidime or ATM), and *E. coli* AR348 (not susceptible to ceftazidime, ATM, or CZA-ATM).

Whole-Genome Sequencing and Analysis

Genomic DNA was extracted from the *E. coli* isolates using the PowerSoil Kit (QIAGEN, Inc., Valencia, CA, USA). WGS was conducted using both Illumina iSeq 100 i1 Reagent v2 (300-cycle) short-read sequencing (Illumina, San Diego, CA, USA) and long-read Nanopore (Oxford Nanopore Technologies, Oxford, UK), as previously described [30]. As further discussed in the results, because the antimicrobial resistance markers were identical in both isolates, WGS sequencing analysis focused on comparing the patient's isolate with the *E. coli* ATCC 25922 reference genome and an *E. coli* clinical isolate of the same sequence type (ST) from another patient containing a bla_{NDM-5} gene (exhibiting susceptibility to cefiderocol and CZA-ATM) [27]. Multiple sequence alignment was used to identify missense mutations resulting in changes to amino acid composition that may have contributed to elevated cefiderocol and CZA-ATM MICs. Efforts focused on examining for insertions, deletions, and frameshift mutations in *ftsI* (gene encoding PBP3). Sequencing results were also interrogated for known markers that would translate to relevant enzymatic changes impacting the MICs of these agents such as porin mutations (ie, OmpC, OmpF), the AcrAB-TolC multidrug-resistant efflux pump, other PBPs, and iron transport-related proteins (eg, BaeS, CirA, FecA, FepA, FhuA). Bioinformatics analyses were performed by Ares Genetics. Sequencing reads and de novo whole-genome assemblies were deposited to NCBI under BioProject PRJNA918544.

RESULTS

Antimicrobial Susceptibility Testing

AST results were identical for isolates 1 and 7 (Table 1). MICs for colistin and tigecycline were both ≤1 μg/mL. Because of concerns for nephrotoxicity with colistin in a kidney transplant recipient and the limited urinary penetration of tigecycline, these agents were disregarded as treatment agents. The zone of inhibition around the cefiderocol disk was 6 mm; resistance was defined as zone diameters of ≤8 mm [25].

The disk approximation method suggested enhanced activity with CZA-ATM based on visual inspection of the increased zone of inhibition around ATM in the presence of CZA (Figure 1). However, the broth disk elution method (repeated 3 times) demonstrated turbidity at the breakpoint, indicating resistance to this combination (Figure 2) [28]. Isolates were sent to the Maryland Health Department Laboratory for aztreonam-avibactam (ATM-AVI) MIC testing [31], and both isolates were identified as having an MIC of 16/4 μg/mL. ATM-AVI activity was extrapolated from the ATM CLSI breakpoints (≤4 μg/mL), as there are currently no CLSI or FDA interpretive criteria for ATM-AVI.

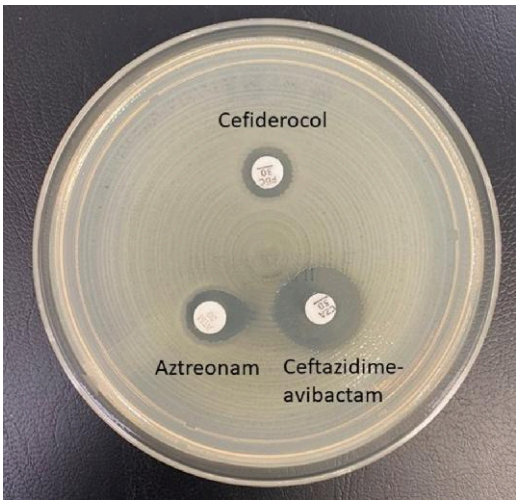

Figure 1. Disk approximation method evaluating for increased activity of ceftazidime-avibactam and aztreonam, compared to aztreonam alone.

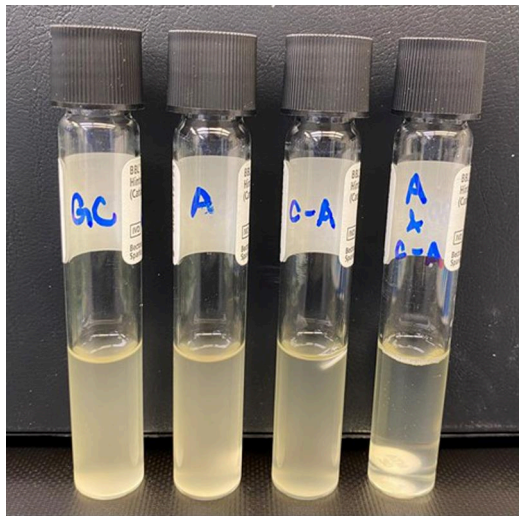

Figure 2. Broth disk elution results testing for susceptibility of (*A*) growth control in the absence of any disks, (*B*) aztreonam (30 µg), (*C*) ceftazidime-avibactam (30/20 µg), and (*D*) the combination of ceftazidime-avibactam (30/20 µg) and aztreonam (30 µg), inoculated with the patient's clinical *Escherichia coli* isolate, from left to right.

AST testing results for cefepime-taniborbactam, cefepime-zidebactam, and meropenem-xeruborbactam were as follows: 16/4 µg/mL, ≤0.25/0.25 µg/mL, and ≤0.25/8 µg/mL. Although neither CLSI nor FDA susceptibility criteria have been established for these 3 agents, the cefepime-zidebactam and meropenem-xeruborbactam MICs would presumably be within the susceptible range, based on current susceptibility criteria for cefepime and meropenem. The cefepime-taniborbactam MIC was 16/4 µg/mL. Although it is unknown if this MIC will be categorized as susceptible or not susceptible when breakpoints are established, the cefepime-taniborbactam MIC is in the resistant range for cefepime (ie, ≥16 µg/mL).

WGS Analysis

Illumina and Nanopore generated high-quality assemblies with at least 78× coverage. The *E. coli* isolate belonged to ST167 and contained a single copy of a $bla_{\text{NDM-5}}$ gene identified on a plasmid belonging to the IncFIA/IncFIB/IncFIC replicon groups. The insertion sequence IS*Aba*125 was upstream of the $bla_{\text{NDM-5}}$ gene.

The plasmid harboring the $bla_{\text{NDM-5}}$ gene possessed a series of other antimicrobial resistance genes including $bla_{\text{TEM-1}}$ (penicillins and early-generation cephalosporins), *dfA12* (trimethoprim), *aadA2* (aminoglycosides), *sul1* (sulfamethoxazole), *rmtB* (aminoglycosides), and *macB* (macrolides). A single copy of a $bla_{\text{CMY-59}}$ gene (expanded-spectrum cephalosporins) was separately harbored on an IncI-γ replicon type. When compared with the genome of another ST167 *E. coli* clinical isolate containing a $bla_{\text{NDM-5}}$ gene and not exhibiting resistance to cefiderocol and the combination of CZA-ATM [27], a 12–base pair insertion in *ftsI*, which translated to a 4–amino acid duplication in PBP3, was identified (ie, tyrosine-arginine-isoleucine-asparagine [YRIN]) at position 338. Frameshift mutations were identified in the *cirA* iron transport gene, leading to a truncated CirA protein.

DISCUSSION

We report the case of a patient who traveled to India to receive a renal transplant and became colonized with an NDM-producing *E. coli* isolate with a mutant PBP3 in conjunction with CirA deficiency and CMY production that exhibited resistance to both cefiderocol and CZA-ATM, leaving no viable treatment options for his urinary tract infection. We believe he became colonized with this isolate in India as his first post-transplant infection (isolate 1) had an identical resistance profile and identical antimicrobial resistance markers as his most recent infection (isolate 7), and he had no previous exposure to CZA, CZA-ATM, or cefiderocol before isolate 1, arguing against incremental accumulation of acquired resistance mechanisms from antibiotic exposure. Rather, he likely was colonized with a high-risk *E. coli* clone known to simultaneously harbor and retain multiple resistance markers as it spread across populations, as further described below. To our knowledge, this is the first clinical case of a patient in the United States harboring an NDM-producing isolate exhibiting resistance to all available β-lactam treatment regimens. The isolate's unexpected resistance to both cefiderocol and CZA-ATM was likely due to a combination of factors including (1) a modified PBP3 (increased MICs to both regimens), (2) truncated iron-binding protein (increased MIC to cefiderocol), and (3) a bla_{CMY} gene (increased MIC to CZA-ATM).

PBPs are responsible for cross-linking peptidoglycan, a critical component of the bacterial cell wall that imparts both bacterial structure and protection. ATM is highly specific to PBP3 but PBP3 also a target of other β-lactams such ceftazidime, cefepime, and cefiderocol [16, 32, 33]. PBP3 mutations generally raise MICs to all of these agents, but not to carbapenems, which predominantly target PBP1A, PBP1B, and PBP2 [34–36]. However, the combination of PBP3 mutations and bla_{NDM} genes has the potential to confer broad β-lactam resistance to all available β-lactams. PBP3 resistance is generally mediated through a 4–amino acid insertion due to a 12–base pair duplication (YRIN) or the same duplication with a single mismatch (YRIK/YRIP) after position 333 [20, 37]. These insertions impact the accessibility of various β-lactam agents to the transpeptidase pocket of PBP3 [37]. Resistance to ATM-AVI (and, by extension, CZA-ATM) is increasingly being reported in diverse STs of *E. coli* in Asia [38, 39] and Europe [40, 41] containing PBP3 mutations in conjunction with bla_{NDM} genes. Transformation studies indicate that PBP3 insertions identical to what was observed in our patient's isolate can lead to a 4-fold increase in cefiderocol MICs but may not cause frank resistance, suggesting that they likely have additive value in contributing to cefiderocol resistance [39].

A mutant CirA iron transport protein was likely also a factor in the cefiderocol resistance exhibited by our patient's isolate. It was anticipated that the redundancy of the TonB-dependent iron transport system would protect cefiderocol from frank resistance. Since its clinical use, however, reports have been conflicting on the association of isolated mutant iron transport on cefiderocol resistance [33, 39, 42–44]. In knockout experiments, deletion of the *cirA* gene in an ATCC 25922 *E. coli* isolate did not increase the MIC of cefiderocol [39]. However, others have demonstrated cefiderocol resistance in the setting of heterogenous CirA mutations, but generally in conjunction with NDM production [42–44]. The presence of a mutant CirA likely led to an incremental increase in cefiderocol MICs in our patient's isolate.

Finally, the presence of bla_{CMY} genes has been linked to elevated ATM-AVI MICs (and by extension CZA-ATM) [19–22]. Three *E. coli* isolates with 4 amino acid insertions in PBP3 recovered from hospital sewage in China were observed to carry a bla_{CMY-42} gene on an IncI-γ plasmid [20]. Cloning and plasmid-curing experiments indicated that CMY-42 production or amino acid insertions in PBP3 alone did not result in resistance to ATM-AVI, but when both were present, ATM-AVI MICs increased from 1/4 to 32/4 μg/mL. This is plausible as alterations in PBP3 reduce the amount of ATM reaching its target, making it vulnerable to hydrolysis from CMY enzymes. There has been some speculation that the combination of CZA-ATM may retain activity against NDM-producing isolates even in situations when the combination of ATM-AVI may not be active. First, the addition of ceftazidime (ie, CZA-ATM as opposed to ATM-AVI) increases the potential for targeting more PBPs, although PBP3 remains the primary site of action for ceftazidime [45]. Additionally, it is hypothesized that ceftazidime may serve as a "decoy" that is hydrolyzed by serine β-lactamases (eg, AmpC enzymes) traveling with NDM enzymes, enabling greater amounts of ATM to reach PBP3. However, CZA-ATM MICs are only ∼1 dilution lower compared with ATM-AVI MICs in the setting of PBP3 alterations and CMY enzymes, which falls within the accepted standard error of AST testing [20].

Regardless of the likely culprits of the pan-β-lactam resistance observed, this case is worrisome given that *E. coli* ST167 clinical isolates harboring bla_{NDM-5} genes are increasingly being recognized as an international high-risk clone [41, 42, 44, 46–50]. *E. coli* ST167 have been associated with unique virulence factors (eg, novel capsular synthesis gene clusters) [48]; the combination of resistance and virulence makes them ripe for global dissemination. Moreover, the bla_{NDM-5} gene has been identified on a variety of replicon types including IncF, IncX3, IncH, IncL/M, and IncA/B/C, enhancing the dissemination capacity of NDM-producing *E. coli* ST167 [47, 49]. The presence of PBP3 insertions, mutant CirA proteins, and expression of bla_{CMY} in NDM-5-producing isolates—which have been identified as a combination of resistance markers in *E. coli* ST167 isolates—almost ensures resistance to cefiderocol and CZA-ATM.

The increasing prevalence of NDM-producing Enterobacterales we will likely witness in upcoming years underpins the importance of accurate approaches to susceptibility testing of CZA-ATM. We used 3 different approaches in this case: the disk approximation method, the broth disk elution method, and ATM-AVI MIC testing. Even though the first approach has been most commonly employed, methods are not standardized, and the results are prone to subjective interpretations [27, 51–57]. In contrast, the broth disk elution method offers a practical, MIC-based approach for AST determination of CZA-ATM. Data evaluating 61 carbapenem-resistant Enterobacterales isolates from the CDC-FDA bank at 3 clinical microbiology laboratories indicated 98% categorical agreement of the broth disk elution method with BMD, a reference method for susceptibility testing, making this a promising approach for standardizing susceptibility testing of the CZA-ATM combination [28].

In summary, our patient's *E. coli* isolate was likely resistant to CZA-ATM and cefiderocol due to the presence of a PBP3 insertion in the background of NDM production, a truncated CirA protein, and production of a CMY-type β-lactamase. To our knowledge, this is the first reported case of an NDM-producing Enterobacterales clinical isolate in the United States exhibiting resistance to all available β-lactam agents. This case underscores the importance of a standardized and validated approach to susceptibility testing to CZA-ATM as the commonly used disk approximation method is prone to

misinterpretation. Three upcoming β-lactam-β-lactamase inhibitor combinations (ie, cefepime-taniborbactam, cefepime-zidebactam, and meropenem-xeruborbactam) with in vitro activity against NDM-producing isolates may be promising future treatment options for NDM-producing isolates. There are concerns, however, that at least 1 of these agents (ie, cefepime-taniborbactam) may not be able to remain active in the presence of antimicrobial resistance markers identified in high-risk ST167 *E. coli* clones [22].

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Patient consent. Informed written consent was provided by the patient.

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