Clinical Infectious Diseases

BRIEF REPORT

Monitoring Ceftazidime-Avibactam and Aztreonam Concentrations in the Treatment of a Bloodstream Infection Caused by a Multidrug-Resistant *Enterobacter* sp. Carrying Both *Klebsiella pneumoniae* Carbapenemase–4 and New Delhi Metallo-β-Lactamase–1

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(See the Editorial Commentary by Shields and Doi on pages 1099–101.)

In an infection with an *Enterobacter* sp. isolate producing *Klebsiella pneumoniae* Carbapenemase–4 and New Delhi Metallo-β-Lactamase–1 in the United States, recognition of the molecular basis of carbapenem resistance allowed for successful treatment by combining ceftazidime-avibactam and aztreonam. Antimicrobial synergy testing and therapeutic drug monitoring assessed treatment adequacy.

Keywords. ceftazidime-avibactam; aztreonam; carbapenemase; NDM; KPC.

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The spread of antimicrobial resistance among Gram-negative bacilli via mobile genetic elements is a major public health threat that persists despite advances in antimicrobial stewardship and infection control. Many resistance determinants are disseminated via plasmid-mediated transmission; among these, carbapenemase genes are some of the most concerning. Infections caused by carbapenemase-producing *Enterobacteriaceae* (CPE) represent a major therapeutic challenge to clinicians and are significantly associated with higher morbidity and mortality [1]. *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM) are 2 of the more frequently encountered carbapenemases worldwide. KPC was first reported in 1996 and represents the most prevalent carbapenemase in the United States [1, 2].

In contrast, NDM was initially reported in the United States by the Centers for Disease Control and Prevention in 2010 [3]. At present, rapid global dissemination of bla_{NDM} has occurred via mobile genetic elements with diverse replicon types. This spread represents a clinical challenge, as these plasmids frequently carry resistance determinants to other antibiotic classes, including aztreonam (ATM), aminoglycosides, and quinolones. ATM is the only commercially available monobactam that is not hydrolyzed by NDM or other metallo-β-lactamase (MBLs). However, *Enterobacteriaceae* that harbor bla_{NDM} often express either extended-spectrum β-lactamases, such as CTX-M-15, which hydrolyzes ATM, and carbapenemases like OXA-48. Of note, infections with an isolate that produces both KPC (which also hydrolyzes ATM) and NDM are rarely reported.

A potential treatment regimen for NDM-producing *Enterobacteriaceae* that also contain other serine β-lactamases is the combination of ATM with ceftazidime-avibactam (CAZ-AVI) [4]. This combination introduces a β-lactamase inhibitor (AVI) that "protects" ATM from hydrolysis by the broad range of extended-spectrum β-lactamases and AmpCs that frequently accompany NDM-producing strains [5]. Bacterial killing is likely achieved by the action of ATM, a monobactam that is not hydrolyzed by NDM [6].

Herein, we report our experience using CAZ-AVI with ATM to treat an infection caused by an *Enterobacter* isolate harboring both bla_{KPC} and bla_{NDM} in a 4-year-old child suffering from a hematological malignancy. Given the limited pharmacodynamic (PD) and pharmacokinetic (PK) information in acutely infected pediatric patients with NDM-producing organisms, antibiotic synergy testing and therapeutic drug monitoring (TDM) were conducted. *Enterobacter hormaechei* subsp. *hoffmannii* strain Eh1 was isolated from blood cultures of a 4-year-old boy whose past medical history is significant for B-cell precursor acute lymphoblastic

leukemia. Chemotherapy and transplant regimens are available in the Supplementary Methods.

At 3 days following stem cell infusion, the patient developed fever (39.3°C), diarrhea, and pruritus. A physical examination revealed a diaphoretic 20.5 kg child with an elevated heart rate and significant skin breakdowns in the right axilla and perianal region. Blood samples and perianal swabs were obtained for bacterial culture. Piperacillin-tazobactam and vancomycin were initiated empirically. Laboratory testing revealed leukopenia (0.1×10^9 per liter), severe neutropenia (absolute neutrophil count, 3 cells per μl), thrombocytopenia (14×10^9 per liter), and a creatinine clearance of 77.44 ml/min. Piperacillin-tazobactam was replaced by meropenem as the patient remained persistently febrile.

The isolate was identified by subculture and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as *E. hormaechei*. Antimicrobial susceptibility testing (AST) revealed resistance to all penicillins, cephalosporins, carbapenems, aminoglycosides, quinolones, and ATM (Table 1). Therapy with CAZ-AVI was initiated; subsequent additional testing revealed resistance to CAZ-AVI (minimal inhibitory concentration [MIC] > 256/4 μg/ml), meropenem-vaborbactam (MIC > 256/8 μg/ml), and plazomicin (MIC > 256 μg/ml). Using a Verigene BC GN microarray, KPC and NDM carbapenemases were identified; these results prompted the addition of ATM to CAZ-AVI [4]. Perianal swab cultures yielded *E. hormaechei* with the same resistance profile as the blood isolate. Synergy testing confirmed susceptibility to CAZ-AVI in the presence of ATM (Supplementary Figure 1S). TDM of postinfusion CAZ-AVI and ATM serum concentrations was performed to ensure sufficient levels of both antibiotics throughout the dosing interval [7–9]. CAZ-AVI was administered as a 50 mg/kg prolonged 3-hour infusion every 8 hours. The ATM dose was 50 mg/kg every 8 hours (Supplementary Methods). The patient achieved clinical and microbiologic cure, completing a 2-week course without relapse.

METHODS

The initial AST was performed using a disk diffusion assay, MicroScan (Beckman Coulter), and gradient diffusion strips (bioMerieux and Liofilchem) according to Clinical and Laboratory Standards Institute guidelines. A determination of antimicrobial resistance genes was established using the Verigene BC GN microarray (Luminex). Whole-genome sequencing (WGS) was conducted using Illumina and MinION platforms.

A checkerboard broth microdilution assay determined the CAZ-AVI and ATM MICs (Supplementary Figure 1S). The fractional inhibitory concentration (FIC) was calculated to evaluate the magnitude of synergy [8]. See the Supplementary Methods for TDM, WGS, plasmid replicon typing, conjugation, FIC, and polymerase chain reaction methods.

RESULTS

WGS and *in silico* multilocus sequence typing identified Eh1 as *E. hormaechei* subsp. *hoffmannii* ST78, an *Enterobacter cloacae* complex, high-risk international clone. Illumina and MinION Unicycler assemblies revealed a circular 4.85 Mbp chromosome and 4 circular plasmids. A 108 kb incompatibility (Inc) F-family plasmid, designated as pEM861_2, was subtyped as Y4:A-:B36 and harbored $bla_{\text{NDM-1,}}$ *rmtC*, and *sul1* genes (Supplementary Table 1S; Supplementary Figure 1S). A 160 kb IncF-family plasmid, designated as pEM861_1, contained a putative IncFIB replicon and carried $bla_{\text{KPC-4}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$, *sul1*, and several genes allowing for the enzymatic modification of other antimicrobial classes.

Escherichia coli J53 transconjugants were successfully obtained. Both IncFII and IncFIB amplicons were identified in transconjugant colonies. The presence of $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-4}}$ within transconjugant isolates was confirmed by multiplex polymerase chain reaction. AST of the *E. coli* J53 transconjugant carrying both carbapenemases demonstrated the same antimicrobial resistance profile as Eh1 (Table 1). Using checkerboard analyses (Supplementary Figure 1S), the calculated FIC for CAZ-AVI and ATM was 0.038. Free concentrations of CAZ and ATM were measured above 2 g/ml during the dosing interval. The AVI concentration was ≥2.5 μg/ml for approximately 50% of the dosing interval (Supplementary Table 2S; Supplementary Figure 2S).

DISCUSSION

A 4-year-old immunocompromised child who developed a bloodstream infection with a KPC-4– and NDM-1–producing multidrug-resistant *Enterobacter* isolate was successfully treated using a combination of CAZ-AVI and ATM. The activity of this drug combination was confirmed by synergy testing, and the adequacy of antibiotic treatment was assessed by TDM.

The acquisition of $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-4}}$ on transmissible plasmids represents a potential epidemiologic threat in the United States. This sentinel identification of KPC and NDM production by a single isolate warrants additional concern, as international travel was not documented.

Recent studies have demonstrated the synergistic in vitro and clinical activity of ATM and CAZ-AVI against MBL-producing organisms, including *Enterobacteriaceae, Pseudomonas*, and *Stenotrophomonas* [4, 10, 11]. A summary of previously reported *Enterobacteriaceae* isolates carrying both bla_{KPC} *and* $bla_{\text{NDM-1}}$ is provided in Supplementary Table 3S. *Enterobacter* spp. were notably involved in 6 out of 10 earlier reports. In all but 2 isolates, bla_{NDM} and bla_{KPC} plasmids were non-IncF replicon types. In some instances, bla_{KPC}- and bla_{NDM}-containing plasmids were not conjugative. Note that bla_{KPC} is typically carried on plasmids of various Inc groups, including IncFIB, which harbored $bla_{\text{KPC-4}}$ in our strain.

Table 1. Minimal Inhibitory Concentrations of Select Antibiotics Comparing Strain Eh1, *Escherichia coli* J53, and *Escherichia coli* J53 Transconjugants Carrying 1 or 2 Plasmids

	Eh1	*E. coli* J53	*E. coli* J53: pKPC4_EM861_1 and pNDM1_EM861_2	*E. coli* J53: pNDM1_EM861_2
Antibiotic	MIC, μg/ml	MIC, μg/ml	MIC, μg/ml	MIC, μg/ml
Ceftazidime	>128	<.5	>128	>128
Imipenem	>8	<.5	32	16
Meropenem	>32	<.5	>32	8
Ceftazidime-avibactam	>256/4	<.5/4	>256/4	>256/4
Ceftazidime-avibactam + aztreonam	2/4 + 2	<.5/4 + .5	2/4 + 2	1/4 + 1
Ceftazidime + aztreonam	>128 + 128	<.5 + .5	>128 + 128	.5 + .5
Ciprofloxacin	>2	…	…	…
Amikacin	>32	…	…	…
Gentamicin	>8	…	…	…
Tobramycin	>8	…	…	…
Plazomicin	>256	…	…	…
Colistin	.125	…	…	…
Cefepime	>16	…	…	…
Piperacillin-tazobactam	>64/4	…	…	…
Aztreonam	>16	…	…	…
Ertapenem	>4	…	…	…
Meropenem-vaborbactam	>256/8	…	…	…

Abbreviations: *E. coli*, *Escherichia coli*; MIC, minimal inhibitory concentration.

CAZ-AVI was approved for the treatment of CPE infections, including KPC-producing organisms. Clinical evidence has subsequently shown improved outcomes with CAZ-AVI, compared to colistin, in the treatment of KPC-producing *K. pneumoniae* [12]. However, NDM is not inhibited by AVI and hydrolyzes all β-lactams except for the monobactam ATM [5]. Unfortunately, plasmids that encode bla_{NDM-1} frequently harbor additional resistance determinants, including other β-lactamases that hydrolyze ATM.

TDM was introduced into the plan of care to maintain synergistic concentrations of the ATM and CAZ-AVI combination. A PK/PD target of 100% free time (fT) > MIC was achieved for CAZ (trough level 7.929 μg/ml) and ATM (trough level 13.492 μg/ml). Similarly, AVI concentrations exceeded 2.5 μg/ml for ~50% of the dosing interval (Supplementary Table 2S; Supplementary Figure 2S). Although a definitive PK/PD target for AVI in combination with CAZ and ATM has not yet been determined, analogous inferences can be extrapolated from dose fractionation studies of AVI with other antibiotics. The percent time that free concentrations exceed a threshold concentration value (%$ft > C_T$) is described as the critical PK/PD index, while the magnitude of the C_T threshold is dependent on the accompanying β-lactam [7–9]. Further studies are needed to determine the required PK/PD parameters for CAZ, ATM, and AVI when used in combination; however, the concentrations obtained in this patient appeared to be sufficient.

In summary, the Centers for Disease Control and Prevention has recognized carbapenem-resistant *Enterobacteriaceae* as an urgent national threat. Our report draws attention to an *Enterobacter* isolate carrying bla_{NDM-1} and bla_{KPC-4} on conjugative plasmids. Given the current lack of therapeutic options, the potential for horizontal transmission in the United States is worrisome from a public health perspective. Infections caused by CPE are associated with delays in prescribing effective antimicrobial therapy, poor outcomes, and higher mortality rates [1]. This case highlights that combining ATM and CAZ-AVI, supported by synergy testing and TDM, is a viable and successful strategy until newer antimicrobial agents are developed against NDM-producing organisms.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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