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SUSCEPTIBILITY

In Vitro Activity of Ceftazidime-Avibactam against Carbapenem-Resistant and Hypervirulent *Klebsiella pneumoniae* Isolates

Fangyou Yu,[a,b] Jingnan Lv,[b] Siqiang Niu,[c] Hong Du,[d] Yi-Wei Tang,[e,j] Robert A. Bonomo,[f,g,h,k,l] Barry N. Kreiswirth,[i] Liang Chen[i]

[a]Department of Clinical Laboratory, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai, China

[b]Department of Laboratory Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

[c]Department of Laboratory Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

[d]Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[e]Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, USA

[f]Case VA Center for Antimicrobial Resistance and Epidemiology (CARES), Case Western Reserve University, Cleveland, Ohio, USA

[g]Research Service, Louis Stokes Cleveland VA Medical Center, Cleveland, Ohio, USA

[h]Department of Medicine, Case Western Reserve University, Cleveland, Ohio, USA

[i]Public Health Research Institute Tuberculosis Center, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA

[j]Department of Pathology and Laboratory Medicine, Weill Medical College of Cornell University, New York, New York, USA

[k]Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio, USA

[l]Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio, USA

ABSTRACT Carbapenem-resistant and hypervirulent *Klebsiella pneumoniae* (CR-hvKp) strains have emerged while antimicrobial treatment options remain limited. Herein, we tested the *in vitro* activity of ceftazidime-avibactam and other comparator antibiotics against 65 CR-hvKp isolates. Ceftazidime-avibactam, colistin, and tigecycline are highly active *in vitro* against CR-hvKp isolates (MIC_{90}, $\leq$1 μg/ml), including *K. pneumoniae* carbapenemase 2 (KPC-2)-producing ST11 CR-hvKp. On the basis of previous clinical experience and the *in vitro* data presented herein, we posit that ceftazidime-avibactam is a therapeutic option against CR-hvKp infections.

KEYWORDS ceftazidime-avibactam, susceptibility, carbapenem resistance, hypervirulent *Klebsiella pneumoniae*

A singular population of carbapenem-resistant and hypervirulent *Klebsiella pneumoniae* (CR-hvKp) strains have been increasingly reported in recent years, predominantly from East Asia, and specifically, from China (1, 2). These strains are commonly hypervirulent and multidrug resistant, capable of causing serious infections in the hospitalized patients as well as in healthy individuals in the community. Infections caused by these strains are usually associated with high mortality, partially due to the limitation in effective antibiotic treatment options. A recent study from China reported a fatal outbreak caused by *K. pneumoniae* carbapenemase 2 (KPC-2)-producing epidemic ST11 *K. pneumoniae* strains with increased virulence, as a result of the acquisition of a pLVPK-like virulence plasmid (1). The emergence of these multidrug and hypervirulent organisms calls for the application of novel antibiotics that can efficiently

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Address correspondence to Liang Chen, Chen11@njms.rutgers.edu.

F.Y., J.L., and S.N. contributed equally to this work.

control these infections and lower the mortality, morbidity, and the overall burden of these spreading strains.

As a novel cephalosporin–β-lactamase inhibitor combination, ceftazidime-avibactam was approved by the United States Food and Drug Administration (U.S. FDA) for the treatment of complicated intraabdominal infections, complicated urinary tract infections, hospital-acquired bacterial pneumonia, and ventilator-associated bacterial pneumonia (www.fda.gov). The combination of ceftazidime and avibactam has broad spectrum activity against Ambler class A extended-spectrum β-lactamases (ESBLs) (e.g., TEM, SHV, and CTX-M types), KPC carbapenemases, class C (AmpC) and some class D (e.g., OXA-48 carbapenemase) β-lactamase producers, but not active against class B metallo-β-lactamases (3) and KPC enzymes with substitutions in the Ω-loop (e.g., D179Y substitution) (4, 5). Here, we determined the *in vitro* activity of ceftazidime-avibactam against CR-hvKp isolates to examine whether this novel β-lactam–β-lactamase inhibitor combination will be promising in treating infections caused by these life-threating pathogens.

Currently, a consensus phenotypic or genotypic definition of hvKp is not present, although clinically, this designation has been largely linked to a hypermucoviscosity phenotype (determined by string test) (6). Most hvKps have a common array of virulence genes, including mucoid phenotype regulator genes *rmpA* and *rmpA2*, and several siderophore-mediated iron acquisition operons (1, 7). Other studies showed that aerobactin (encoded by the *iucABCD-iutA* operon), but not yersiniabactin (encoded by the *ybt* operon), salmochelin (encoded by the *iroBCDN* operon), or enterobactin (encoded by the *ent* operon), was more likely associated with hypervirulence in *K. pneumoniae* strains (8). Recent studies showed that the hypermucoviscosity phenotype is insufficient in determining hypervirulence in *K. pneumoniae* (2), while a combination of phenotypic and genotypic characterizations may better microbiologically identify an hvKp strain. In this study, we define an hvKp strain on the basis of the demonstration of a positive string test (hypermucoviscosity) and coharboring of the genes *rmpA* (*rmpA* or *rmpA2*) and *iutA*. The string test was conducted as previously described (9) by stretching a bacterial colony on a 5% sheep blood agar plate, and the formation of a viscous string >5 mm in length was considered positive.

Sixty-five unique (one isolate per patient) CR-hvKp isolates, collected from two hospitals in Eastern China between 2014 and 2016, were included in this study. These isolates were collected from sputum (50.8% [33/65]), urine (16.9% [11/65]), blood (15.4% [10/65]), pus (6.2% [4/65]), ascites (4.6% [3/65]), and other sources (6.2% [4/65]). All 65 isolates were hypermucoviscous (string test, >5 mm) and were positive for *rmpA-rmpA2* and *iutA* by PCR analysis (1). Susceptibility testing was initially done by Vitek 2 Compact (bioMérieux) and interpreted using CLSI guidelines (10). These isolates ($n = 65$) were uniformly resistant to ertapenem, ceftazidime, ceftriaxone, cefepime, piperacillin-tazobactam, ciprofloxacin, and levofloxacin, 98.5% ($n = 64$) were resistant to imipenem, 96.9% ($n = 63$) to aztreonam, 81.5% ($n = 53$) to gentamicin, 69.2% ($n = 45$) to amikacin, and 66.2% ($n = 43$) to trimethoprim-sulfamethoxazole. Multilocus sequence typing (11) showed that 92.3% of the isolates belong to ST11 (60/65), and the remaining five isolates were ST268 ($n = 2$), ST65 ($n = 1$), ST412 ($n = 1$), and ST595 ($n = 1$). All 65 isolates harbored bla_{KPC-2}.

Among the 65 isolates, PCR and Sanger sequencing revealed that all except two isolates carried bla_{CTX-M} (59 $bla_{CTX-M-65}$, 3 $bla_{CTX-M-3}$, and 1 $bla_{CTX-M-14}$). SHV- or TEM-type ESBLs and AmpC genes were not identified. Sequencing of the outer membrane porin genes *ompK35* and *ompK36* (12) showed that all 60 ST11 strains contain a mutant OmpK35, with a premature stop codon at amino acid position 73, as well as a mutant OmpK36, due to the glycine and aspartic acid duplication at amino acid 134 (inserted GD amino acids [aa]134 and 135). The remaining five non-ST11 isolates carry wild-type OmpK35 and OmpK36. Among the 60 ST11 isolates, 34 have the capsular polysaccharide *wzi* allele 64 (*wzi64*) (corresponding with capsular type KL64), and the other 26 isolates have *wzi209* (corresponding with capsular KL47) (13). These two capsular types were previously described as two major ST11 clades in China (14). The original KPC-2

TABLE 1 *In vitro* susceptibility of different antibiotics against CR-hvKp isolates

Antimicrobial agent	Resistance (% [*n*])	MIC (μg/ml)		
		Range	50%	90%
All isolates ($n = 65$)				
Meropenem	65 (100)	4 to ≥256	128	≥256
Imipenem	64 (98.5)	2 to ≥256	128	≥256
Doripenem	64 (98.5)	1 to ≥256	64	128
Ertapenem	64 (98.5)	1 to ≥256	128	≥256
Aztreonam	63 (96.9)	8 to >64	>64	>64
Ceftazidime	65 (100)	16 to ≥256	128	≥256
Ceftazidime-avibactam	0 (0)	<0.125 to 1	1	1
Colistin	0 (0)	<0.125 to 1	0.25	0.5
Tigecycline	0 (0)	0.5 to 1	1	1
ST11 isolates ($n = 60$)				
Meropenem	60 (100)	16 to ≥256	≥256	≥256
Imipenem	60 (100)	16 to ≥256	≥256	≥256
Doripenem	60 (100)	16 to ≥256	64	128
Ertapenem	60 (100)	16 to ≥256	128	≥256
Aztreonam	60 (100)	32 to >64	>64	>64
Ceftazidime	60 (100)	16 to ≥256	≥256	≥256
Ceftazidime-avibactam	0 (0)	<0.125 to 1	1	1
Colistin	0 (0)	<0.125 to 1	0.25	0.5
Tigecycline	0 (0)	0.5 to 1	1	1

ST11 hvKp strains carried capsular type KL47 (*wzi209*) (1), while in this study, we found another capsular type KL64 (*wzi64*) in CR-hvKp ST11 strains, suggesting the plasmid-borne virulence genes have spread to another major clade in ST11. The five non-ST11 isolates carry *wzi72* (ST65, $n = 1$), *wzi95* (ST268, $n = 2$), *wzi206* (ST412, $n = 1$), and *wzi251* (ST595, $n = 1$), corresponding to capsular type K2, K20, K57, and K16, respectively.

In vitro susceptibility of 65 CR-hvKp isolates against ceftazidime-avibactam and some relevant comparator agents was evaluated by broth microdilution according to CLSI-approved methodologies (10). Avibactam was tested at a fixed concentration of 4 μg/ml. The ceftazidime-avibactam MIC of ≤8 μg/ml was regarded as susceptible, while an MIC of >8 μg/ml was defined as resistant (10). MICs were determined in duplicates on two separate days. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the quality control strains with each experiment (10). CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility breakpoints (for colistin and tigecycline) were used to determine susceptibility/resistance levels. The results are shown in Table 1.

In this study, the CR-hvKp isolates showed high-level resistance to four tested carbapenems, especially KPC-2-producing ST11 isolates (Table 1). The MIC_{50} values in ST11 isolates were ≥256, ≥256, 128, and 64 μg/ml for imipenem, meropenem, ertapenem, and doripenem, respectively. In addition, all isolates were resistant to ceftazidime, and 96.9% ($n = 63$) were resistant to aztreonam. Adding avibactam (4 μg/ml) significantly lowered the ceftazidime MICs (range, <0.125 to 1 μg/ml) more than 128-fold, rendering 100% activity of ceftazidime-avibactam against the CR-hvKp isolates. Importantly, the efficacy of avibactam added to ceftazidime was not affected by outer membrane permeability, and the MICs in ST11 OmpK35/36 mutant isolates were lowered more than 256-fold (range, <0.125 to 1 μg/ml). The results suggest that ceftazidime-avibactam may have potent *in vitro* activity against CR-hvKp isolates, especially for the KPC-2-procuding ST11 isolates. Two other "last-resort" antibiotics, colistin and tigecycline, showed comparable activities against CR-hvKp, with $MIC_{90}/_{50}$s of 0.5/0.25 μg/ml for colistin and 1/1 μg/ml for tigecycline, respectively.

To our knowledge, this is the first study to examine the activities of ceftazidime-avibactam against CR-hvKp clinical isolates. Our results showed that ceftazidime-avibactam, colistin, and tigecycline remain highly active against CR-hvKp, including the KPC-2-producing ST11 hvKp isolates, which are increasingly described in China (1).

However, given the rapid spread of *mcr-1* plasmids conferring colistin resistance, reported in China among animal and clinical *Enterobacteriaceae* isolates (15), the concern is that the treatment arsenal for CR-hvKp may be easily compromised. In addition, the original KPC-2 ST11 hvKp outbreak study showed that despite being *in vitro* susceptible to tigecycline and colistin, long-term treatment with colistin or tigecycline (alone or in combination with several other antibiotics) was not able to eradicate KPC-2-producing ST11 hvKp isolates, resulting in fatal outcomes (1). A recent prospective, multicenter observational study (Consortium on Resistance Against Carbapenems in Klebsiella and other *Enterobacteriaceae* [CRACKLE]) showed that compared with colistin, ceftazidime-avibactam treatment has a lower mortality of adjusted 30 days all-cause hospital mortality and may be a preferred alternative to colistin in the treatment of KPC-producing carbapenem-resistant *Enterobacteriaceae* infections (16). Similarly, another study showed that ceftazidime-avibactam treatment of carbapenem-resistant *K. pneumoniae* bacteremia was associated with higher rates of clinical success and survival than other regimens, including those for colistin (17). Taken together, our *in vitro* activity study suggested ceftazidime-avibactam may be a reasonable choice in the treatment of CR-hvKp isolates, particularly for the KPC-2-producing ST11 hvKp isolates. A further clinical evaluation of ceftazidime-avibactam treatment efficacy is warranted.

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