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ORIGINAL RESEARCH

In vitro Activity of Ceftazidime-Avibactam and Aztreonam-Avibactam Against Carbapenem-resistant *Enterobacteriaceae* Isolates Collected from Three Secondary Hospitals in Southwest China Between 2018 and 2019

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Chunhong Zou,[1,2,*] Jie Wei,[3,*] Baoju Shan,[4,5,*] Xian Chen,[6] Deqiang Wang,[2,7] Siqiang Niu[1]

[1]Department of Laboratory Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing, People's Republic of China; [2]College of Laboratory Medicine, Chongqing Medical University, Chongqing, People's Republic of China; [3]Department of Laboratory Medicine, West China Second University Hospital, Key Laboratory of Obstetric & Gynecologic and Pediatric Diseases and Birth Defects of Ministry of Education, Sichuan University, Chengdu, People's Republic of China; [4]Pediatric Research Institute; Ministry of Education Key Laboratory of Child Development and Disorders; National Clinical Research Center for Child Health and Disorders (Chongqing), China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Children's Hospital of Chongqing Medical University, Chongqing, People's Republic of China; [5]Chongqing Key Laboratory of Pediatrics, Children's Hospital of Chongqing Medical University, Chongqing, People's Republic of China; [6]Department of Clinical Laboratory, The Affiliated Hospital of Qingdao University, Qingdao, People's Republic of China; [7]The Key Laboratory of Molecular Biology of Infectious Diseases Designated by the Chinese Ministry of Education, Chongqing Medical University, Chongqing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Siqiang Niu
Department of Laboratory Medicine, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing 400016, People's Republic of China
Tel +86-23-8901 2742
Fax +86-23-8901 2513
Email siqiangniu@cqmu.edu.cn

Purpose: To assess the antimicrobial activities of ceftazidime/avibactam (CAZ/AVI) and aztreonam/avibactam (ATM/AVI) against carbapenem-resistant *Enterobacteriaceae* (CRE) isolates collected from three secondary hospitals in Southwest China between 2018 and 2019.

Materials and Methods: A total of 120 unique CRE clinical isolates were collected and carbapenemase genes were detected using PCR. Antimicrobial susceptibility was determined using standard broth microdilution method and the results were interpreted according to CLSI breakpoints.

Results: The 120 carbapenem-resistant strains included 92 *Klebsiella pneumoniae*, 10 *Escherichia coli*, 10 *Enterobacter cloacae*, five *Klebsiella aerogenes*, and three *Klebsiella oxytoca* isolates. Seventy-four percent of these 120 CRE isolates were collected from patients located in non-ICUs; 65.0% of these CRE isolates were collected from male patients; and 34.2% of these isolates were isolated from respiratory tracts. Four different carbapenemase genes were identified among 103 carbapenemase-producing *Enterobacteriaceae* (CPE) isolates, including $bla_{\text{KPC-2}}$ (n=77), $bla_{\text{NDM-1}}$ (n=16), $bla_{\text{NDM-5}}$ (n=12) and $bla_{\text{IMP-4}}$ (n=2). Overall, 21.7%, 37.5%, 40.8%, 75.0%, and 100% of the CRE strains were susceptible to levofloxacin, trimethoprim/sulfamethoxazole, amikacin, CAZ/AVI, and ATM/AVI, respectively. In addition, antimicrobial susceptibility testing showed that 96.7% isolates (n=116) were resistant to aztreonam, and the addition of avibactam (4 mg/L) significantly reduced the MICs of those aztreonam-resistant isolates by more than 128-fold (range: ≤0.125–4 mg/L), and 90.0% (n=108) of total 120 isolates were inhibited at ATM/AVI concentration ≤1 mg/L.

Conclusion: Our study revealed significant antimicrobial resistance among the CRE isolates against some commonly used antibiotics in three secondary Chinese hospitals. ATM/AVI exhibited potent activity against CRE isolates, including double carbapenemase-producing isolates, whereas CAZ/AVI was active against all KPC producers.

Keywords: carbapenem-resistant *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae*, ceftazidime/avibactam, aztreonam/avibactam

Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) infections have become a serious clinical concern and are associated with high morbidity and mortality, as they

greatly limit therapeutic options.[1,2] CRE are listed as an urgent threat by the CDC of US, and have been categorized in the critical and highest priority group of pathogens by the WHO.[3,4]

Carbapenem resistance in *Enterobacteriaceae* can be caused by different mechanisms, of which the most common are the production of carbapenemases and the outer membrane porin dysfunction with extended-spectrum β-lactamases (ESBLs) and/or hyperproduction of AmpC cephalosporinase.[5,6] Three major classes of carbapenemases are associated with the spread of carbapenem-resistant *Enterobacteriaceae*: Ambler class A (eg KPC), Ambler class B (eg, NDM, IMP and VIM), and Ambler class D (eg OXA-48-like) carbapenemases. KPC and OXA-48-like enzymes have serine-based hydrolytic activity, while metallo-β-lactamase (MBL) enzymes (encompass the Ambler class B enzymes) require the presence of metal for their activity.[5,6]

With the advent of new effective therapeutic options for CRE infections, such as the novel β-lactam/β-lactamase inhibitors ceftazidime/avibactam (CAZ/AVI) (active against AmpC, ESBL, KPC and OXA-48-like producers) and aztreonam/avibactam (ATM/AVI) (active against AmpC, ESBL, KPC, MBL, and OXA producers), the therapeutic recipe might be personalized based on the antimicrobial susceptibility profiles and molecular resistance phenotypes.[7–10]

The CAZ/AVI combination was approved by the US Food and Drug Administration in 2015 to treat complicated intra-abdominal infections, complicated urinary tract infections, hospital-acquired bacterial pneumonia, and ventilator-associated bacterial pneumonia.[11,12] This combination has broad-spectrum activity against CRE isolates.[13] However, this combination is not active against MBLs and KPC enzymes with substitutions (eg D179Y substitution).[14,15]

ATM is the only clinically used β-lactam stable to MBL hydrolysis, although it is easily inactivated by ESBLs, KPCs, and AmpC. As MBLs-carrying *Enterobacteriaceae* may frequently harbor additional ATM-inactivating β-lactamases, the activity of ATM against these isolates is often mitigated. However, the addition of avibactam to aztreonam make this combination active against KPC, AmpC, MBL, and OXA producers.[16,17] Consequently, ATM/AVI has been proposed to treat infections due to MBL producers.

Recent study showed that the carbapenem resistance rates and CRE species containing various carbapenemases varied in different geographical regions.[18] Currently, most studies on CRE were conducted in large tertiary hospitals in large cities in China, the antimicrobial resistance status in secondary or community hospitals remains rarely explored. In addition, several studies have described the in vitro activity of CAZ/AVI and ATM/AVI against clinical CRE isolates in large tertiary hospitals in China, however, their activities against CRE isolates from secondary hospitals were largely unknown. Here, we conducted a multicenter study in Southwest China to evaluate the antibacterial effect of CAZ/AVI and ATM/AVI on clinically isolated CRE bacteria collected from three secondary hospitals.

Materials and Methods

Bacterial Strains

A total of 120 nonduplicate clinical CRE isolates that were part of the routine hospital laboratory procedure causing infectious diseases were collected between 1 January 2018 and 31 December 2019 in three secondary hospitals in Chongqing, Southwest China. Species were identified using a VITEK® MS system (bioMérieux, France). In this study, CRE was defined as: resistant to any carbapenem antimicrobials (ie MICs of ≥2 μg/mL against ertapenem or ≥4 μg/mL against meropenem or imipenem.

Screening of Carbapenemase Genes

PCR was performed to screen the carbapenemase-encoding genes, including bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM} and bla_{OXA-48}-like. Positive PCR products were sequenced by Sanger sequencing (Sangon Biotech) and the sequences were blasted in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The primers used for detecting these carbapenemase genes have been reported previously.[18,19]

In vitro Antimicrobial Susceptibility Testing

The MICs of CRE strains were determined using standard broth microdilution method and were interpreted according to CLSI criteria.[20] For CAZ/AVI and ATM/AVI testing, AVI was tested at a fixed concentration of 4 mg/L, while CAZ and ATM was added at different concentrations, respectively. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality

control strains. MICs were determined in triplicate on two separate days.

Results

Bacterial Isolates and Patient Information

A total of 120 CRE isolates were collected from three secondary hospitals between 1 January 2018 and 31 December 2019 in Chongqing, Southwest China. The highest proportion of isolates was collected from Hospital A (40.8%) (Table 1). The majority of isolates were collected from patients located in non-ICUs (74.2%) and 65.0% of isolates were collected from male patients. In addition, most isolates were collected from adult patients (90.8%) (data not shown).

The CRE isolates were mainly isolated from respiratory tracts (n=41, 34.2%), urinary tracts (n=24, 20.0%) and blood culture (n=19, 15.8%). *K. pneumoniae* (n=92, 76.7%), *E. coli* (n=10, 8.3%) and *E. cloacae* (n=10, 8.3%) were the most common species. *K. pneumoniae and E. coli* isolates were mostly from respiratory tracts, while *E. cloacae* isolates were more frequently isolated from urinary tracts (data not shown).

Molecular Characteristics of CRE

Among the 120 CRE isolates, 85.8% (n=103) were CPE. Among the 103 CPE isolates, 99 (96.1%) were single carbapenemase-producers while four (3.9%) carried two carbapenemase genes. Notably, three *K. pneumoniae* strains were found to co-carry two carbapenemase genes (two with bla_{KPC-2} and bla_{NDM-1}, and one with bla_{NDM-1} and bla_{IMP-4}), whilst one *E. cloacae* isolate co-harbored the bla_{KPC-2} and bla_{NDM-1}. Among the carbapenemase genes detected, bla_{KPC-2} (n=77, 74.8%) was the most prevalent (Table 2). However, no isolates were found to harbo r bla_{VIM} or bla_{OXA-48}-like. There were some differences in the proportion of carbapenemase genes by hospital, and the above 4 carbapenemase genes were prevalent in Hospital A and Hospital B, while only bla_{KPC-2} was found in Hospital C (Table 2).

Table 2 Prevalence of Carbapenamase Genotype by Hospital

Gene	Total (n)	Hospital A (n)	Hospital B (n)	Hospital C (n)
bla_{KPC-2}	74	34	14	26
bla_{KPC-2}, bla_{NDM-1}	3	2	1	0
bla_{NDM-1}	12	4	8	0
bla_{NDM-1}, bla_{IMP-4}	1	1	0	0
bla_{IMP-4}	1	0	1	0
bla_{NDM-5}	12	8	4	0

Antimicrobial Susceptibility

These 120 CRE isolates were tested against clinically used antibiotics. The results showed that these isolates had high resistance rates against most common clinically used antibiotics tested (Table 3). According to CLSI criteria,[20] over 80% isolates were resistant to imipenem and meropenem, and over 60% isolates were resistant to gentamicin, ciprofloxacin, levofloxacin and sulfamethoxazole-trimethoprim, and the most active compounds were CAZ/AVI (75.0% susceptible) and ATM/AVI (100.0% susceptible). The addition of AVI significantly lowered the ceftazidime MICs (range: 0.125–4 mg/L) by more than 128-fold, rendering 100% activity of CAZ/AVI against all the KPC-producers. A total of 29 isolates were resistant to CAZ/AVI and they were all MBL-producers. Furthermore, addition of AVI significantly lowered the aztreonam MICs (range: ≤0.125–4 mg/L) by more than 128-fold against all the 120 CRE isolates tested.

Discussion

Carbapenem-resistant *Enterobacteriaceae* strains have become a serious global public health threat.[21] In China, the first strain harboring bla_{KPC} was reported in 2007, and

Table 1 Species Distribution Among CRE and CPE

Species	CRE	CPE by Hospital (n)			
		Total	Hospital A	Hospital B	Hospital C
K. pneumoniae	92	80	39	18	23
E. coli	10	8	4	4	0
E. cloacae	10	9	6	3	0
K. aerogenes	5	3	0	0	3
K. oxytosus	3	3	0	3	0
Total	120	103	49	28	26

Table 3 In vitro Susceptibility of Different Antibiotics Against 120 CRE Isolates

Antimicrobial Agent	Total n	Resistance n (%)	MIC (mg/L)		
			Range	MIC50	MIC90
Ceftazidime	120	120 (100.0)	16–≥128	≥128	≥128
Ceftazidime-avibactam	120	30 (25.0)	0.125–>256	1	256
Aztreonam	120	116 (96.7)	≤1–≥128	≥128	≥128
Aztreonam-avibactam	120	0 (0)	≤0.125–4	≤0.125	1
Ertapenem	120	120 (100.0)	4–512	256	512
Imipenem	120	103 (85.8)	0.5–512	64	256
Meropenem	120	98 (81.7)	0.25–256	64	128
Gentamicin	120	78 (65.0)	≤1–≥128	≥128	≥128
Amikacin	120	71 (59.2)	≤2–≥64	≥64	≥64
Levofloxacin	120	94 (78.3)	≤0.125–≥8	4	≥8
Ciprofloxacin	120	94 (78.3)	≤0.125–≥16	8	≥16
Sulfamethoxazole-trimethoprim	120	75 (62.5)	≤0.5–≥16	8	≥16

since then, bla_{KPC-2} has become the predominant carbapenemase gene and widely spread in China.[18] Similarly, since the first description of China NDM-1-producing strain in 2010, the bla_{NDM} gene has been recovered from various regions in China and been found in the various species of *Enterobacteriaceae*, including *E. coli, K. pneumoniae, E. cloacae, K. oxytoca, E. aerogenes*, and *C. freundii*.[18,19,22]

Avibactam is a non-β-lactam-β-lactamase inhibitor that inhibits the activities of Ambler class A -lactamases (eg, ESBLs, KPC), class C β-lactamases, and some class D β-lactamases, and avibactam restores the in vitro activity of both ceftazidime and aztreonam against KPC-producing *K. pneumoniae* isolates, including, in the case of ATM/AVI, activity against isolates that coproduced MBLs, and avibactam in combination with ceftazidime or aztreonam has the potential to be an agent to treat infections caused by multidrug-resistant bacteria producing the groups of β-lactamases compared with piperacillin-tazobactam and other agents.[13,16,17] In addition, our results are similar to those of studies previously reported for the action of avibactam.[16–18]

In this observational study, we investigated the molecular mechanism of the CRE isolates collected from three secondary Southwest Chinese hospitals, and further assessed the in vitro antimicrobial activities of CAZ/AVI and ATM/AVI against these CRE isolates, while most of the other studies focused on CRE isolates from tertiary hospitals in China.[18,19,22,23] Our study revealed three interesting findings.

First, the CRE isolates were detected from five different *Enterobacteriaceae* species. *K. pneumoniae* was the most common CRE species, accounting for 76.7% (92/120) of all CRE isolates. In addition, the CRE isolates were most isolated from respiratory tracts (n=41, 34.2%).

Second, diverse types of carbapenemases were identified, including KPC-2, NDM-1, NDM-5, and IMP-4. Of the 120 CRE isolates, 103 (85.8%) were found to produce carbapenemases. Among these 103 CPE strains, KPC-2 was the dominant carbapenemase (77/103, 74.8%) and was primarily found in *K. pneumoniae* (73/77, 94.8%). Notably, PCR failed to screen any carbapenemase genes among 17 out of 120 CRE strains, including 12 *K. pneumoniae*, two *E. coli*, two *K. aerogenes* and one *E. cloacae*, suggesting that other mechanisms may have contributed to the carbapenem resistance among these 17 isolates tested.

Third, this study demonstrated that CAZ/AVI exhibited potent activity against all the KPC producers tested in our study. Although some reports have documented cases of CAZ/AVI resistance to in KPC-2-producing *K. pneumoniae* isolates in China (with ESBL overexpression and OMP loss),[22] our study showed that all KPC producers were highly sensitive to CAZ/AVI. Meanwhile, all the studied CRE isolates, including the MBL producers, were highly sensitive to ATM/AVI in vitro.

Taken together, our study revealed significant antimicrobial resistance of the CRE isolates against the most commonly used antibiotics in three secondary Southwest Chinese hospitals. ATM/AVI exhibited potent activity against all CRE isolates, including MBL-producing isolates, whereas CAZ/AVI was active against all KPC producers. The clinical application of these new agents should be personalized and standardized to limit misuse and avoid the emergence of resistance.

Ethical Approval

Not required.

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Disclosure

The authors report no conflicts of interest in this work.

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