이학석사 학위논문

In vitro activity of ceftazidime-avibactam and aztreonam-avibactam against multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli*

다제내성 *Klebsiella pneumoniae*와 *Escherichia coli* 에 대한 ceftazidime-avibactam과 aztreonam-avibactam의 감수성 연구

울 산 대 학 교 대 학 원

의 과 학 과

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지 도 교 수　정 용 필

이 논문을 이학석사 학위 논문으로 제출함

2020년 2월

울 산 대 학 교　대 학 원

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이승철의 이학 석사학위 논문을 인준함

심사위원 이 상 오 (인)
심사위원 성 홍 섭 (인)
심사위원 정 용 필 (인)

울 산 대 학 교 대 학 원

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Abstract

Background: Increasing resistance to broad-spectrum cephalosporin and carbapenem in *Enterobacteriaceae* makes it challenging to select appropriate antibiotics. There are limited treatment options for these pathogens. Avibactam, new β-lactamase inhibitor, with other β-lactams, can overcome resistance due to various β-lactamases. My study aimed to evaluate *in vitro* activity of ceftazidime-avibactam and aztreonam-avibactam and their inoculum effect in multidrug-resistant (MDR) *Enterobacteriaceae* including carbapenem-resistant *Enterobacteriacae* (CRE). The study also assessed their *in vitro* activity according to resistance mechanism against β-lactam having these MDR pathogens.

Methods: A total of 228 non-repetitive, consecutive extended-spectrum β-lactam-resistant *Escherichia. coli* and *Klebsiella pneumoniae* blood isolates (120 and 108 isolates, respectively) were prospectively collected from blood cultures in Asan Medical Center from Jan 2017 and May 2018. To better define the inhibitory profile of study antibiotics, 81 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates (25 and 56 isolates, respectively) isolated from various clinical specimens were collected from Jan 2011 to Oct 2018 and separately tested. *In vitro* susceptibilities to ceftazidime, aztreonam, meropenem, ceftazidime-avibactam, aztreonam-avibactam, colistin, and tigecycline were evaluated by the broth microdilution reference method using standard and high inocula. Phenotypic determination of resistance mechanism to β-lactam and PCR for the detection of carbapenemase genes were performed in CRE.

Results: All 228 study blood isolates were resistant to cefotaxime; 26 (11%) were non-susceptible to carbapenem, and only three (1%) were carbapenemase-producing *Enterobacteriaceae* (CPE) in *K. pneumoniae*. Ceftazidime-avibactam and aztreonam-avibactam exhibited excellent *in vitro activity* against study blood isolates; MIC_{50}/MIC_{90} were 0.5/2 μg/mL and 0.125/0.5 μg/mL, respectively. Ninety-nine percent of blood isolates were susceptible (MIC≤ 8 μg/mL) to ceftazidime-avibactam, and when the aztreonam-avibactam-susceptible breakpoint of 8 μg/mL was applied, 99% of isolates were susceptible to aztreonam-avibactam. Ceftazidime-avibactam and aztreonam-avibactam were more active against *E. coli* than against *K. pneumoniae*. The positive rates of inoculum effect for ceftazidime-avibactam,

aztreonam-avibactam, and meropenem were 22%, 30%, and 38%, respectively. *K. pneumoniae* exhibited significantly higher rates of the inoculum effect on ceftazidime-avibactam, aztreonam-avibactam, and meropenem than *E. coli*. Ceftazidime-avibactam and aztreonam-avibactam showed relatively good susceptibilities in 81 CRE isolates; 73% of CRE isolates were susceptible to ceftazidime-avibactam, and 95% of isolates had aztreonam-avibactam MICs of ≤8 μg/mL. The resistance rate to tigecycline was high (75%), whereas that to colistin was 13%. When comparing non-carbapenemase producing (non-CP) CRE and CPE, ceftazidime-avibactam was more active against non-CP CRE (MIC_{50}/MIC_{90}, 2/16 μg/mL vs. 4/≥512 μg/mL), and aztreonam-avibactam is more active against CPE (MIC_{50}/MIC_{90}, 0.5/8 μg/mL vs. 0.25/1 μg/mL). The positive rates of inoculum effect for ceftazidime-avibactam and aztreonam-avibactam were 18% and 47%, respectively.

Conclusions: Ceftazidime-avibactam was a reasonable choice to overcome extended-spectrum β-lactam-resistant isolates; however, it had weak activity against CRE, especially against CPE. Aztreonam-avibactam was more active *in vitro* against extended-spectrum β-lactam-resistant and CRE isolates than ceftazidime-avibactam. However, due to its substantial inoculum effect in CRE, a possibility of aztreonam-avibactam treatment failure should be considered in the high inoculum infection.

(Keywords: tmultidrug-resistant enterobacteriaceae, ceftazidime-avibactam, aztreonam-avibactam, CRE)

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Introduction

Bacteremia caused by extended-spectrum β-lactam (third-generated cephalosporin)-resistant *Enterobacteriaceae* infections is a concern for global human health. Increasing resistance to broad-spectrum cephalosporins in *Enterobacteriaceae* makes it difficult to select appropriate antibiotics. There were new problems that carbapenem-resistant isolates appeared and spreading worldwide[1-3]. There are limited treatment options for these pathogens, and colistin is the most frequently used one. However, colistin is hard to be maintained, especially in critically ill patients due to its high renal toxicity[4]. For these reasons, there were some efforts to solve these problems by combining avibactam, new β-lactamase inhibitor, with other β-lactam antibiotics. Ceftazidime-avibactam, which targets extended-spectrum β-lactamase (ESBL)-producing or AmpC β-lactamase-producing strains and carbapenem-resistant *Enterobacteriaceae* (CRE), has been extensively studied[5-7]. Ceftazidime-avibactam was initially an effective antibiotic that can overcome ESBLs, AmpC β-lactamase (AmpC), *Klebsiella pneumoniae* producing carbapenemases (KPCs), and OXA-48, but multidrug-resistant (MDR) bacteria resistant to it gradually began to emerge; it is not active against metallo-β-lactamase (MBL) such as NDM-1[8].

Aztreonam, a monobactam, is a unique agent among currently used β-lactams, in that it is stable to hydrolysis by MBLs. However, aztreonam is easily inactivated by ESBLs, AmpC, and KPCs. *Enterobacteriaceae* carrying MBL also commonly carry these β-lactamases, that inactivate aztreonam, negating the activity of aztreonam against MBL. Over time, the susceptibility of *Enterobacteriaceae* to aztreonam has been reduced. When combined with avibactam, aztreonam can inhibit cell wall synthesis in MBL-producing bacteria, despite the presence of co-carried β-lactamases. Thus, aztreonam-avibactam can have an advantage over ceftazidime-avibactam in the treatment against MBL-producing strains. However, there are limited data on the susceptibility of aztreonam-avibactam to MDR pathogens or CRE. β-lactam antibiotics are known to have an inoculum effect against gram-negative bacteria with variable extents. The inoculum effect is a laboratory phenomenon described as a significant increase in the minimal inhibitory concentration (MIC) of an antibiotic when the number of

bacteria inoculated is increasing[9]. In clinical situations, treatment with an antibiotic with the inoculum effect can fail in a high bacterial burden infection such as an abscess.

My study aimed to evaluate *in vitro* activity of ceftazidime-avibactam and aztreonam-avibactam and their inoculum effect in MDR *Enterobacteriaceae* including CRE. The study also assessed their *in vitro* activity according to resistance mechanism against β-lactam having these MDR pathogens.

Materials and Methods

1. Bacterial isolates

A total of 228 non-repetitive, consecutive extended-spectrum β-lactam (third-generation cephalosporin)-resistant *Escherichia. coli* and *K. pneumoniae* blood isolates (120 and 108 isolates, respectively) were prospectively collected from blood cultures in Asan Medical Center, a 2700-bed, university-affiliated tertiary-care teaching hospital in the Republic of Korea from Jan 2017 and May 2018. To better define the inhibitory profile of study antibiotics, 81 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates (25 and 56 isolates, respectively) isolated from various clinical specimens were collected from Jan 2011 to Oct 2018 and separately tested. Species identification and initial antimicrobial susceptibilities were determined by the MicroScan Walk-Away plus System using Neg Combo Panel Type 72 (Dade Behring Inc., West Sacramento, CA). The isolates were classified into four groups according to the phenotype of their β-lactamases produced; 1) ESBL producer, 2) AmpC producer, 3) ESBL and AmpC coproducer 4) carbapenemase producer.

2. Antibiotic susceptibility test and resistance investigation

In vitro susceptibilities to ceftazidime, aztreonam, meropenem, ceftazidime-avibactam, aztreonam-avibactam, colistin, and tigecycline were evaluated in triplicate by the broth microdilution (BMD) reference method using standard inocula as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines[10]. Each strain stored at -80 ° C were streaked on an agar plate and incubated 24 hours before the experiment, and then placed the colony directly into the test tube and measured 0.5 McFarland standard. Ceftazidime, aztreonam, meropenem, tigecycline, and colistin were purchased from Sigma-Aldrich (St. Louis, MO, USA), and avibactam was purchased from Adooq Bioscience (Irvine, CA, USA). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as a reference strain. All results determined with these strains were within the CLSI quality control ranges. All BMD results except colistin and tigecycline were interpreted according to the standard criteria of the CLSI guideline, and those of colistin and tigecycline were interpreted according to the 2019

EUCAST susceptibility breakpoints. Antimicrobial ranges tested and expressed in μg/mL were as follows: ceftazidime (0.06–256), aztreonam (0.06–256), ceftazidime-avibactam (0.015/4–256/4), aztreonam-avibactam (0.015/4–256/4), meropenem (0.015–128), colistin (0.25–128), and tigecycline (0.03–256).

The isolates were confirmed for the presence of ESBLs by the MicroScan ESBL detection test (included Neg Combo Panel Type 72) using cefotaxime and ceftazidime alone and in combination with clavulanic acid. For the isolates in which the presence of ESBLs was not confirmed by the MicroScan ESBL detection test, further double-disk synergy test using cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg) and amoxicillin plus clavulanate (20 μg and 10 μg each) was performed[11, 12]. The isolates, non-susceptible to cefoxitin (MIC > 8 μg/mL), was considered a surrogate marker for the presence of high-level production of AmpC and were further characterized by the AmpC confirmatory test using cefoxitin and cloxacillin[13]. Cefepime, ceftazidime, cefotaxime, and amoxicillin-clavulanic acid disc were purchased from Bio-Rad (Hercules, CA, USA), and cefoxitin disc was commercially obtained from Oxoid (Basingstoke, UK). Modified carbapenem inactivation method (mCIM) was conducted when isolates were suspicious for carbapenemase production based on imipenem or meropenem MICs ≥2 μg/mL or ertapenem MIC ≥1 μg/mL (using 10 μg meropenem discs) according to the CLSI guidelines[14]. Genes for KPC, VIM, NDM, and OXA-48-like carbapenemases were sought by in-house multiplex PCR in all carbapenemase-producing isolates, which were identified by mCIM. The sequence of primers used for this study was as follows.

Table 1. PCR primers used for the detection of carbapenemase genes

Primer	Primer sequence	Product size (bp)	Reference
KPC forward	5'-ATGTCACTGTATCGCCGTCT-3'	893	Schechner *et al.* (2009)[15]
KPC reverse	5'-TTTTCAGAGCCTTACTGCCC-3'		
NDM-1 forward	5'-GAATGTCTGGCAGCACACTT-3'	480	Du *et al.* (2013)[16]
NDM-1 reverse	5'-TTGGCCTTGCTGTCCTTGAT-3'		
OXA-48 forward	5'-GCTTGATCGCCCTCGATT-3'	281	Dallenne *et al.* (2010)[17]
OXA-48 reverse	5'-GATTTGCTCCGTGGCCGAAA-3'		
VIM forward	5'-GATGGTGTTTGGTCGCATA-3'	390	
VIM reverse	5'-CGAATGCGCAGCACCAG-3'		

3. Determination of inoculum effect

To determine whether there was an inoculum effect, the MICs of each β-lactam were determined using high inocula (1×10^7 CFU/mL)[18]. An inoculum effect was defined as an eightfold or greater increase in the MIC when tested with the higher inoculum[19].

Results

1. Susceptibility of extended-spectrum β-lactam-resistant *E. coli* and *K. pneumoniae* blood isolates and inoculum effect

All 228 study blood isolates were resistant to cefotaxime by the MicroScan panel; 26 (11%) isolates were non-susceptible to carbapenem, and only three (1%) were carbapenemase-producing *Enterobacteriaceae* (CPE) in *K. pneumoniae*. Most isolates were resistant to ceftazidime and aztreonam (78% and 87%, respectively). However, ceftazidime-avibactam and aztreonam-avibactam exhibited good *in vitro activity* against study blood isolates; MIC_{50}/MIC_{90} were 0.5/2 μg/mL and 0.125/0.5 μg/mL, respectively (Table 2). Ninety-nine percent of isolates were susceptible (MIC≤ 8 μg/mL) to ceftazidime-avibactam, and when the aztreonam-avibactam-susceptible breakpoint of 8 μg/mL was applied, 99% of isolates were susceptible to aztreonam-avibactam. The MICs distributions of study antibiotics in each *E. coli* and *K. pneumoniae* are shown in Table 3 and 4. *K. pneumoniae* had higher MIC_{50}/MIC_{90} values of ceftazidime-avibactam and aztreonam-avibactam than *E. coli* (1/4 μg/mL and 0.12/1 μg/mL vs. 0.25/1 μg/mL and 0.12/0.25 μg/mL, respectively). Ceftazidime-avibactam and aztreonam-avibactam were more active against *E. coli* than against *K. pneumoniae*. Among 228 blood isolates, only two isolates (0.9%) in *K. pneumoniae* were resistant to ceftazidime-avibactam; one was CPE, and the other was non-carbapenemase-producing CRE (non-CP-CRE). In addition, two isolates in *K. pneumoniae* and one in *E. coli* had aztreonam/avibactam MICs of ≥16 μg/mL; all were non-CP-CRE.

At high inocula, MIC_{50} and MIC_{90} values of ceftazidime-avibactam increased from 0.5 to 1 μg/mL and from 2 to 8 μg/mL, respectively; those of aztreonam-avibactam, from 0.125 to 0.25 μg/mL and from 0.5 to 64 μg/mL, respectively; those of meropenem, from 0.03 to 0.125 μg/mL and from 0.25 to 16 μg/mL. Hence, 8% and 21% of isolates became resistant to ceftazidime-avibactam and meropenem, respectively, at high inocula; 15% of isolates were aztreonam-avibactam MICs of ≥16 μg/mL. The positive rates of inoculum effect for ceftazidime-avibactam, aztreonam-avibactam, and meropenem were 22%, 30%, and 38%, respectively. Table 5 shows differences in the inoculum effect between *E. coli* and *K. pneumoniae*. *K.*

pneumoniae exhibited significantly higher rates of the inoculum effect on ceftazidime-avibactam, aztreonam-avibactam, and meropenem than *E. coli*.

Table 2. Antimicrobial susceptibility of extended-spectrum β-lactam resistant *E. coli* and *K. pneumoniae* isolates to five antimicrobial agents (n=228)

Antimicrobial agent	Number of isolates (cumulative %) with indicated MICs (µg/mL)															MIC (µg/mL)[a]		S n(%)[b]
	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	MIC_{50}	MIC_{90}	
CAZ						5 (2.2)	11 (7.0)	12 (12.3)	21 (21.5)	22 (31.1)	34 (46.1)	21 (55.3)	22 (64.9)	24 (75.4)	56 (100)	64	≥512	28 (12.3)
CAZ-AVI	1 (0.4)		8 (3.9)	65 (32.4)	72 (64.0)	47 (84.6)	22 (94.3)	9 (98.2)	2 (99.1)	1 (99.6)			1 (100)			0.5	2	226 (99.1)
ATM					4 (1.8)		5 (4.0)	5 (6.2)	16 (13.2)	20 (21.9)	25 (32.9)	37 (49.1)	33 (63.6)	83[c] (100)		128	≥512	14 (6.2)
ATM-AVI	2 (0.9)	42 (19.3)	109 (67.1)	48 (88.2)	9 (92.1)	6 (94.7)	6 (97.4)	3 (98.7)		2 (99.6)	1 (100)					0.125	0.5	NA
MEM	143 (62.7)	49 (84.2)	12 (89.5)	10 (93.9)	5 (96.1)		1 (96.5)	2 (97.4)	2 (98.2)		1 (98.7)	3[c] (100)				0.03	0.25	219 (96.1)

S, susceptible; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; ATM, aztreonam; ATM-AVI, aztreonam-avibactam; MEM, meropenem

[a] 50 and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

[b] CLSI susceptibility breakpoints were used: ceftazidime, ≤4 µg/mL; ceftazidime-avibactam, ≤8/4 µg/mL; aztreonam, ≤4 µg/mL; meropenem, ≤1µg/mL; no breakpoint criteria have been defined for aztreonam-avibactam.

[c] MIC is greater than or equal to the indicated value.

Table 3. Antimicrobial susceptibility of extended-spectrum β-lactam resistant *E. coli* isolates to five antimicrobial agents (n=120)

Antimicrobial agent	Inoculum size	Number of isolates (cumulative %) with indicated MICs (μg/mL)															MIC (μg/mL)		S
		≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	MIC_{50}	MIC_{90}	n (%)
CAZ	Standard						5 (4.2)	11 (13.3)	5 (17.5)	18 (32.5)	17 (46.7)	28 (70.0)	14 (81.7)	15 (94.2)	3 (96.7)	4 (100)	32	128	21 (17.5)
	High						1 (0.8)	3 (3.3)	6 (8.3)	7 (14.2)	12 (24.2)	12 (34.2)	12 (44.2)	14 (55.8)	22 (74.2)	31 (100)	128	≥512	10 (8.3)
CAZ-AVI	Standard	1 (0.8)		7 (6.7)	56 (53.3)	42 (88.3)	12 (98.3)		1 (99.2)	1 (100)							0.25	1	120 (100)
	High			1 (0.8)	30 (25.8)	54 (70.8)	22 (89.2)	1 (90.0)		4 (93.3)	5 (97.5)	2 (99.2)				1 (100)	0.5	2	112 (93.3)
ATM	Standard					1 (0.8)		4 (4.2)	4 (7.5)	14 (19.2)	17 (33.3)	18 (48.3)	28 (71.7)	19 (87.5)	15[a] (100)		64	≥256	9 (7.5)
	High								1 (0.8)		4 (4.2)	7 (10.0)	7 (15.8)	23 (35.0)		78 (100)	≥512	≥512	1 (0.8)
ATM-AVI	Standard	1 (0.8)	26 (22.5)	67 (78.3)	21 (95.8)	1 (96.7)	1 (97.5)	1 (98.3)	1 (99.2)		1 (100)						0.12	0.25	NA
	High		13 (10.8)	63 (63.3)	25 (84.2)	3 (86.7)	2 (88.3)		1 (89.2)	1 (90.0)	2 (91.7)	1 (92.5)	3 (95.0)	2 (96.7)	1 (97.5)	3 (100)	0.12	8	NA
MEM	Standard	102 (85.0)	11 (94.2)	3 (96.7)	1 (97.5)	2 (99.2)			1 (100)								0.03	0.06	119 (99.2)
	High	25 (20.8)	64 (74.2)	13 (85.0)	2 (86.7)	8 (93.3)			5 (97.5)	2 (99.2)		1 (100)					0.06	0.5	112 (93.3)

[a] MIC is greater than or equal to the indicated value

Table 4. Antimicrobial susceptibility of extended-spectrum β-lactam-resistant *K. pneumoniae* isolates to five antimicrobial agents (n=108)

Antimicrobial agent	Inoculum size	Number of isolates (cumulative %) with indicated MICs (μg/mL)															MIC (μg/mL)		S n (%)
		≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	MIC_{50}	MIC_{90}	
CAZ	Standard								7 (6.5)	3 (9.3)	5 (13.9)	6 (19.4)	7 (25.9)	7 (32.4)	21 (51.9)	52 (100)	256	≥512	7 (6.5)
	High								1 (0.9)			2 (2.8)	1 (3.7)	2 (5.6)	4 (9.3)	98 (100)	≥512	≥512	1 (0.9)
CAZ-AVI	Standard			1 (0.9)	9 (9.3)	30 (37.0)	35 (69.4)	22 (89.8)	8 (97.2)	1 (98.1)	1 (99.1)			1 (100)			1	4	103 (98.1)
	High				5 (4.6)	19 (22.2)	17 (38.0)	26 (62.0)	20 (80.6)	11 (90.7)	4 (94.4)	2 (96.3)	2 (98.1)		2 (100)		2	8	98 (90.7)
ATM	Standard					3 (2.8)		1 (3.7)	1 (4.6)	2 (6.5)	3 (9.3)	7 (15.7)	9 (24.1)	14 (37.0)	68[a] (100)		≥512	≥512	5 (4.6)
	High						1 (0.9)									107 (100)	≥512	≥512	1 (0.9)
ATM-AVI	Standard	1 (0.9)	16 (15.7)	42 (54.6)	27 (79.6)	8 (87.0)	5 (91.7)	5 (96.3)	2 (98.1)		1 (99.1)	1 (100)					0.12	1	NA
	High		5 (4.6)	17 (20.4)	15 (34.3)	9 (42.6)	2 (44.4)	4 (48.1)	5 (52.8)	29 (79.6)	3 (82.4)	3 (85.2)	1 (86.1)	2 (88.0)	6 (93.5)	7 (100)	4	256	NA
MEM	Standard	41 (38.0)	38 (73.1)	9 (81.5)	9 (89.8)	3 (92.6)		1 (93.5)	1 (94.4)	2 (96.3)		1 (97.2)	3[a] (100)				0.06	0.5	100 (92.6)
	High	4 (3.7)	6 (9.3)	10 (18.5)	5 (23.1)	28 (49.1)	16 (63.9)	13 (75.9)	4 (79.6)		9 (88.0)	3 (90.7)	10[a] (100)				1	32	69 (63.9)

[a] MIC is greater than or equal to the indicated value

Table 5. Positive rates of inoculum effect for extended-spectrum β-lactam-resistant isolates

	Number of isolates (%) of positive inoculum effect[a]		
Isolate	Ceftazidime-avibactam	Aztreonam-avibactam	Meropenem
Total	51 (22.4)	68 (29.8)	84 (37.5)
E. coli	10 (8.3)	12 (10)	15 (12.5)
K. pneumoniae	22 (20.4)	56 (51.9)	69 (66.3)[b]

[a] Inoculum effect was defined as an eightfold or greater increase in MIC on testing with the higher inoculum

[b] Four isolates, which could not be evaluated because of off-scale MICs, were excluded.

2. Susceptibility of 81 CRE isolates isolated from various clinical specimens and inoculum effect

All 81 study isolates of *E. coli* and *K. pneumoniae* were resistant to ertapenem, imipenem, or meropenem by the Microscan panel; 85% of isolates were resistant to meropenem in BMD, and 43% were CPE. Ceftazidime-avibactam and aztreonam-avibactam showed relatively good susceptibility in CRE; 73% of CRE isolates were susceptible to ceftazidime-avibactam, and 95% of isolates had aztreonam-avibactam MICs of ≤8 μg/mL (Table 6). The resistance rate to tigecycline was high (75%), whereas that to colistin was 13%. Most of the tigecycline-resistant isolates and colistin-resistant isolates were *K. pneumoniae* (Table 7 and 8).

At high inocula, MIC_{50} of ceftazidime-avibactam increased from 4 to 8 μg/mL, and its MIC90 were ≥512 μg/mL; those of aztreonam-avibactam increased from 0. 5 to 4 μg/mL and from 4 to 256 μg/mL, respectively. Hence, 42% of CRE isolates became resistant to ceftazidime-avibactam, at high inocula; 44% of isolates exhibited aztreonam-avibactam MICs of ≥16 μg/mL. The positive rates of inoculum effect for ceftazidime-avibactam and aztreonam-avibactam were 18% and 47%, respectively (Table 9). *K. pneumoniae* exhibited significantly higher rates of the inoculum effect on ceftazidime-avibactam and aztreonam-avibactam than *E. coli*.

Table 6. Antimicrobial susceptibility of carbapenem-resistant *E. coli* and *K. pneumoniae* isolates to seven antimicrobial agents (n=81)

Antimicrobial agent	Number of isolates (cumulative %) with indicated MICs (μg/mL)														MIC (μg/mL)		S
	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	MIC_{50}	MIC_{90}	n (%)[a]
CAZ					1 (1.2)	1 (2.5)	1 (3.7)	1 (4.9)		2 (7.4)	1 (8.6)	10 (21.0)	17 (42.0)	47 (100)	≥512	≥512	3 (3.6)
CAZ-AVI				2 (2.5)	13 (18.5)	25 (49.4)	13 (65.4)	6 (72.8)	7 (81.5)	1 (82.7)				14 (100)	4	≥512	59 (72.8)
ATM					4 (4.9)	1 (6.2)		1 (7.4)			3 (11.1)	2 (13.6)	8 (23.5)	62 (100)	≥512	≥512	5 (6.2)
ATM-AVI	3 (3.7)	1 (4.9)	27 (38.3)	20 (63.0)	15 (81.5)	3 (85.2)	6 (92.6)	2 (95.1)	2 (97.5)	1 (98.8)		1 (100)			0.5	4	NA
MEM			3 (3.7)	3 (7.4)	2 (9.9)	4 (14.8)	4 (19.8)	12 (34.6)	15 (53.1)	11 (66.7)	12 (81.5)	4 (86.4)	11[b] (100)		16	≥256	8 (9.9)
CST			19 (23.5)	51 (86.4)			2 (88.9)	2 (91.4)	3 (95.1)			3 (98.8)	1[b] (100)		0.5	8	70 (86.4)
TGC		2 (2.5)	9 (13.6)	9 (24.7)	18 (46.9)	14 (64.2)	17 (85.2)	7 (93.8)	2 (96.3)	1 (97.5)	1 (98.8)		1 (100)		2	8	20 (24.7)

[a] CLSI susceptibility breakpoints were used: ceftazidime, ≤4 μg/mL; ceftazidime-avibactam, ≤8/4 μg/mL; aztreonam, ≤4 μg/mL; meropenem, ≤1μg/mL; no breakpoint criteria have been defined for aztreonam-avibactam. 2019 EUCAST susceptibility breakpoints were used for colistin and tigecycline: colistin, ≤2 μg/mL; tigecycline, ≤0.5 μg/mL.

[b] MIC is greater than or equal to the indicated value

Table 7. Antimicrobial susceptibility of carbapenem-resistant *E. coli* isolates to seven antimicrobial agents (n=25)

Antimicrobial agent	Inoculum size	Number of isolates (cumulative %) with indicated MICs (μg/mL)														MIC (μg/mL)		S
		0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	MIC_{50}	MIC_{90}	n (%)
CAZ	Standard						1 (4.0)				2 (12.0)		1 (16.0)	5 (36.0)	16 (64.0)	≥512	≥512	1 (4.0)
	High											1 (4.0)		3 (16.0)	21 (100)	≥512	≥512	0 (0.0)
CAZ-AVI	Standard					5 (20.0)	6 (44.0)	4 (60.0)	2 (68.0)	3 (80.0)					5 (100)	4	≥512	17 (68.0)
	High					4 (16.0)	4 (32.0)	3 (44.0)	5 (64.0)	2 (72.0)	1 (76.0)				6 (100)	8	≥512	16 (64.0)
ATM	Standard					1 (4.0)	1 (8.0)		1 (12.0)			3 (24.0)	1 (28.0)	2 (36.0)	16 (100)	≥512	≥512	2 (8.0)
	High					1 (4.0)	1 (8.0)					1 (12.0)		2 (20.0)	20 (100)	≥512	≥512	2 (8.0)
ATM-AVI	Standard	3 (12.0)		7 (40.0)	5 (60.0)	2 (68.0)	1 (72.0)	3 (84.0)	2 (92.0)		1 (96.0)		1 (100)			0.5	8	NA
	High		2 (8.0)	5 (28.0)	5 (48.0)	1 (52.0)	4 (68.0)	3 (80.0)	1 (84.0)		2 (92.0)			1 (96.0)	1 (100)	1	32	NA
MEM	Standard			1 (4.0)		2 (12.0)	2 (20.0)	1 (24.0)	8 (56.0)	5 (76.0)	2 (84.0)	2 (92.0)	1 (96)	1[a] (100)		8	64	3 (12.0)
	High				1 (4.0)	1 (8.0)	2 (16.0)	1 (20.0)	6 (44.0)	5 (64.0)	3 (76.0)	3 (88.0)		3[a] (100)		16	≥256	2 (8.0)
CST	Standard			11 (44.0)	13 (96.0))			1 (100)								0.5	0.5	24 (96.0)
TGC	Standard		2 (8.0)	9 (44.0)	7 (72.0)	3 (84.0)	1 (88.0)	1 (92.0)		1 (96.0)	1 (100)					0.5	4	18 (72.0)

[a] MIC is greater than or equal to the indicated value

Table 8. Antimicrobial susceptibility of carbapenem-resistant *K. pneumoniae* isolates to seven antimicrobial agents (n=56)

Antimicrobial agent	Inoculum size	Number of isolates (cumulative %) with indicated MICs (μg/mL)													MIC (μg/mL)		S
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	MIC_{50}	MIC_{90}	n (%)
CAZ	Standard				1 (1.8)		1 (3.6)	1 (5.4)			1 (7.2)	9 (23.3)	12 (44.7)	31 (100)	≥512	≥512	2 (3.6)
	High				1 (1.8)			1 (3.6)				1 (5.4)	1 (7.2)	52 (100)	≥512	≥512	1 (1.8)
CAZ-AVI	Standard			2 (3.6)	8 (17.9)	19 (51.8)	9 (67.9)	4 (75.0)	4 (82.1)	1 (83.9)				9 (100)	2	≥512	42 (75.0)
	High				2 (3.6)	7 (16.1)	14 (41.1)	8 (55.4)	5 (64.3)	1 (66.1)	1 (67.9)	5 (77.8)	4 (84.9)	9 (100)	8	≥512	31 (55.4)
ATM	Standard				3 (5.4)							1 (7.2)	6 (17.9)	46 (100)	≥512	≥512	3 (5.4)
	High				1 (1.8)				1 (3.6)			1 (5.4)	1 (7.2)	52 (100)	≥512	≥512	1 (1.8)
ATM-AVI	Standard	1 (1.8)	20 (37.5)	15 (64.3)	13 (87.5)	2 (91.1)	3 (96.5)		2 (100)						0.5	2	NA
	High		9 (16.1)	5 (25.0)	1 (26.8)	6 (37.5)	1 (39.3)	2 (42.9)	3 (48.2)	17 (78.6)	2 (82.1)	1 (83.9)	7 (96.4)	2 (100)	32	256	NA
MEM	Standard		2 (3.6)	3 (9.0)		2 (12.6)	3 (17.9)	4 (25.0)	10 (42.9)	9 (58.9)	10 (76.8)	3 (82.1)	10[a] (100)		32	≥256	5 (9.0)
	High			1 (1.8)		3 (7.2)	1 (9.0)	4 (16.1)	8 (30.4)	6 (41.1)	6 (51.8)	10 (69.6)	17[a] (100)		64	≥256	1 (1.8)
CST	Standard		8 (14.3)	38 (82.1)			1 (83.9)	2 (87.5)	3 (92.9)			3 (98.2)	1[a] (100)		0.5	16	46 (82.1)
TGC	Standard			2 (3.6)	15 (30.4)	13 (53.6)	16 (82.1)	7 (94.6)	1 (96.4)		1 (98.2)		1 (100)		2	8	2 (3.6)

[a] MIC is greater than or equal to the indicated value

Table 9. Positive rates of inoculum effect for carbapenem-resistant isolates

	Number of isolates (%) of positive inoculum effect[a]	
Isolate	Ceftazidime-avibactam	Aztreonam-avibactam
Total	12/67 (17.9)	38/81 (46.9)
E. coli	2/20 (10)	2/25 (8)
K. pneumoniae	10/47 (21.3)	36/56 (64.3)

[a] Inoculum effect was defined as an eightfold or greater increase in MIC on testing with the higher inoculum

3. Susceptibility and inoculum effect according to resistance type in 81 CRE isolates

The MIC distributions in CRE against ceftazidime-avibactam and aztreonam-avibactam according to resistance mechanism are shown in Table 10. Ceftazidime-avibactam was more active against non-CP CRE than against CPE (MIC_{50}/MIC_{90}, 2/16 μg/mL vs. 4/≥512 μg/mL). Although aztreonam-avibactam had good *in vitro* activity against CRE isolates, it is more active against CPE than against non-CP CRE (MIC_{50}/MIC_{90}, 0.25/2 μg/mL vs. 0.5/16 μg/mL). However, the positive rate of the inoculum effect for aztreonam-avibactam was high in both non-CP CRE and CPE (Table 11).

Table 10. Antimicrobial susceptibility of carbapenem-resistant *E. coli* and *K. pneumoniae* isolates according to resistance mechanism and inoculum

Mechanism (n)	Antimicrobial agent	Inoculum size	Cumulative % with indicated MICs (μg/mL)														MIC (μg/mL)	
			0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	MIC_{50}	MIC_{90}
Non-CP CRE (46)	CAZ-AVI	Standard				4.3	23.9	56.5	69.6	80.4	95.7	97.8				100	2	16
		High					10.9	23.9	45.7	58.7	71.7	76.1	78.3	89.1	95.7	100	8	256
	ATM-AVI	Standard	2.2	4.3	26.1	52.2	69.6	73.9	87.0	91.3	95.7	97.8		100			0.5	8
		High		2.2	13.0	23.9		37.0	43.5	45.7	50.0	78.3		80.4	93.5	100	16	256
ESBL (30)	CAZ-AVI	Standard				6.7	16.7	50.0	63.3	80.0	93.3	96.7				100	2	16
		High					10.0	23.3	33.3	53.3	66.7	73.3	76.7	90.0	93.3	100	8	128
	ATM-AVI	Standard	3.3	6.7	30.0	60.0	73.3		86.7	93.3	100						0.5	8
		High		3.3	16.7	30.0		33.3	40.0	43.3	50.0	73.3		76.7	93.3	100	16	256
AmpC (2)	CAZ-AVI	Standard					100										-	-
		High					50.0								100		-	-
	ATM-AVI	Standard				50.0	100										-	-
		High						50.0				100					-	-
ESBL +AmpC (7)	CAZ-AVI	Standard					28.6	85.7	100								2	4
		High						14.3	85.7					100			4	128
	ATM-AVI	Standard			14.3	42.9	71.4	100									1	2
		High						42.9				100					32	32
CPE (35)	CAZ-AVI	Standard					11.4	40.0	60.0	62.9						100	4	≥512
		High					2.9	17.1	37.1	57.1	60.0				62.9	100	8	≥512
	ATM-AVI	Standard	5.7		54.3	77.1	97.1	100									0.25	1
		High		2.9	28.6	42.9	48.6	60.0	62.9	68.6	71.4	88.6	94.3		100		2	64
KPC (17)	CAZ-AVI	Standard					11.8	58.8	82.4							100	2	≥512
		High						11.8	41.2	76.5					82.4	100	8	≥512
	ATM-AVI	Standard	5.9		58.8	82.4	100										0.25	1
		High			35.3	47.1	58.8	64.7		70.6		94.1			100		1	32
NDM-1 (11)	CAZ-AVI	Standard						9.1	18.2	27.3						100	≥512	≥512
		High						9.1	18.2		27.3					100	≥512	≥512
	ATM-AVI	Standard	9.1		54.5	63.6	90.9	100									0.25	1
		High		9.1	36.4	54.5		81.8					100				0.5	64

Table 11. Positive rates of inoculum effect for carbapenem-resistant isolates according to the resistance mechanism

	Number of isolates (%) of positive inoculum effect						
Antimicrobial agent	**Non-CP CRE**	ESBL	AmpC	ESBL +AmpC	**CPE**	KPC	NDM-1
CAZ-AVI	10/45 (22.2)	7/29 (24.1)	1/2 (50.0)	1/7 (14.2)	2/22 (9.1)	2/14 (14.3)	0/3
ATM-AVI	23/46 (50.0)	17/30 (56.7)	1/2 (50.0)	4/7 (57.1)	15/35 (42.9)	6/17 (35.3)	3/11 (27.3)

Discussion

This study tested *in vitro* activity of new antibiotics combining new β-lactamase inhibitor, avibactam with ceftazidime or aztreonam to overcome β-lactamase expressing extended-spectrum β-lactam resistant isolates and carbapenem-resistant isolates including CPE. Ceftazidime-avibactam and aztreonam-avibactam were more potent than ceftazidime or aztreonam similar to previous studies[20]. Most extended-spectrum β-lactam-resistant isolates and carbapenem-resistant isolates were susceptible to ceftazidime-avibactam (99%, 73% respectively). Meropenem was susceptible against 96% of extended-spectrum β-lactam resistant isolates. These results suggest that ceftazidime-avibactam can effectively inhibit extended-spectrum β-lactam-resistant isolates and that the antimicrobial activity of ceftazidime-avibactam is as effective as meropenem. However, ceftazidime-avibactam did not inhibit some carbapenem-resistant isolates, most of which were CPE isolates, including NDM-1 expressing isolates. Recent studies showed that avibactam protects β-lactams from hydrolysis by β-lactamases such as class A (e.g., KPCs), class C (e.g., CMY) and some class D (e.g., OXA-48) enzymes. In the present study, ceftazidime-avibactam did not inhibit class B metallo-β-lactamase such as NDM-1 (MIC: ≥512 μg/mL), similar to previous studies[8, 21].

The combination of aztreonam and avibactam presented a novel approach to the treatment of infections caused by pathogens containing various β-lactamases, including isolates carrying Metallo-β-lactamase. In this study, I used the provisional breakpoint of aztreonam-avibactam as 8 μg/mL because the breakpoint of aztreonam-avibactam has not been suggested by CLSI or EUCAST. The reason for setting the provisional breakpoint of aztreonam-avibactam to 8 μg/mL was that the ceftazidime-avibactam is administered at 2 g q8hr of ceftazidime component, resulting in higher breakpoint (8 μg/mL)than ceftazidime alone; breakpoint determined at 1 g q8hr. The breakpoint of aztreonam-avibactam could be set to 8 μg/mL, like ceftazidime-avibactam if aztreonam component is determined to be administered at 2 g q8hr. If the breakpoint of aztreonam-avibactam was considered to be 8 mg/mL, 99% of all extended-spectrum β-lactam resistant isolates were susceptible and 95% of carbapenem-resistant isolates were susceptible to aztreonam-avibactam. The MIC_{50}/MIC_{90} values of aztreonam-

avibactam in carbapenem-resistant the provisional was 0.5/4 μg/mL, which was similar to that of colistin (0.5/8 μg/mL). This result suggested that aztreonam-avibactam has higher antimicrobial activity than ceftazidime-avibactam against CRE and that it was comparable to colistin.

Previous studies have not studied much about the inoculum effect of avibactam combining antibiotics. In the present study, I observed changes in MICs of ceftazidime-avibactam and aztreonam-avibactam according to the inoculum size. The same definition of inoculum effect as previous studies was used in the study[19]. In the present study, *K. pneumoniae* had higher rates of inoculum effect than *E. coli*. The inoculum effect in *E. coli* was not significantly different between extended-spectrum β- lactam-resistant isolates and CRE isolates (8% vs. 10% in ceftazidime-avibactam; 10% vs. 8% in aztreonam-avibactam). However, in *K. pneumoniae*, aztreonam-avibactam had a higher rate of inoculum effect in CRE isolates (64%) than in extended-spectrum β- lactam-resistant isolates (52%). This data suggests that aztreonam-avibactam is more affected by inoculum size than ceftazidime-avibactam in *K. pneumoniae* (especially in CRE).

My study has several limitations. First, since clinical isolates were collected in a single tertiary center, they may not fully reflect *in vitro* susceptibilities to study antibiotics of MDR isolates in Korea. Second, other *Enterobacteriaceae* such as *Enterobacter* spp. and *Serratia* spp. can exhibit different susceptibility profiles and inoculum effect from *K. pneumoniae* and *E. coli*. Hence, multicenter studies including various *Enterobacteriaceae* species are needed to generalize our findings.

In conclusion, ceftazidime-avibactam was a reasonable choice to overcome extended-spectrum β-lactam-resistant isolates; however, it had weak activity against CRE, especially against CPE. Aztreonam-avibactam was more active *in vitro* against extended-spectrum β-lactam-resistant and CRE isolates than ceftazidime-avibactam. However, due to its substantial inoculum effect in CRE, a possibility of aztreonam-avibactam treatment failure should be considered in the high inoculum infection.

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국문요약

연구배경: 장내세균에서 광범위한 세팔로스포린계열 항생제나 카바페넴 계열 항생제에 대한 내성이 증가하고 있고, 이로 인해 적절한 항생제 선택이 어려워지고 있다. 이러한 문제들을 극복하기 위해 새로운 β-lactamase inhibitor 인 avibactam 을 다른 β-lactam 과 병합하여 사용함으로써 다양한 β-lactamase 로 인한 내성 문제를 해결할 수 있었다. 본 연구는 ceftazidime-avibactam 과 aztreonam-avibactam 의 *in vitro* 에서의 활성과 카바페넴계열 항생제 내성 장내세균(CRE)를 포함한 다제내성 장내세균에서의 inoculum effect 를 평가하는 것을 목표로 했다. 또한 ceftazidime-avibactam 과 aztreonam-avibactam 의 *in vitro* 에서의 활성도를 다제내성 장내세균이 보유중인 β-lactam 계열 항생제에 대한 내성 기전별로 구분해서 평가하였다.

실험 방법: 2011 년 1 월부터 2018 년 5 월까지 서울아산병원에 등록된 228 명의 중복되지 않은 균혈증 환자로부터 3 세대 세팔로스포린 내성인 120 개의 *Escherichia coli* 와 108 개의 *Klebsiella pneumoniae* 균주를 수집했고, 카바페넴 내성균에 대한 자료 확보를 위해 추가로 2011 년 1 월부터 2018 년 10 월까지 81 명의 환자로부터 혈액과 여려 임상 검체로부터 카바페넴 내성인 25 개의 *E. coli* 와 56 개의 *K. pneumoniae* 를 수집했다. 각 균주들로 ceftazidime, ceftazidime-avibactam, aztreonam, aztreonam-avibactam, meropenem, colistin, tigecycline 에 대해 broth microdilution (BMD) 검사를 시행하였고 inoculum effect 를 확인하기 위해 균접종량을 높인 BMD 검사도 시행하였다. 카바페넴 내성 균주들의 β-lactam 계열 항생제 내성 기전은 PCR 을 사용하여 검증하였다.

실험결과: 228개의 균혈증 균주들은 모두 cefotaxime에 내성이었다. 26개(11%)의 균주가 카바페넴에 비감수성이었고 *K. pneumoniae*에서 3개의 균주가 carbapenemase-producing *Enterobacteriaceae* (CPE)였다. 99%의 균혈증 균주는 ceftazidime-avibactam에 대해 감수성이었고, aztreonam-avibactam의 감수성 기준을 8 μg/mL로 설정할 경우 전체 균주의 99%가 감수성이었다. Ceftazidime-avibactam과 aztreonam-avibactam은 공통적으로 *K. pneumoniae*보다 *E. coli*에서 더 높은 활성도를 나타냈다. Ceftazidime-avibactam, aztreonam-avibactam, meropenem의 inoculum effect

양성 비율은 각각 22%, 30%, 38%였고, *K. pneumoniae*가 *E. coli*보다 ceftazidime-avibactam, aztreonam-avibactam, meropenem 모두에서 더 높은 접종원 효과 양성률을 보였다. Ceftazidime-avibactam과 aztreonam-avibactam은 81개의 CRE 균주에서도 좋은 효과를 보였다. CRE 균주 81개중 73%가 ceftazidime-avibactam에 대해 감수성을 나타냈고, aztreonam-avibactam의 감수성 기존을 8 μg/mL로 설정할 경우 95%가 감수성을 나타냈다. CRE 균주에 대한 tigecycline과 colistin의 내성률은 각각 75%와 13%였다. Carbapenemase를 생성하지 않는 CRE (non-CP CRE)와 carbapenemase를 생성하는 균(CPE)을 서로 비교할 경우, ceftazidime-avibactam은 CPE보다 non-CP CRE에서 더 높은 활성도를 보였고(MIC_{50}/MIC_{90}, 2/16μg/mL vs. 4/≥512 μg/mL), aztreonam-avibactam은 CPE에서 더 높은 활성도를 보였다(MIC_{50}/MIC_{90}, 0.5/8μg/mL vs. 0.25/1 μg/mL). CRE에서 ceftazidime-avibactam과 aztreonam-avibactam의 inoculum effect 양성률은 각각 18%와 47%였다.

결론: Ceftazidime-avibactam 은 3 세대 세팔로스포린 내성 균주 치료에 효과적인 항생제 선택이 될 수 있다. 하지만 CRE 균주에 대해서는 낮은 활성도를 보였고 특히 CPE 의 경우에는 더 낮은 경향을 보였다. Aztreonam-avibactam 의 경우 ceftazidime-avibactam 보다 CRE 포함하는 모든 균주에서 더 좋은 항균효과를 나타냈다. 하지만 aztreonam-avibactam 은 CRE 에서 상당한 inoculum effect 를 보이기 때문에 높은 균 부담이 있는 감염에서는 치료 실패의 가능성이 있어 신중한 선택이 필요하다.

(주요단어: 다제내성 장내세균, ceftazidime-avibactam, aztreonam-avibactam, 카바페넴 내성 장내세균)