In Vitro Activities and Inoculum Effects of eftazidime-Avibactam and Aztreonam-Avibactam against Carbapenem-Resistant Enterobacterales Isolates from South Korea

Taeun Kim 1, Seung Cheol Lee 2, Moonsuk Bae 2, Heungsup Sung 3, Mi-Na Kim 3, Jiwon Jung 2, Min Jae Kim 2, Sung-Han Kim 2, Sang-Oh Lee 2, Sang-Ho Choi 2, Yang Soo Kim 2, and Yong Pil Chong 2*

1 Division of Infectious Diseases, Department of Medicine, Nowon Eulji University Hospital, Seoul 01830, Korea; sleepju@naver.com
2 Department of Infectious Diseases, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, Korea; sclee628@naver.com (S.C.L.); carukeion@gmail.com (M.B.); trueblue27@naver.com (J.J.); nahani99@gmail.com (M.J.K.); kimsunghanmd@hotmail.com (S.-H.K.); solemd@amc.seoul.kr (S.-O.L.); sangho@amc.seoul.kr (S.-H.C.); yskim@amc.seoul.kr (Y.S.K.)
3 Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, Korea; sung@amc.seoul.kr (H.S.); mnkim@amc.seoul.kr (M.-N.K.)

* Correspondence: drchong@amc.seoul.kr; Tel.: +82-2-3010-3306; Fax: +82-3010-3306
**Supplemental Table S1.** Antimicrobial susceptibility of carbapenem-resistant *E. coli* and *K. pneumoniae* isolates to seven antimicrobial agents (n=81).

| Antimicrobial agent | Inoculum size | No. of isolates (cumulative %) with indicated MICs (μg/mL) | MIC (μg/mL) | S (n %) |
|---------------------|---------------|------------------------------------------------------------|-------------|---------|
|                     |               | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | ≥512 | MIC<sub>90</sub> | MIC<sub>100</sub> | |
| CAZ                 | Standard      | 1 | 1 | 1 | 1 | 2 | 1 | 10 | 17 | 47 | ≥512 | ≥512 | 3 | 
|                     | High          | 1 | (1.2) | (2.5) | (3.7) | (4.9) | (7.4) | (8.6) | (21.0) | (42.0) | (100) | ≥512 | ≥512 | (3.7) |
| CAZ-AVI             | Standard      | 2 | 1 | 2 | 3 | 6 | 13 | 16 | 7 | 14 | 4 | ≥512 | 59 | |
|                     | High          | 6 | (2.5) | (18.5) | (49.4) | (65.4) | (72.8) | (81.5) | (82.7) | (100) | 15 | 8 | ≥512 | (58.0) |
| ATM                 | Standard      | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 2 | 8 | 62 | ≥512 | ≥512 | 5 | (6.2) |
|                     | High          | 2 | (2.5) | (3.7) | (4.9) | (7.4) | (11.1) | (13.6) | (23.5) | (100) | 72 | ≥512 | ≥512 | 3 | (3.7) |
| ATM-AVI             | Standard      | 3 | (3.7) | (4.9) | (38.3) | (63.0) | (81.5) | (85.2) | (92.6) | (95.1) | (97.5) | (98.8) | (100) | 0.5 | 4 | NA<sup>b</sup> |
|                     | High          | 2 | (2.5) | (19.8) | (31.1) | (34.6) | (46.9) | (51.9) | (55.6) | (59.3) | (82.7) | (85.2) | (86.4) | (96.3) | (100) | 4 | 256 | NA<sup>b</sup> |
| MEM                 | Standard      | 3 | (3.7) | (7.4) | (9.9) | (14.8) | (19.8) | (34.6) | (53.1) | (66.7) | (81.5) | (86.4) | (100) | 16 | ≥256 | 8 | (9.9) |
|                     | High          | 2 | (2.5) | (3.7) | (9.9) | (12.3) | (24.7) | (40.7) | (51.9) | (63.0) | (75.3) | (100) | 32 | ≥256 | 3 | (3.7) |
| CST                 | Standard      | 19 | (23.5) | (86.4) | (88.9) | (91.4) | (95.1) | (98.8) | (100) | 0.5 | 8 | (86.4) |
|                     | High          | 2 | (2.5) | (13.6) | (24.7) | (46.9) | (64.2) | (85.2) | (93.8) | (96.3) | (97.5) | (98.8) | (100) | 2 | 8 | 20 | (24.7) |

MIC, minimum inhibitory concentration; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; ATM, aztreonam; ATM-AVI, aztreonam-avibactam; MEM, meropenem; CST, colistin; TGC, tigecycline; NA, not available.<sup>a</sup> CLSI susceptibility breakpoints were used: ceftazidime, ≤4 μg/mL; ceftazidime-avibactam, ≤8/4 μg/mL; aztreonam, ≤4 μg/mL; meropenem, ≤1μg/mL; 2019 EUCAST susceptibility breakpoints were used for colistin and tigecycline: colistin, ≤2 μg/mL; tigecycline, ≤0.5 μg/mL.<sup>b</sup> Not available because no breakpoint criteria have been defined for aztreonam-avibactam.<sup>c</sup> MIC is greater than or equal to the indicated value.
**Supplemental Table S2.** Ceftazidime-avibactam and aztreonam-avibactam MIC distribution according to meropenem, colistin and tigecycline susceptibility pattern in carbapenem-resistant isolates.

|                         | Susceptible (n = 70) | Resistant (n = 11) | \( P \) value | Susceptible (n = 20) | Resistant (n = 61) | \( P \) value |
|------------------------|----------------------|--------------------|---------------|----------------------|-------------------|---------------|
| **Ceftazidime-avibactam MIC** |                      |                     |               |                      |                   |               |
| S (≤8 µg/mL)           | 50 (71.4)            | 9 (81.8)           | .47           | 14 (70.0)            | 45 (73.8)         | .74           |
| R (>8 µg/mL)           | 20 (28.6)            | 2 (18.2)           | .50           | 6 (30.0)             | 16 (26.2)         | .99           |
| **Aztreonam-avibactam MIC** |                      |                     |               |                      |                   |               |
| Lower MIC (≤8 µg/mL)   | 67 (95.7)            | 10 (90.9)          | .50           | 19 (95.0)            | 58 (95.1)         | .99           |
| Higher MIC (>8 µg/mL)  | 3 (4.3)              | 1 (9.1)            | .37           | 1 (5.0)              | 3 (4.9)           |               |

Data are presented as the number of isolates with the corresponding percentage shown in parentheses. *Ceftazidime-avibactam CLSI breakpoints: S ≤8/4 µg/mL, R >8/4 µg/mL.* **Colistin EUCAST breakpoints: S ≤2 µg/mL, R >2 µg/mL.*** Tigecycline EUCAST breakpoints: S ≤0.5 µg/mL, R >0.5 µg/mL.
**Supplemental Table S3.** MIC distributions of ceftazidime-avibactam and aztreonam-avibactam for carbapenem-resistant *E. coli* and *K. pneumoniae* (n=81).

| AZT/AVI, MIC (µg/mL) | CAZ-AVI MIC (µg/mL) No. of isolates (no. of CPE) with indicated MIC | Grand total (No. of CPE) |
|----------------------|---------------------------------------------------|-------------------------|
|                      | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | >256 |                               |
| 0.06                 | 1   | 1 |   |   |   |   |   |   | 2   |     | 2    | 3 (2)                           |
| 0.125                | 1   | 0 |   |   |   |   |   |   |     | 2    |     | 1 (0)                          |
| 0.25                 | 1   | 8 | 9 | 6 | 1 |   |   |   | 8   | 8    | 27   | 17 (1)                         |
| 0.5                  | 3   | 9 | 6 | 1 |   |   |   |   |     | 1    |     | 20   | 8 (8)                           |
| 1                    | 1   | 4 | 5 | 4 | 1 | 2 |   |   |     | 2    | 15   | 7 (2)                          |
| 2                    | 1   | 0 | 1 |   |   |   |   |   |     | 3    |     | 3 (1)                          |
| 4                    | 1   | 1 | 3 | 0 | 1 |   |   |   |     | 6    |     | 0 (6)                          |
| 8                    | 1   |   |   |   | 1 |   |   |   |     | 2    |     | 0 (2)                          |
| 16                   |     |   |   |   |   | 1 |   |   |     | 2    |     | 0 (2)                          |
| 32                   |     |   |   |   |   |   |   |   | 1    |     |     | 1 (0)                          |
| 64                   |     |   |   |   |   |   |   |   |     |     | 1    |     |
| 128                  |     |   |   |   |   |   |   |   |     |     | 1    |     |
| Grand total (No. of CPE) | 2   | 13 | 25 | 13 | 6 | 1  |   |   | 7   | 1   | 14 | 13 (1) | 81 (81) |
Supplemental Table S4. Resistance mechanisms of carbapenem-resistant E. coli (n=25) and K. pneumoniae (n=56).

| Mechanism      | E. coli, n (%) | K. pneumoniae, n (%) | Total, n (%) |
|----------------|----------------|----------------------|--------------|
| Non-CP-CRE     | 18 (72.0)      | 28 (50.0)            | 46 (100)     |
| ESBL           | 13 (52.0)      | 17 (30.4)            | 30 (65.2)    |
| AmpC           | 1 (4.0)        | 1 (1.8)              | 2 (4.3)      |
| ESBL+AmpC      | 0              | 7 (12.5)             | 7 (15.2)     |
| Others         | 4 (16.0)       | 3 (5.4)              | 7 (15.2)     |

CP-CRE: 7 (28.0) 28 (50.0) 35 (100)
KPC: 2 (8.0) 15 (26.8) 17 (48.6)
NDM: 4 (16.0) 7 (12.5) 11 (31.4)
OXA-48-like: 1 (4.0) 3 (5.4) 4 (11.4)
VIM: 0 1 (1.8) 1 (2.9)
Others: 0 2 (3.6) 2 (5.7)

Data are presented as the number of isolates with the corresponding percentage shown in parentheses. Non-CP-CRE, non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae.
### Supplemental Table S5. Antimicrobial susceptibility and positive rate of inoculum effect of carbapenem-resistant isolates according to resistance mechanism.

| Species (n) | Mechanism (n) | Antimicrobial agent | Inoculum size | MIC (µg/mL) | S (%) | No. of isolates (%) with inoculum effect |
|-------------|---------------|---------------------|---------------|-------------|-------|------------------------------------------|
| *E. coli* (25) | Non-CRE (18) | CAZ-AVI | Standard | 2 | 16 | ≥512 | 13 (77.8) | 1/17 (5.9) |
| | CRE (18) | CAZ-AVI | High | 4 | ≥512 | 1 to ≥512 | 13 (72.2) | |
| | | ATM-AVI | Standard | 0.5 | 32 | 0.06 to 128 | NA | 1/18 (5.6) |
| | | | High | 0.5 | 256 | 0.125 to ≥512 | NA | |
| | ESBL (13) | CAZ-AVI | Standard | 2 | 16 | 1 to ≥512 | 11 (84.6) | 1/12 (8.3) |
| | | ATM-AVI | High | 8 | ≥512 | 1 to ≥512 | 10 (76.9) | |
| | | | Standard | 0.5 | 8 | 0.06 to 8 | NA | 1/13 (7.7) |
| | AmpC (1) | CAZ-AVI | High | - | - | 1 | 1 (100) | 0 |
| | | ATM-AVI | Standard | - | - | 0.5 | NA | 0 |
| | CRE (7) | CAZ-AVI | High | ≥512 | ≥512 | 1 to ≥512 | 3 (42.9) | 1/3 (33.3) |
| | | ATM-AVI | Standard | 0.25 | 2 | 0.06 to 2 | NA | 1/7 (14.3) |
| | | | High | 1 | 32 | 0.125 to 2 | NA | |
| | KPC (2) | CAZ-AVI | Standard | - | - | 1 to 4 | 2 (100) | 1/2 (50.0) |
| | | ATM-AVI | High | - | - | 4 to 8 | 2 (100) | |
| | NDM (4) | CAZ-AVI | Standard | ≥512 | ≥512 | ≥512 | 0 | ND |
| | | ATM-AVI | High | 0.25 | 2 | 0.06 to 2 | NA | 0 |
| *K. pneumoniae* (56) | Non-CRE (28) | CAZ-AVI | Standard | 2 | 16 | 0.5 to 32 | 23 (82.1) | 9/28 (32.1) |
| | | ATM-AVI | High | 8 | 256 | 1 to 256 | 14 (50.0) | 22/28 (78.6) |
| | ESBL (17) | CAZ-AVI | Standard | 4 | 16 | 0.5 to 32 | 13 (76.5) | 6/17 (35.3) |
| | | ATM-AVI | High | 16 | 128 | 1 to 256 | 6 (35.3) | |
| | AmpC (1) | CAZ-AVI | High | - | - | 1 | 1 (100) | 1/1 (100) |
| | | ATP-AVI | Standard | 0.5 | 1 | 256 | NA | |
| | | | High | - | - | 32 | NA | 1/1 (100) |
| | ESBL + AmpC (7) | CAZ-AVI | Standard | 2 | 4 | 1 to 4 | 7 (100) | 1/7 (14.3) |
| | | ATM-AVI | High | 4 | 128 | 2 to 128 | 6 (85.7) | |
| | CRE (28) | CAZ-AVI | Standard | 4 | ≥512 | 1 to ≥512 | 19 (67.9) | 1/19 (5.3) |
| | | ATM-AVI | High | 8 | ≥512 | 2 to ≥512 | 17 (60.7) | |
| | KPC (15) | CAZ-AVI | Standard | 2 | ≥512 | 2 to ≥512 | 12 (80.0) | 1/12 (8.3) |
| | | ATM-AVI | High | 8 | ≥512 | 8 to ≥512 | 11 (73.3) | |
| | NDM (7) | CAZ-AVI | Standard | 2 | ≥512 | ≥512 | 3 (42.9) | 0/3 |
| | | ATM-AVI | High | 0.5 | 32 | 0.25 to 256 | NA | |
| | | | Standard | 0.25 | 1 | 0.25 to 1 | NA | 3/7 (42.9) |

Non-CP-CRE, non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae; CAZ-AVI, ceftazidime-avibactam; ATM-AVI, aztreonam-avibactam, NA, not available; ND, not determined.\(^a\)

CLSI susceptibility breakpoints were used: ceftazidime, ≤4 µg/mL; ceftazidime-avibactam,
≤8/4 μg/mL; aztreonam, ≤4 μg/mL; meropenem, ≤1μg/mL; 2019 EUCAST susceptibility breakpoints were used for colistin and tigecycline: colistin, ≤2 μg/mL; tigecycline, ≤0.5 μg/mL.