No. 421

# *In vitro* and *in vivo* synergistic antimicrobial activities of the combinations of meropenem, colistin, tigecycline, and ceftolozane/tazobactam against carbapenem-resistant *Klebsiella pneumoniae*

Young Kyung Yoon*, Jeong Yeon Kim, Jin Woong Suh, Jang Wook Sohn
Institute of Emerging Infectious Diseases, Division of Infectious Diseases, Department of Internal Medicine, Korea University College of Medicine, Seoul, Republic of Korea

Young Kyung Yoon, M.D., Ph.D.

Division of Infectious Diseases, Department of Internal Medicine,
Korea University Anam Hospital, Korea University College of Medicine,
73, Inchon-ro, Seongbuk-gu, Seoul 02841, Republic of Korea
Tel: +82-2-920-5341, Fax: +82-2-920-5616
E-mail: young7912@korea.ac.kr

## BACKGROUND

➢ **Carbapenem-resistant *Klebsiella pneumoniae* (CRKp)**
- Increasingly public health issue due to the limited effectiveness of new antimicrobials or other factors such as treatment cost
- Spread of CRKp in a hospital environment makes infection control difficult
- Pathogen of critical priority in the global priority list of multidrug-resistant bacteria and has urged the development of new antibiotics provided by the WHO

➢ **Combination antimicrobial therapy**
- Alternative interim strategy for effectively managing CRAB infections
- Broadening the spectrum of activity, minimizing the development of antimicrobial resistance, and synergistically inactivating microorganisms

## Aims of Study

➢ The aim of this study was to evaluate the *in vitro* and *in vivo* activities of various antimicrobial combinations against carbapenem-resistant *Klebsiella pneumoniae* (CRKp).

## MATERIALS and METHODS

➢ **Study population**
❖ Clinical isolates of CRKp from nonduplicate patients with CRKp bacteremia
❖ A 1,048-bed tertiary care hospital in Seoul, Republic of Korea

➢ **Bacterial isolates and antimicrobial susceptibility testing**
❖ Identification and antimicrobial susceptibility testing of CRKp
1) MicroScan Pos Combo Panel Type 6 automated system (Baxter Diagnostics, West Sacramento, CA, USA)
2) Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany)
❖ Minimum inhibitory concentration (MIC)
: Broth microdilution method using Cation-adjusted Mueller–Hinton II broth (CA-MHB) (Becton Dickinson & Co., Sparks, MD, USA)
: Geometric twofold serial dilutions were performed according to the CLSI recommendations

➢ **Checkerboard assays for synergy testing**
❖ Panels of 96-well microtiter plates based on the MIC of each antibiotic, as determined using broth microdilution.
❖ Inoculum consisted of two-fold diluted 0.5 McFarland turbidity standard (100 μL) prepared using CA-MHB (final inoculum concentration: 5 × 105 CFU/mL in each well)
❖ Fractional inhibitory concentration index (FICI) = [(MIC of drug A in combination)/(MIC of drug A alone)] + [(MIC of drug B in combination)/(MIC of drug B alone)]
❖ Interpretation of the FICI was as follows: FICI ≤ 0.5, synergistic; 0.5 < FICI ≤ 1, additive; 1 < FICI ≤ 4, indifferent; and FICI > 4, antagonistic

➢ **Time-kill assay for synergy testing**
❖ 100-μL aliquots were obtained from each tube at 0, 2, 4, 8, 12, and 24 h of incubation and serially diluted in saline for the determination of viable counts.
❖ The bactericidal activity of single antibiotics or combinations was defined as a decrease of ≥ 3 log10 in 24 h compared with the number of viable cells at the initial time point.
❖ A synergistic effect was defined as a decrease of ≥ 2 log10 CFU/mL within 24 h when the antibiotics in combinations were compared with the most active individual drug at different time points.
❖ An increase of > 2 log10 was considered to indicate antagonism.
❖ Indifference was defined as any outcome that did not meet the criteria for either synergy or antagonism

➢ **Mouse model of CRKp infection**
❖ Injection of CRE (200 μL of $10X10^8$ CFUs/ml) in the center of the mouse abdomen with Insulin Syringe at a depth of 90° and 4 mm
❖ Antibiotics are injected 20 min after inoculation. Assuming the mouse's weight is 20 g, dilution of the corresponding amount of antibiotic in 200 μL of NS and injection of 50 μL at 30 min intervals 4 times in different areas
❖ Antibiotic combination for mouse model
: Combination with the most promising therapeutic effect in checkerboard assays and time-kill assays
: Meropenem + colistin, meropenem + ceftolozane/tazobactam, colistin + ceftolozane/tazobactam

| Dose (mg/kg) | MEM | C/T | RIF | CST |
|---|---|---|---|---|
| High | 20 | 100 | 10 | 40 |
| Middle | 10 | 50 | 5 | 20 |
| Low | 5 | 25 | 2.5 | 10 |

## RESULTS

**Table 1. Distributions of MICs (ug/mL) of CRKp. Gray color indicates resistance**

| CRKp | MEM | C/T | TZP | CAZ | FEP | FOF | ETP | CST | AMK | TGC | MIN | RIF | CIP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 16 | 64 | >256 | >128 | >128 | >256 | 64 | 128 | 2 | 1 | 32 | 128 | 128 |
| 27 | 16 | 64 | >256 | >128 | >128 | >256 | 64 | 128 | 2 | 1 | 32 | 64 | 128 |
| 28 | 4 | 128 | >256 | >128 | >128 | >256 | 32 | 4 | 0.125 | 0.25 | 2 | 128 | 32 |
| 29 | 128 | 128 | >256 | >128 | >128 | >256 | >128 | 8 | 2 | 2 | 32 | 128 | 128 |
| 30 | 8 | 32 | >256 | 64 | 128 | >256 | 64 | 16 | 2 | 1 | 16 | 128 | 64 |
| 32 | 8 | 128 | >256 | 128 | 64 | >256 | 16 | 1 | 2 | 1 | 16 | 128 | 64 |
| 39 | 16 | 64 | >256 | >128 | 128 | >256 | 32 | 1 | >128 | 2 | 32 | 128 | 64 |
| 41 | 8 | 32 | >256 | 32 | 16 | >256 | 64 | 16 | 1 | 0.5 | 8 | 128 | 0.125 |
| 42 | 16 | 32 | >256 | 64 | 128 | >256 | 64 | 16 | 1 | 8 | 128 | >128 | 128 |
| 44 | 64 | 64 | >256 | 64 | 128 | >256 | >128 | 16 | 1 | 0.5 | 16 | 128 | 8 |
| 47 | 4 | 64 | >256 | >128 | >128 | >256 | 128 | 2 | 2 | 8 | >128 | 128 | 128 |

➢ Abbreviation: AMK, Amikacin; CAZ, Ceftrazidime;CIP, Ciprofloxacin; CST, Colistin; C/T, Ceftolozane/Tazobactam
➢ Of 11 CRKP isolates, 10 carried the blaKPC-2-type carbapenem-hydrolyzing enzymes except 1 blaNDM-1-type.

**Table 2. Results of checkerboard assay for two-drug combinations against CRKp**

| CRKp | MEM + RIF | MEM + C/T | MEM + CST | MEM + TGC | CST + C/T | CST + TGC |
|---|---|---|---|---|---|---|
| 15 | ADD. | SYN. | IND. | ADD. | ADD. | ADD. |
| 27 | SYN. | SYN. | IND. | ADD. | SYN. | SYN. |
| 28 | ADD. | IND. | SYN. | ADD. | ADD. | IND. |
| 29 | ADD. | ADD. | SYN. | IND. | ADD. | ADD. |
| 30 | IND. | SYN. | SYN. | IND. | ADD. | ADD. |
| 32 | SYN. | SYN. | ADD. | SYN. | ADD. | SYN. |
| 39 | SYN. | SYN. | SYN. | ADD. | ADD. | SYN. |
| 41 | SYN. | ADD. | SYN. | ADD. | SYN. | SYN. |
| 42 | SYN. | SYN. | ADD. | ADD. | ADD. | ADD. |
| 44 | SYN. | ADD. | SYN. | ADD. | ADD. | IND. |
| 47 | SYN. | SYN. | IND. | ADD. | SYN. | SYN. |
| **Summary** | | | | | | |
| SYN. | 7 | 7 | 6 | 1 | 3 | 5 |
| ADD. | 3 | 3 | 2 | 8 | 8 | 4 |
| IND. | 1 | 1 | 3 | 2 | 0 | 2 |
| ANT. | 0 | 0 | 0 | 0 | 0 | 0 |

➢ Checkerboard assay showed that *in vitro* synergistic activity (∑FICI ≤ 0.5) against CRKp isolates was the highest for the meropenem-ceftolozane/tazobactam combination (63.6%), the meropenem-rifampin (63.6%), followed by meropenem-colistin (54.5%), and colistin-tigecycline (45.5%).

**Table 3. Results of checkerboard assay for two-drug combinations against CRKp**

| CRKp (X 1.0) | MEM + RIF | MEM + C/T | MEM + CST | MEM + TGC | CST + C/T | CST + TGC |
|---|---|---|---|---|---|---|
| 15 | SYN. | IND. | SYN./B. | IND. | SYN./B. | IND. |
| 29 | SYN. | IND. | SYN./B. | IND. | SYN. | SYN. |
| 30 | SYN. | SYN. | SYN./B. | IND. | SYN./B. | SYN. |
| 39 | SYN./B. | SYN./B. | SYN./B. | IND. | SYN./B. | IND. |
| 44 | SYN./B. | SYN./B. | SYN./B. | IND. | SYN./B. | SYN./B. |
| 47 | SYN./B. | SYN./B. | SYN./B. | IND. | SYN./B. | SYN./B. |

➢ For the time-kill assay (X 1.0) performed using an antibiotic concentration of 1 × MIC, *in vitro* synergistic and bactericidal effects against the 6 CRKp isolates were most frequently observed for the meropenem-colistin (40% and 100%, respectively), colistin-ceftolozane/tazobactam (100% and 83%, respectively), and meropenem-rifampin (100% and 50%, respectively), and meropenem-ceftolozane/tazobactam (67% and 50%, respectively).

| CRKp (X 0.5) | MEM + RIF | MEM + C/T | MEM + CST | MEM + TGC | CST + C/T | CST + TGC |
|---|---|---|---|---|---|---|
| 15 | SYN. | IND. | SYN./B. | IND. | SYN./B. | IND. |
| 29 | SYN. | IND. | SYN./B. | IND. | SYN. | SYN. |
| 30 | SYN. | SYN. | SYN./B. | IND. | SYN./B. | SYN. |
| 39 | SYN./B. | SYN./B. | SYN./B. | IND. | SYN./B. | IND. |
| 44 | SYN./B. | SYN./B. | SYN./B. | IND. | SYN./B. | SYN./B. |
| 47 | SYN./B. | SYN./B. | SYN./B. | IND. | SYN./B. | SYN./B. |

**Table 4. Survival rates for single agents in mouse model of CRKp infection**

| Single-agents (%) | DD | MEM | C/T | RIF | CST |
|---|---|---|---|---|---|
| High | 100% | 100% | 20% | 100% | 100% |
| Middle | 100% | 60% | 0% | 100% | 80% |
| Low | 40% | 80% | 20% | 60% | 60% |

**Table 5-1. Survival rates for antibiotic combinations in mouse model of CRKp infection according to the dose**

| Survival rates (%) | 0H | 12H | 24H | 36H | 48H |
|---|---|---|---|---|---|
| CRE control | 100 | 60 | 40 | 20 | 20 |
| CST+C/T H-H | 100 | 100 | 100 | 100 | 100 |
| CST+C/T H-L | 100 | 100 | 100 | 80 | 80 |
| CST+C/T L-H | 100 | 100 | 100 | 100 | 100 |
| CST+C/T L-L | 100 | 100 | 100 | 80 | 80 |
| CRE control | 100 | 80 | 60 | 40 | 40 |
| MEM+C/T H-H | 100 | 100 | 100 | 100 | 100 |
| MEM+C/T H-L | 100 | 100 | 100 | 100 | 100 |
| MEM+C/T L-H | 100 | 100 | 80 | 80 | 80 |
| MEM+C/T L-L | 100 | 100 | 100 | 100 | 100 |
| CRE control | 100 | 80 | 60 | 40 | 40 |
| MEM+CST H-H | 100 | 100 | 100 | 100 | 100 |
| MEM+CST H-L | 100 | 100 | 100 | 100 | 100 |
| MEM+CST L-H | 100 | 100 | 100 | 100 | 100 |
| MEM+CST L-L | 100 | 100 | 100 | 100 | 100 |

**Table 5-2. Summary of survival rates for antibiotic combinations in mouse model of CRKp infection according to the dose**

| Combination (%) | MEM + C/T | MEM + CST | CST + C/T |
|---|---|---|---|
| H - H | 100% | 100% | 100% |
| H - L | 100% | 100% | 80% |
| L - H | 80% | 100% | 100% |
| L - L | 100% | 100% | 80% |

## Conclusions

❖ **In conclusion, the present *in vivo* study demonstrated that the low-dose combination of colistin with other antibiotics may be a promising alternative to nephrotoxic high-dose colistin alone for treating CRKp infections.**
❖ **However, it was difficult to predict this possibility in *in vitro* tests, and even there is great discordance of antimicrobial synergistic activities between the checkerboard microdilution and time-kill assays.**