Triple combination of Tigecycline-Colistin-Meropenem Against Carbapenemase Producing *Klebsiella pneumoniae* Isolates in an *In Vitro* PK-PD Model

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INTRODUCTION AND PURPOSE

In absence of effective therapeutic approaches against carbapenamase producing *Klebsiella pneumoniae* (CP-Kp) isolates, combination therapy is often used with the hope to increase antibacterial activity. Tigecycline has a broad spectrum of *in vitro* activity against a variety of tetracycline-susceptible and tetracycline-resistant bacterial strains. Meropenem possesses some activity against CP-Kp and colistin is often used as the last resort to combat CP-Kp caused infections.

We therefore assessed the triple and double combination of standard dosing regimens of tigecycline, colistin and meropenem against CP-Kp isolates in an *in vitro* PK-PD model.

METHODS

***In vitro* PK-PD model.** One wild-type (Tzan59) and 2 VIM producing CP-Kp isolates Sec2 and Sec4 with CLSI MICs 0.125, 1, and 2 mg/l for tigecycline, 0.5, 0.25 and 0.5 mg/l for colistin and 0.06, 16 and 256 mg/l for meropenem, respectively were used at 10^7CFU/ml in an in vitro PK-PD model against the double and triple combination of tigecycline, colistin and meropemen. Another 4 CP-Kp isolates 1780, 1781, 1782 and EUG with various resistance mechanisms (1 KPC+VIM, 1 NDM, 1 KPC and 1 KPC+VIM+SHV-5) and tigecycline/colistin/meropenem MICs of 0.5/4/64, 4/32/512, 1/2/256, 1/2/128 mg/l, respectively were tested using a two compartment PK-PD dialysis/diffusion closed model **(Figure 1)**.

Pharmacokinetics. The human plasma concentration-time profiles of 100mg q12 tigecycline, 4.5MU q12 colistin and 1g q8 of meropenem dosing regimens were simulated for 48h targeting tigecycline, colistin and meropenem free serum maximum concentrations of 0.3, 1.6 and 60 mg/l with half-lives 14h, 12h and <2h, respectively **(Figure 2)**.

The *K. pneumoniae* clinical isolates were inoculated in the internal compartment and drugs were injected in both compartments and incubated at 37°C on a magnetic stirrer for 48h.

Drug levels were determined with microbiological agar diffusion assays. Bacterial growth was assessed by quantitative cultures estimating the $\log_{10}$CFU/ml.

Figure 1. *In vitro* PK-PD dialysis/diffusion closed model.

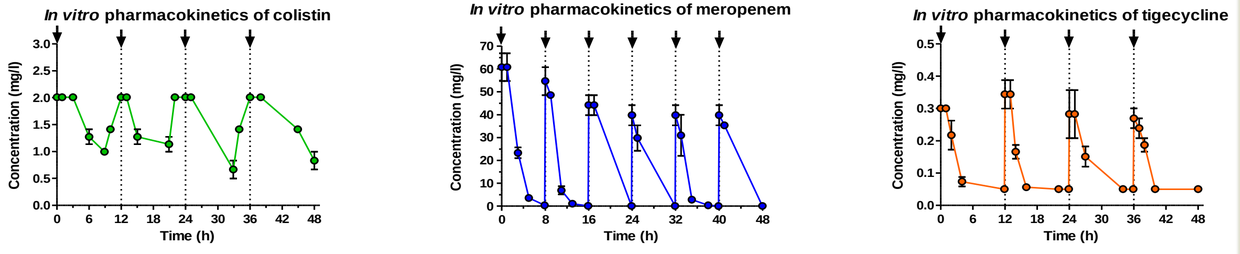

Figure 2. *In vitro* pharmacokinetics of colistin, meropenem and tigecycline simulating free serum concentrations. The arrows indicate the time points where drug was added in the *in vitro* model simulating q12 administrations of tigecycline and colistin and q8 administration of meropenem.

RESULTS

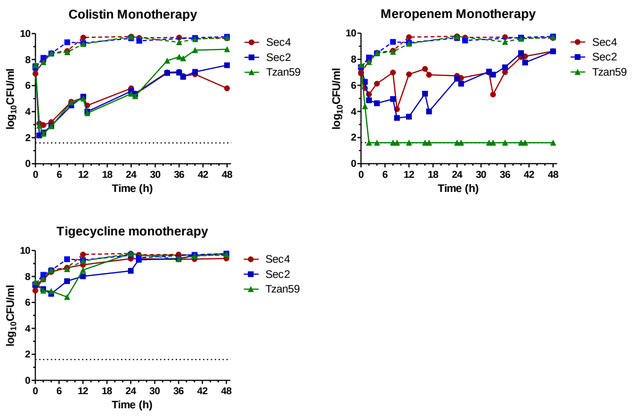

Figure 3. Time kill curves of monotherapies against 3 *K. pneumoniae* strains (1 wild type TZAN59 and 2 VIM producing SEC2 and SEC4) with meropenem MIC 0,06, 16 and 256 mg/l, respectively.

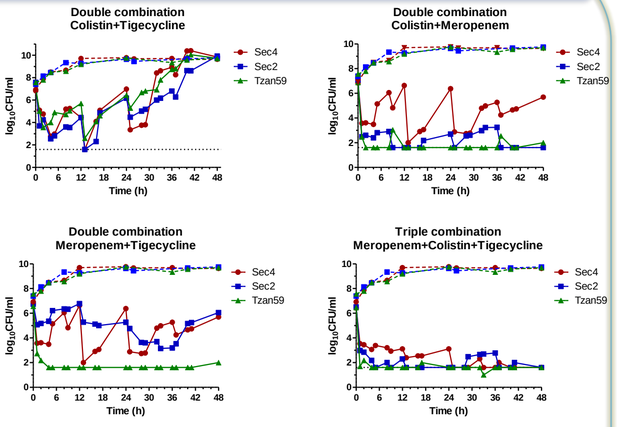

Figure 4. Time kill curves of double and triple combinations against 3 *K. pneumoniae* strains (1 wild type TZAN59 and 2 VIM producing SEC2 and SEC4) with meropenem MIC of 0,06, 16 and 256 mg/l, respectively.

- **Monotherapy.** Among monotherapy regimens, only meropenem was effective against the wild-type isolate with MIC 0.06 mg/l **(Figure 3)**.
- **Double combinations.** Among double combinations against the 2 VIM producing isolates (SEC2, SEC4), colistin+tigecycline increased CFU/ml counts by $2\log_{10}$ whereas meropenem+tigecycline combination therapy decreased them by $1\log_{10}$ **(Figure 4)**. Colistin+ meropenem combination decreased by 1 and $5\log_{10}$ the CFU/ml counts of the isolates with meropenem MIC 256 and 16 mg/l, respectively.
- **Triple combination.** The triple combination decreased by 5 $\log_{10}$ CFU/ml counts of both VIM producing isolates. Against the other 4 isolates with mixed resistance mechanisms, a 3 and 5 $\log_{10}$ CFU/ml reduction was observed against the isolates with meropenem MICs 256 and 64-128mg/l, respectively whereas a $2\log_{10}$CFU/ml increase was found for the isolate with meropenem MIC 512 mg/l **(Figure 5)**.

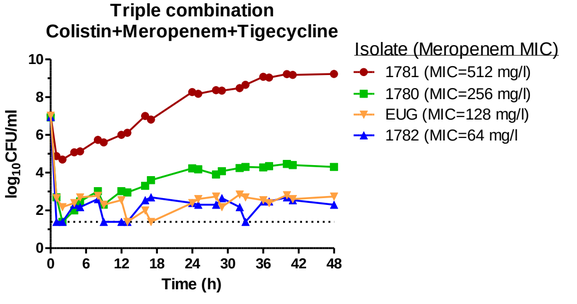

Figure 5. Time kill curves of the triple combination against 4 *K. pneumoniae* strains (1782, EUG, 1780 and 1781) with different resistance mechanisms.

CONCLUSIONS

- ✓ The double combination of colistin with meropenem was effective against CP-Kp isolates with meropenem MICs up to 16 mg/l.
- ✓ For isolates with meropenem MICs up to 256 mg/l the triple combination of colistin, meropenem and tigecycline was effective.