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Interactions of tigecycline with colistin, gentamicin or meropenem against tigecycline resistant carbapenemase producing *Klebsiella pneumoniae* isolates

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Abstract

Objective: To evaluate the *in vitro* effectiveness of binary combinations of tigecycline with colistin, gentamicin or meropenem against carbapenemase-producing *Klebsiella pneumoniae* isolates, resistant to tigecycline. **Methods:** Time-kill methodology was used. Synergy was defined as a ≥2-$\log_{10}$ decrease in CFU/ml between the combination and the most active single agent at various time intervals (1, 3, 5 and 24h) whereas antagonism as an ≥2-$\log_{10}$ increase. All other interactions were considered indifferent. Clinically achievable fixed agent concentrations were used. **Results:** A total of 24 KpC+ strains with a tigecycline MIC range of 4-12 mg/l were used and 48 combinations between tigecycline and either colistin, gentamicin or meropenem were evaluated. The combination of tigecycline/colistin exhibited synergy for 41.7% of colistin resistant isolates mostly at 5h and for 25% of colistin susceptible isolates at 24h. The combination of tigecycline with gentamicin was synergistic for 10% of the gentamicin resistant isolates and 25% of the susceptible isolates. Antagonism was observed in 10% of all tigecycline/colistin combinations. **Conclusions:** There is a possibility of synergism between tigecycline and colistin against KpC+ isolates resistant to both agents. This warrants further investigation, in light of the therapeutic inadequacy against such strains.

Background

Over the last decade, carbapenemase producing *Klebsiella pneumoniae* isolates have become increasingly common worldwide, while infections related to them have been associated with therapeutic failures and high mortality rates. Many of the circulating strains exhibit not only multi-drug (MDR) but sometimes extensively-drug (XDR) or even pan-drug resistance. Tigecycline remains one of our last resort drugs, but when resistance occurs, leaves us with very few therapeutic options. The objective of this study was to assess the *in vitro* effectiveness of binary combinations of tigecycline with colistin, gentamicin or meropenem against tigecycline resistant KpC+ isolates, often resistant to the aforementioned agents.

Methods

- Susceptibility testing was performed by the semi-automated VITEK 2 system (bioMerieux) as a screening method.
- MICs for all antimicrobials involved were confirmed by Etest (bioMerieux) according to manufacturer instructions.
- Resistance mechanisms to carbapenems were phenotypically detected according to the EUCAST guidelines.
- Interactions between tigecycline and colistin, gentamicin or meropenem were assayed by the time-kill methodology. Interactions were assessed at various time intervals (1,3,5 and 24 h). Clinically achievable fixed agent concentrations were used.
- Synergy was defined as a ≥2-$\log_{10}$ decrease in CFU/ml between the combination and the most active single agent. Antagonism was defined as a ≥2-$\log_{10}$ increase in CFU/ml between the combination and the most active single agent. All other interactions were characterized as indifferent.

Results

- 24 carbapenemase producing *Klebsiella pneumoniae* isolates were used.
- All isolates were resistant to tigecycline with MICs 4 – 12 mg/L.
- A total of 48 binary combinations were evaluated: 20 combinations of tigecycline with colistin (12 against colistin resistant and 8 against colistin susceptible isolates), 18 combinations with gentamicin (10 against gentamicin resistant and 8 against gentamicin susceptible isolates) and 10 combinations with meropenem (MICs to meropenem were 4-64 mg/L).
- The combination of tigecycline/colistin exhibited synergy in 5 of the 12 (41.7%) colistin resistant isolates mostly at 5h and in 2 of the 8 (25%) colistin susceptible isolates at 24h.

Figure 1. Time killing curves of tigecycline and colistin combination against resistant to both agents isolates and against one susceptible to colistin isolate

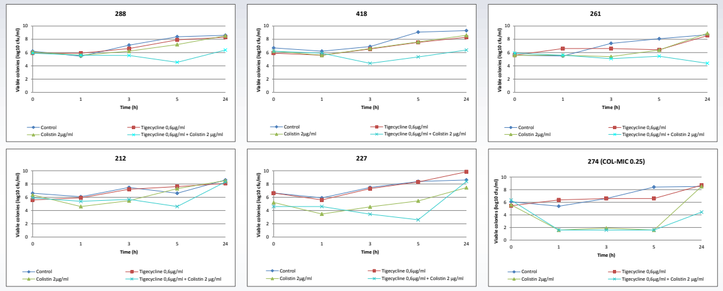

Table 1. TIG and COL MICs (mg/L) of resistant isolates and results of time-kill curves of the combination at concentrations 0.6mg/L TIG and 2mg/L COL

Strain	TIG MIC	COL MIC	Synergy	Δ $\log_{10}$ CFU/ml		
				3h	5h	24h
418	4	12	3h, 5h, 24h	-2.2	-2.3	-2.2
212	6	12	5h	0.2	-2.7	-0.2
288	6	12	5h, 24h	-0.6	-2.7	-2.2
259	6	12	-	-0.1	-1.0	-1.7
315	8	12	-	0.3	-1.9	-1.4
424	6	16	-	0.1	0.4	-0.6
265	6	16	-	-0.6	2.5	-0.5
347	8	16	-	1.1	0.5	-0.8
261	8	16	24h	-0.3	-0.9	-4.2
262	8	24	-	-0.4	-0.3	0.0
227	6	32	5h	-1.1	-2.8	0.9
385	8	32	-	-0.3	0.4	0.5
INDIFFERENCE				11/12 (91.7%)	7/12 (58.3%)	9/12 (75%)
SYNERGY				1/12 (8.3%)	4/12 (33.3%)	3/12 (25%)
ANTAGONISM				0/12 (0%)	1/12 (8.3%)	0/12 (0%)

*Values represent the change in CFU/ml after 3, 5 and 24 hours exposure to the combination TIG and COL compared to the most active single agent. Negative values indicate a decrease in colony count.
White color indicates indifferent effect, green indicates synergy and orange indicates antagonism.

Results

- The combination of tigecycline/gentamicin exhibited synergy in 1 of the10 (10%) gentamicin resistant isolates and in 2 of the 8 (25%) gentamicin susceptible isolates.
- The combination of tigecycline/meropenem was indifferent for all tested isolates.
- Antagonism was observed between tigecycline and colistin against 1 of the 12 colistin resistant isolates (8.3%), and 1 of the 8 colistin susceptible isolates (12.5%). There was no antagonistic effect between tigecycline and the other antibiotics.

Figure 2. Time killing curves of tigecycline and gentamicin combination against one resistant to both agents isolate and against two susceptible to gentamicin isolates

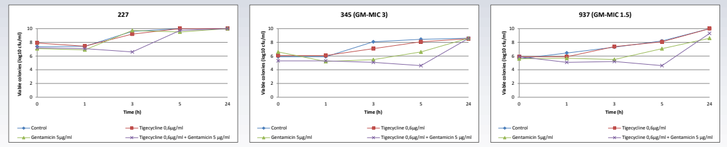

Table 2. TIG and GM MICs (mg/L) of resistant isolates and results of time-kill curves of the combination at concentrations 0.6mg/L TIG and 5mg/L GM

Strain	TIG MIC	GM MIC	Synergy	Δ $\log_{10}$ CFU/ml		
				3h	5h	24h
462	4	32	-	-0.6	-0.1	0.5
395	4	64	-	-1.0	-0.1	1.3
244	12	192	-	-0.9	-0.8	0.0
265	6	512	-	0.6	1.3	0.0
227	6	512	3h	-2.6	0.2	0.0
259	6	512	-	-1.5	0.0	0.0
212	6	512	-	0.8	0.2	0.0
262	8	512	-	0.5	0.0	0.0
261	8	512	-	0.9	0.0	0.0
347	8	512	-	0.0	0.7	0.0
INDIFFERENCE				9/10 (90%)	10/10 (100%)	10/10 (100%)
SYNERGY				1/10 (10%)	0/10 (0%)	0/10 (0%)
ANTAGONISM				0/10 (0%)	0/10 (0%)	0/10 (0%)

*Values represent the change in CFU/ml after 3, 5 and 24 hours exposure to the combination TIG and GM compared to the most active single agent. Negative values indicate a decrease in colony count.
White color indicates indifferent effect and green indicates synergy.

Conclusion

There is a possibility of synergistic interaction of tigecycline and colistin against carbapenemase-producing *K. pneumoniae* strains resistant to both agents. This observation might be important when there are no other therapeutic solutions. It therefore deserves further investigation.

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