

# Colistin-based and colistin-sparing antimicrobial combinations against carbapenem-resistant *Klebsiella pneumoniae*.



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## Objectives

The aim of the study was to evaluate the in-vitro activity of different antimicrobial combinations (with and without colistin) against carbapenem-resistant *Klebsiella pneumoniae*. Furthermore, different methods used for MICs determination of colistin and tigecycline [macro broth dilution (MBD), automated system (VITEK-2) and gradient strip diffusion (E-test)] were compared.

## Methods

A series of carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) collected from hospitalized patients with CR-Kp colonization or infection at the Department of Public Health and Infectious Diseases (Sapienza University of Rome) was included in the study. MICs<sub>50/90</sub> of ertapenem (ERT), doripenem (DOR), meropenem (MEM), colistin (COL), rifampin (RIF) and tigecycline (TIG) were determined by BMD. Checkerboard method was used to evaluate the synergistic activity of COL-based (COL+MEM, COL+DOR, COL+RIF, COL+TIG) and COL-sparing combinations (MEM+ERT). Synergism was defined as FIC index  $\leq 0.5$ . Additionally, killing curves were performed for MEM+ERT.

## Results

Overall, 39 strains from 39 subjects (mean age 57.2 years; 11 F, 28 M) with CR-Kp infection (n=33) or colonization (n=6) were collected. Fluoroquinolones, gentamicin, amikacin and trimethoprim-sulfamethoxazole resistance was observed in 39/39 (100%), 30/39 (76.9%), 33/39 (84.6%) and 37/39 (94.8%) of the strains, respectively. COL and TIG resistance was present in 15/39 (38.4%) and 22/39 (56.4%), in 10/39 (25.6%) and 21/39 (53.8%), in 15/39 (38.4%) and 3/39 (7.6%) throughout VITEK-2, E-test and MBD, respectively. By MBD, MICs<sub>50/90</sub> were 128/512 mcg/ml for both ERT and MEM, 64/128 mcg/ml for DOR and 2/128, 32/512 mcg/mL for COL and RIF, respectively.

Synergy was observed in 17/39 (43.5%), 26/39 (66.6%), 39/39 (100%), 31/39 (79.4%) and 26/39 (66.6%) for MEM+ERT, COL+MEM, COL+RIF, COL+TIG and COL+DOR, respectively. Among the 15/39 (38.4%) COL resistant strains throughout MBD, synergism was detected in 12/15 (80%), 15/15 (100%), 12/15 (80%) and 15/15 (100%) of COL+MEM, COL+RIF, COL+DOR and COL+TIG combinations, respectively.

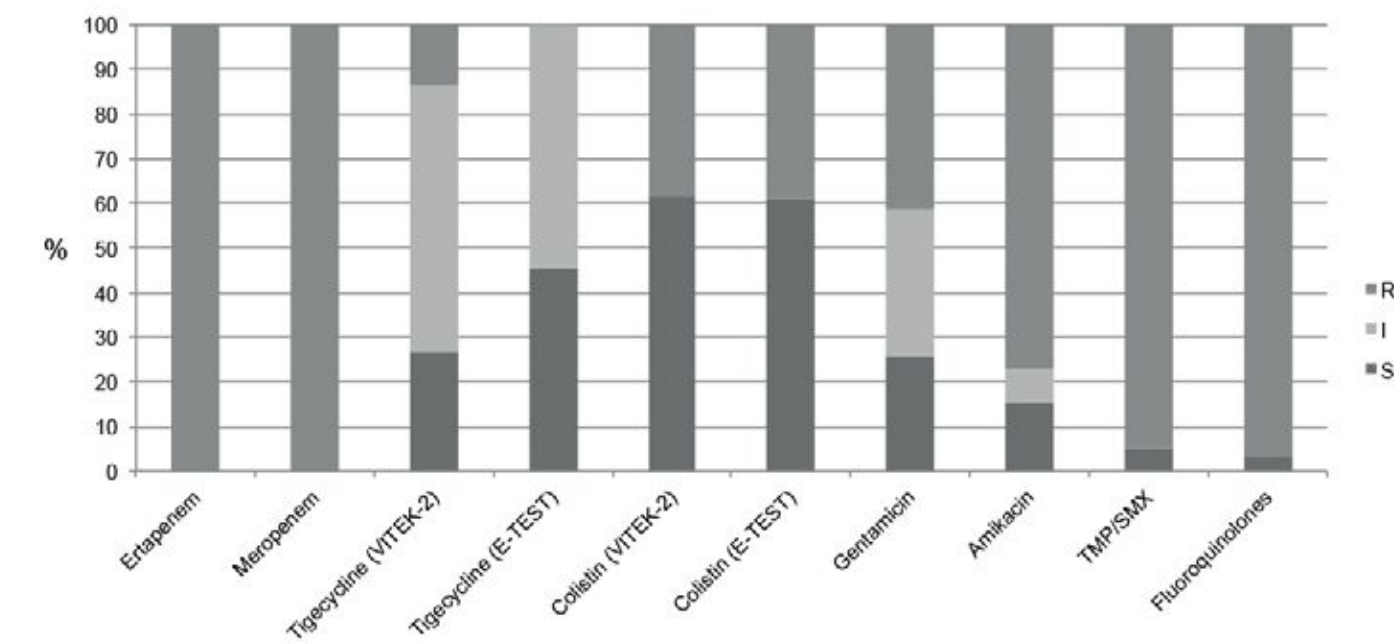
**Table 1.** Comparison between VITEK-2 system and E-test (a), VITEK-2 system and MBD (b), E-test and MBD (c) methods for tigecycline and colistin MIC determination. Concordant values are represented in bold. MBD: macro broth dilution; S: susceptible; I: intermediate; R: resistant.

	VITEK-2		Concordance (k-index)		VITEK-2		Concordance (k-index)		MBD		Concordance (k-index)
	(S), n	(I+R), n			(S), n	(I+R), n			(S), n	(R), n	
<b>a) Tigecycline (n=39)</b>											
E-test (S), n	11	7	0.667 (0.327)								
E-test (I+R), n	6	15									
<b>b) Tigecycline (n=39)</b>											
MBD (S), n	16	20	0.462 (0.02)								
MBD (R), n	1	2									
<b>c) Tigecycline (n=39)</b>											
E-test (S), n	18	0	0.538 (0.133)								
E-test (I+R), n	18	3									
<b>a) Colistin (n=39)</b>											
E-test (S), n	23	6	0.821 (0.596)								
E-test (R), n	1	9									
<b>b) Colistin (n=39)</b>											
MBD (S), n	21	2	0.872 (0.733)								
MBD (R), n	3	13									
<b>c) Colistin (n=39)</b>											
E-test (S), n	22	7	0.795 (0.550)								
E-test (R), n	1	9									

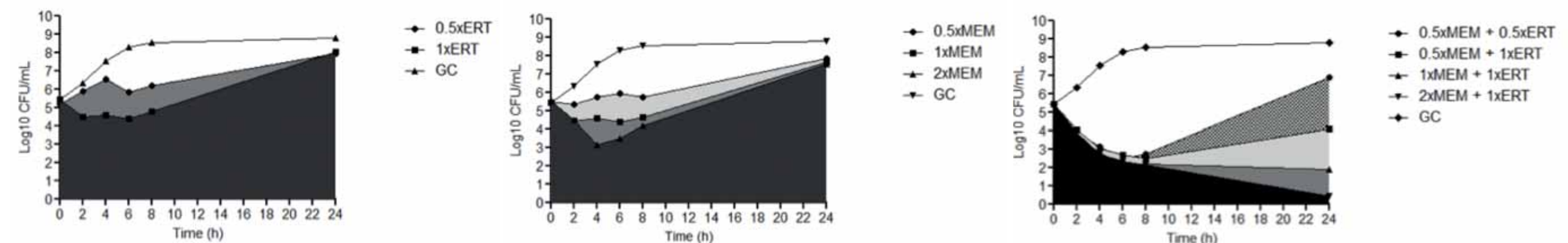
**Table 2.** Synergism of different combinations against CR-Kp throughout checkerboard method. Values are expressed as number and percentage.

	MEM+ERT, n (%) (n=39)	MEM+TIG, n (%) (n=10)*	COL+MEM, n (%) (n=39)	COL+RIF, n (%) (n=34)*	COL+TIG, n (%) (n=39)	COL+DOR, n (%) (n=39)
Total	12/39 (30.7)	1/10 (10)	18/39 (46.1)	33/34 (97)	24/39 (61.5)	17/39 (43.5)
COL-S (n=23)	5/23 (21.7)	1/4 (25)	10/23 (43.4)	19/20 (95)	12/23 (52.1)	9/23 (39.1)
COL-R (n=16)	7/16 (43.7)	0/6 (0)	8/16 (50)	14/14 (100)	12/16 (75)	8/16 (50)

**Figure 1.** Antimicrobial susceptibility of carbapenem-resistant *K. pneumoniae* throughout VITEK-2 system. E-test method was used for tigecycline and colistin susceptibility.



**Figure 2.** Representative areas under the curve (AUCs) of MEM (A) and ERT (B) alone and in combination (C) at different concentrations against 33 carbapenem-resistant *K. pneumoniae* isolates. MEM: meropenem; ERT: ertapenem; GC: growth control.



## Conclusion

Colistin-based regimens showed high levels of synergistic *in-vitro* activity, even in the presence of COL-resistant strains. In case of TIG susceptibility, MBD should be preferred to both VITEK-2 and E-test methods. Synergy testing should be performed whenever a CR-Kp is detected, especially when unconventional therapeutic approaches are considered.