Double-carbapenem regimen, alone or in combination with colistin, in the treatment of infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp)

Dear Editor,

We read with interest the article published by Zhang et al., concerning the emergence of infection caused by a hypervirulent (hv) carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) strain.[1]

Infections caused by CR-Kp represent an emerging threat worldwide due to high mortality rate and limited therapeutic options.[2] Although considered as part of antimicrobial combinations against CR-Kp, colistin use might be limited by its potential nephrotoxicity and resistance; thus, colistin-free unconventional approaches such as the double-carbapenem (DC) regimen have recently been proposed.[3–6]

In the present study, we evaluated the clinical effectiveness and the *in-vitro* activity of the DC regimen [ertapenem (ERT) plus high dose of meropenem (MEM)], alone or in combination with colistin (COL), in the treatment of infections caused by CR-Kp.

Over a 3-years period (2012–2015), we enrolled all consecutive patients with CR-Kp infection treated with the DC regimen, with or without colistin, at the Department of Public Health and Infectious Diseases of "Sapienza" University (Rome) and at the Mediterranean Neurological Institute "Neuromed" (Pozzilli).

The use of these combinations was decided by the attending Infectious Diseases Specialist according to patients' clinical condition, renal function and strains susceptibility pattern. Early response was defined as resolution of signs and symptoms of infection at 5th day of antimicrobial treatment whereas the overall follow-up was set up at 60-days. Patients gave informed written consent.

Clinical samples underwent microbiological procedures as for daily practice. Additional *in-vitro* studies included phenotypic determination of carbapenemases[7] and evaluation of MEM + ERT and COL + MEM + ERT synergism throughout killing studies. In the triple combination, we decided to lower MEM rather than COL or ERT concentrations because in the presence of high MEM MICs, which were far above the achievable concentration of the drug, there was a higher probability of reaching serum MEM concentration with 0.5× MIC and 0.25× MIC concentrations than with 1× MIC. The area under the curve (AUC) of each combination, expressed as mean ± SD, was calculated. The number of CFU/mL was expressed as mean ± standard error of the mean (SEM). For statistical analyses we used GraphPad Prism version 5 (Graphpad Software MacKiev).

A total of 32 patients with CR-Kp infection were enrolled in the study: subjects receiving ERT + MEM (Group A; n = 18) and subjects receiving COL + MEM + ERT (Group B; n = 14). Clinical characteristics of study population are shown in Table 1.

Overall, clinical success was achieved in 75% of subjects. The triple combination therapy was used more frequently in patients with a more severe condition at the time of infection (i.e. septic shock). Although patients in Group B tended to have an earlier clinical response than those in Group A, no statistical difference was found between the 2 groups regarding early response to therapy and mortality at 60-days.

Killing studies were performed on 28 CR-Kp strains (16 from Group A, 12 from Group B) whereas the remaining 4 strains were not available. MICs50/90 of both MEM and ERT were 128 and 256 μg/mL by macrobroth dilution. All the strains were KPC producers. Results of killing studies are shown in Fig. 1.

In the DC group (n = 16 strains, Fig. 1a), bactericidal activity of ERT + MEM was observed in 12/16 (75%) at 8 h and raised to 14/16 (87.5%) at 24 h whereas in the COL + DC group (n = 12 strains, Fig. 1b), the triple combination (COL1× MIC + MEM1× MIC + ERT1× MIC) showed a more rapid bactericidal activity than the double-carbapenem regimen at MEM1× MIC + ERT1× MIC (8/12 *versus* 6/12 at 8 h) and this effect was confirmed even when sub-inhibitory concentrations of drugs were tested. Furthermore, the triple combination at all the tested concentrations was bactericidal at 24 h. Interestingly, the bactericidal effect of the combination COL + MEM + ERT was confirmed even in the presence of COL MIC ≥2 μg/mL.

In the present study, we enrolled patients treated with two types of unconventional regimens (MEM + ERT in 18 patients and COL + MEM + ERT in 14 subjects).

In the *in-vitro* analyses, we showed that the addition of COL to the DC regimen obtained a rapid bactericidal activity, which was maintained up to 24 h. Of note, the activity of this combination was observed even at sub-inhibitory concentrations of the drugs, with no statistically significant differences in the AUCs of the tested concentrations.

Based on our results, the addition of colistin to MEM + ERT might be useful by inducing an earlier antibacterial activity than that obtained with the double-carbapenem regimen alone. This effect might be crucial in the setting of more severe infections, where a rapid antibacterial effect of an antimicrobial regimen is auspicable in order to improve the clinical outcome of the patients, even when the presence of colistin resistance might discourage its use. Our results could lead to the hypothesis that starting with an aggressive treatment of colistin + ertapenem + meropenem followed by a therapeutic switch to a less toxic regimen (i.e. the double-carbapenem regimen) might be a reasonable therapeutic options against systemic infections caused by CR-Kp.[8]

Furthermore, our experiments showed that even in the presence of higher COL MICs (≥2 μg/mL) the combination of COL + MEM + ERT was highly effective. In particular, all the patients with COL resistance (n = 3) had clinical improvement at 60-days of follow-up. In these cases, the detergent-like property of colistin might have a crucial role in facilitating the penetration of the other drugs into the bacteria.[9]

One of the major features of the present study is the presence of both clinical and *in-vitro* results. In an era in

Table 1 General characteristics of study population.

	Study population (n = 32)	DC group[a] (n = 18, Group A)	COL[b] + DC group (n = 14, Group B)	*p*-value
Demographic characteristics				
• Age (years), M ± SD	55.1 ± 15.2	55.6 ± 13.6	54.4 ± 17.5	0.74
• Sex (M:F)	23:9	14:4	9:5	0.45
• APACHE III score, M ± SD	64.9 ± 29.6	50.7 ± 28.1	83.2 ± 20.3	0.001
• ≥2 Comorbidities	14 (43.7)	5 (27.7)	9 (64.2)	0.07
Classification of infection, n (%)				
• CA:HCA:HA	0:6:26	0:6:12	0:0:14	0.0001
• Length of hospitalization before infection, days[c]	17.5 (2–437)	14 (2–65)	23 (5–437)	0.005
Risk factors for infection (previous 12 months), n (%)				
• Previous hospitalization	22 (68.7)	15 (83.3)	7 (50)	0.02
• Intensive care unit	22 (68.7)	10 (55.5)	12 (85.7)	0.06
• Vescical catheter	18 (56.2)	12 (66.6)	6 (42.8)	0.12
• Central venous catheter (CVC)	11 (34.3)	9 (50)	2 (14.2)	0.28
Risk factors for infection (previous 72 h), n (%)				
• Naso-gastric tube	18 (56.2)	6 (33.3)	12 (85.7)	0.004
• Fibrobroncoscopy	14 (43.7)	4 (22.2)	10 (71.4)	0.01
• Parenteral total nutrition	14 (43.7)	4 (22.2)	10 (71.4)	0.01
• CVC	20 (62.5)	7 (38.8)	13 (92.8)	0.002
Previous antibiotic therapy (90 days), n (%)	29 (90.6)	15 (83.3)	14 (100)	0.23
• Cephalosporin	10 (31.2)	3 (16.6)	7 (50)	0.06
• Penicillins	18 (56.2)	7 (38.8)	11 (78.5)	0.03
• Carbapenems	15 (46.8)	6 (33.3)	9 (64.2)	0.45
• Beta-lactams (overall)	27 (84.3)	13 (72.2)	14 (100)	0.05
• Fluoroquinolones	10 (31.2)	5 (27.7)	5 (35.7)	0.71
• Colistin	9 (28.1)	4 (22.2)	5 (35.7)	0.45
CR-Kp rectal colonization, n (%)	27 (96.4)	13/14 (92)[e]	14/14 (100)	0.37
Colistin-resistance, n (%)	11 (34.3)	8 (44.4)	3 (21.4)	0.26
Clinical presentation[d], n (%)				
• Sepsis	5 (15.6)	3 (16.6)	2 (14.2)	0.99
• Severe sepsis	13 (40.6)	7 (38.8)	6 (42.8)	0.99
• Septic shock	8 (25)	2 (11.1)	6 (42.8)	0.09
• Type of infection				
Pneumonia	9 (28.1)	4 (22.2)	5 (35.7)	0.99
EV infection	3 (9.3)	2 (11.1)	1 (7.1)	0.87
UT infection	9 (28.1)	8 (44.4)	1 (7.1)	0.04
CVC infection	6 (18.7)	0 (0)	6 (42.8)	0.003
Primary bacteremia	6 (18.7)	4 (22.2)	2 (14.2)	0.67
• Presence of bacteremic infection	18 (56.2)	8 (44.4)	10 (71.4)	0.16
Definitive therapy, n (%)				
• Early response[e]	23 (71.8)	11 (61.1)	12 (85.7)	0.23
• Length of definitive therapy, days[c]	21 (7–150)	18.5 (7–150)	25 (7–34)	0.04
• Clinical response (days)[c]	4 (2–15)	4 (2–15)	4 (3–12)	0.68
• Microbiological response (days)[f c]	4 (2–17)	3 (2–17)	5 (3–10)	0.43
• Adverse events, n (%)[g]	6 (18.7)	3 (16.6)	3 (21.4)	1.00
Outcome at 60 days, n (%)				
• Exitus	6 (18.7)	3 (16.6)	3 (21.4)	0.99
• Relapse[h]	2 (6.2)	2 (11.1)	0 (0)	0.49
• Improvement	24 (75)	13 (72.2)	11 (78.5)	0.89

DC: double-carbapenem; COL: colistin; CR-Kp: carbapenem-resistant *Klebsiella pneumoniae*; CA: community-acquired; HCA: health-care associated; HA: hospital-acquired; EV: endo vascular; UT: urinary tract; CVC: central venous catheter.

[a] The DC regimen consisted of ERT 1 g (1-h infusion) followed by high dose of MEM (2 g every 8 h, 3-h infusion).

[b] COL administration was 9,000,000 UI as a loading dose followed (after 12 h) by 4,500,000 UI every 12 h.

[c] Data are expressed as median (range).

[d] Clinical presentation (sepsis, severe sepsis and septic shock) was determined according to period guidelines.[10].

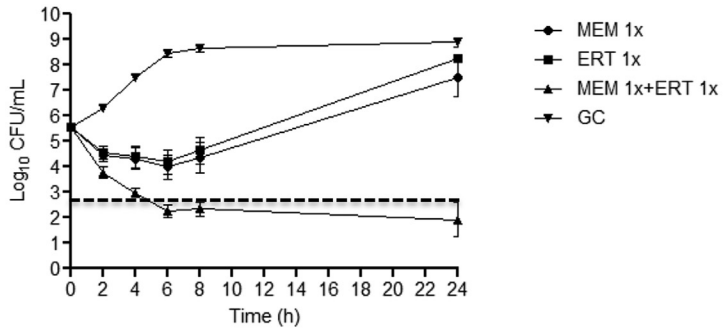
[e] Early clinical response was defined as resolution of signs and symptoms of infections (i.e. defervescence, improvement of clinical conditions, reduction of white blood cells, C-reactive protein) achieved at 5th day of antimicrobial treatment.

[f] Microbiological response was defined as negativity of cultures performed after 5 days of antimicrobial treatment.

[g] Type of adverse events included: mild sodium-disorder (n = 1, Group A), vomiting (n = 1, Group A), seizures (n = 2, 1 Group A, 1 Group B), leukopenia (n = 2, Group B).

[h] Infection relapse was defined as recrudescence of CR-Kp infection within the 60-day of follow up after an initial response.

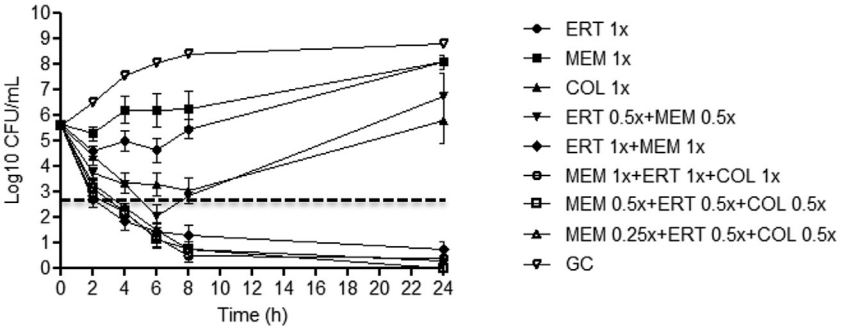
a)



DC group (n=18)*	Bactericidal activity[a] (2h), n (%)	Bactericidal activity[a] (4h), n (%)	Bactericidal activity[a] (6h), n (%)	Bactericidal activity[a] (8h), n (%)	Bactericidal activity[a] (24h), n (%)	Synergistic activity[b] (24h), n (%)	AUC, mean ± SD**
1xMICMEM+ 1xMIC ERT	1 (6.2)	5 (31.2)	8 (50)	12 (75)	14 (87.5)	14 (87.5)	59.9± 25.5

*: killing studies were performed on 16 strains. **: AUC of GC (growth control) was 199.4±11.7.

b)



DC+COL group (n=14)*	Bactericidal activity[a] (2h), n (%)	Bactericidal activity[a] (4h), n (%)	Bactericidal activity[a] (6h), n (%)	Bactericidal activity[a] (8h), n (%)	Bactericidal activity[a] (24h), n (%)	Synergistic activity[b] (24h), n (%)	AUC, mean ± SD**
0.5xMIC MEM +0.5xMIC ERT	0	1 (8.3)	4 (33.3)	6 (16.6)	2 (16.6)	2 (16.6)	103± 29.1
1xMIC MEM + 1xMIC ERT	4 (33)	6 (50)	10 (83.3)	10 (83.3)	11 (91.6)	11 (91.6)	34.9± 25.4[§]
1xMIC MEM+ 1xMIC ERT+ 1xMIC COL	4 (33)	8 (66.6)	9 (75)	12 (100)	12 (100)	n.a.	25.6 ± 18.7[§]
0.5xMICMEM+ 0.5xMIC ERT+ 0.5xMIC COL	2 (16.6)	8 (66.6)	8 (66.6)	11 (91.6)	12 (100)	n.a.	25.2 ± 12.6[§]
0.25xMICMEM+ 0.5xMIC ERT+ 0.5xMIC COL	2 (16.6)	9 (75)	11 (91.6)	11 (91.6)	12 (100)	n.a.	28.7 ± 15.5[§]

*: killing studies were performed on 12 strains.[§]: the difference among tested concentrations were not statistically significant. **AUC of GC (growth control was 195.9± 6.9.

Figure 1 Bactericidal activity and killing studies of double-carbapenem regimen (a) and colistin (COL) plus double-carbapenem regimen (b). DC: double-carbapenem; MEM: meropenem; ERT: ertapenem; GC: growth control; AUC: area under the curve; SD: standard deviation. Dashed line represents bactericidal activity. [a]: bactericidal activity was defined as ≥3-log10 CFU/ml reduction of the initial bacterial count at each time point; [b]: synergistic activity was defined as a ≥100-fold decrease in CFU/mL between the combination and its most active constituent at the same concentration after 24 h.

which traditional antimicrobial susceptibility reports seem to be no longer informative, we believe that performing *in-vitro* synergy studies could represent an additional tool in order to guide treatment decisions and predict the potential clinical efficacy of the chosen combination.

In conclusion, we were able to demonstrate that both the double-carbapenem alone and the double-carbapenem

regimen plus colistin were clinically and microbiologically effective in the treatment of infections caused by CR-Kp, the latter even at sub-inhibitory concentrations. MEM + ERT might be a valid therapeutic option when COL use is discouraged whereas COL + MEM + ERT might be considered in subjects presenting with more severe conditions (i.e. septic shock), where an early clinical response is auspicable.

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Conflict of interest

None declared.

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