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SHORT COMMUNICATION

Clinical and *in vitro* efficacy of colistin plus vancomycin and rifampin against colistin-resistant *Acinetobacter baumannii* causing ventilator-associated pneumonia

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SUMMARY

We present the case of a patient with ventilator-associated pneumonia (VAP) caused by a pan-resistant *Acinetobacter baumannii* successfully treated with the combination colistin plus vancomycin plus rifampin, whose *in vitro* activity was investigated by checkerboard method and killing testing. Furthermore, the serum bactericidal activity (SBA) was assessed.
Our case shows that an innovative regimen consisting of colistin plus antimicrobials active only against Gram-positive microorganisms might represent a valid therapeutic option for severe infections caused by colistin-resistant *A. baumannii*.

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A 77-year-old man was admitted to the Intensive Care Unit (ICU) for an acute subarachnoid hemorrhage. His recent medical history included a diagnosis of culture negative brain abscess treated with a combination therapy of linezolid and meropenem six weeks prior to ICU admission. The patient underwent neurosurgery and mechanical ventilation was started. During the early post-operative period the patient had an acute worsening of respiratory gas exchanges, associated with leukocytosis. Chest radiography showed left pneumonia and tracheal aspirate was positive for Extended Spectrum Beta Lactamases (ESBL)-producing *E. coli*. Thus, therapy with meropenem 1 g every 8 hours was started, with improvement of clinical conditions. However, after 12 days after intubation the patient developed fever and hypotension with sudden worsening of respiratory gases and metabolic acidosis. A CT scan of the chest revealed bilateral multiple areas of consolidation of the lungs. Bronchoalveolar lavage culture was positive for colistin- (COL) and carbapenem-resistant *Acinetobacter baumannii* and methicillin-resistant *S. aureus* (MRSA). Meropenem was discontinuated and therapy with i.v. colistin 9.000.000 UI loading dose followed by 4.500.000 UI every 12 hours plus rifampin (RIF) 600 mg/day plus vancomycin (VAN) 1g loading dose followed by 2 g/day was initiated. Aerosolized COL (1.000.000 UI every 8 hours) was also administered. The clinical conditions of the patient improved. However, a significant increase in creatinine level was observed (up to 3 mg/dL), leading to the reduction of COL dosages to 2.500.000 every 12 hours and discontinuation of VAN after 6 days of therapy. Serum bactericidal activity (SBA) performed at day 3 of therapy was 1:16 (pre-dose) and 1:64 (after dose). COL and RIF were stopped after 2 weeks of therapy, with resolution of the infection and progressive improvement of renal function after 4 weeks. The patient was then transferred to a rehabilitation center 7 weeks after ICU admission.

Considering its challenging antimicrobial susceptibility pattern, the clinical isolate was immediately submitted to additional *in vitro* analyses including synergy testing by both checkerboard method and killing tests.

Minimal inhibitory concentrations (MICs) of COL, VAN and RIF were determined by broth macrodilution method (BMD) in cation-adjusted Mueller Hinton broth (CAMHB) (CLSI, 2012). Furthermore, for COL MIC determination a gradient strip test (E-test) was used. Synergy tests were performed throughout the checkerboard method at different concentrations of COL+RIF, COL+VAN and COL+RIF+VAN combinations. Complete synergism was defined as FIC-index (FICI) ≤0.5. The activity of COL, VAN and RIF, alone and in combination, was also investigated by time-kill studies at a final inoculum of ~$5x10^5$ CFU/ml at the following concentrations: 1xMIC COL, 0.25xMIC VAN, 1xMIC RIF, 1xMIC COL+0.25xMIC VAN, 1xMIC COL+1xMIC RIF, 1xMIC COL+0.25xMIC VAN+ 1xMIC RIF. The VAN concentration used for killing studies reflected the serum trough level achieved during therapy (16 mg/L). Bactericidal activity was defined as ≥3-$\log_{10}$ CFU/ml reduction of the initial bacterial count at each time point whereas 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. The detection limit was 10 CFU/mL. SBA was determined at 3th day of COL+VAN+RIF

Key words:
Acinetobacter baumannii, Multi-drug resistance, Colistin resistance, Ventilator-associated pneumonia, Vancomycin, Rifampin.

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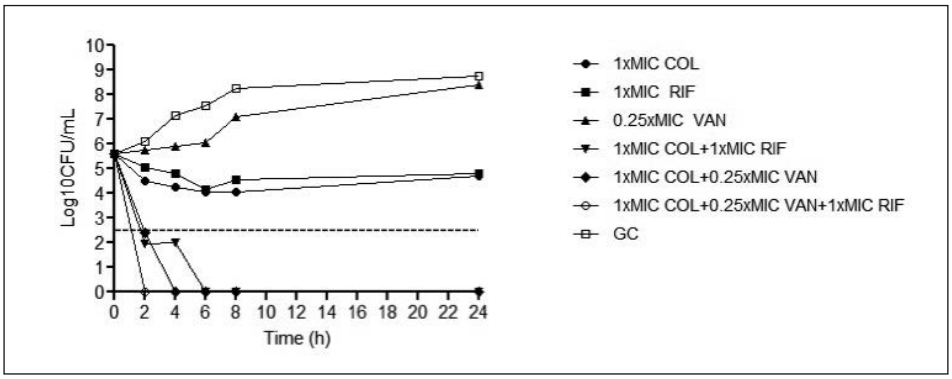

Figure 1 - *Killing studies evaluating the activity of COL, VAN and RIF, alone and in combination at different concentrations, against a colistin- and carbapenem-resistant A. baumannii. COL: colistin; VAN: vancomycin; RIF: rifampin; GC: growth control. Dashed line represents bactericidal activity that occurred at any time point compared to the initial bacterial inoculum.*

therapy by collecting patient's blood 30 min before and 2 h after therapy administration.

Throughout MBD, MICs of COL, RIF and VAN were 4, 2 and 64 mg/L, respectively. By using E-test, COL MIC was 4 mg/L. A complete synergism was observed for COL+VAN and COL+RIF. At killing experiments, COL (1xMIC, 4 mg/L) and RIF (1xMIC, 2 mg/L) alone showed only a slight decrease of CFU/mL whereas VAN (0.25xMIC, 16 mg/L) alone did not have any effect on lowering the bacterial growth (*Figure 1*). On the other hand, when the combinations COL+VAN, COL+RIF and COL+VAN+RIF were tested, a rapid bactericidal activity was observed after 2 h. All tested combinations showed an absence of growth at 6h, which was maintained up to 24 h. In particular, absence of growth was observed at 6 h for COL+RIF combination whereas COL+VAN and COL+VAN+RIF yielded no bacteria at 4 h and 2 h, respectively. All the tested combinations were synergic at 24 h.

Infections caused by *A. baumannii* are associated with a high mortality rate especially in the presence of multidrug resistant (MDR) strains since therapeutic options are severely limited (Maragakis *et al*., 2008; Orsi *et al.*, 2011). Colistin-based combinations have been considered the milestone of the treatment of MDR *A. baumanni* (Motaouakkil *et al.*, 2006; Durante-Mangoni *et al*., 2013) whereas unconventional regimens against carbapenem-resistant *A. baumannii* include the combination of COL plus rifampin (RIF) or glycopeptides (Garnacho-Montero *et al*., 2013; Petrosillo *et al.*, 2014; Ceccarelli *et al*., 2015). However, the emergence of COL resistance represents a real challenge and nowadays insufficient data are available for the treatment of *A. baumannii* infections due to COL-resistant strains (Leite *et al*., 2016).

In the present case, we demonstrated that a regimen consisting of COL plus antimicrobials active only against Gram-positive microorganisms might represent a valid therapeutic option for severe infections caused by COL-resistant *A. baumannii*. The observed clinical effectiveness was confirmed by both SBA (performed at the 3rd day of COL+VAN+RIF therapy) and killing studies, which showed a high and early bactericidal activity for all the tested combinations. Interestingly, COL+VAN was more rapid in reducing the bacterial load than COL+RIF, which has hitherto been considered a primary regimen against carbapenem-resistant *A. baumannii* (Durante-Mangoni *et al*., 2013). The rationale of using COL+VAN or RIF is based on the cell-permeabilizing properties of COL, which might allow the other drug to reach its target at inhibitory concentrations (Gordon *et al*., 2010; Vidaillac *et al*., 2012). Although preliminary *in vitro* data confirmed this effect even in the presence of COL resistance (Vidaillac *et al*., 2012; O'Hara *et al*., 2013; Bae *et al*., 2016), the clinical experience is still limited. Thus, the favorable result of combined clinical and microbiological data in the present case might be of crucial importance for the treatment of severe infections caused by colistin-resistant *A. baumannii*.

Given that both *A. baumannii* and MRSA were isolated from the patient's bronchoalveolar lavage, we should consider that the use of VAN and RIF as part of triple therapy against *A. baumannii* might also have had a role in the containment of *S. aureus* infection.

One of the major concerns regarding the combination COL+VAN resides in its potential nephrotoxicity. Although data are still controversial (Garnacho-Montero *et al*., 2013; Petrosillo *et al*., 2014), our case confirms the potential toxicity of this regimen and we were forced to reduce colistin dosages and to discontinuate VAN after 6 days of therapy. This latter aspect should be considered, especially if concomitant multiple nephrotoxic drugs are administered. If renal failure is a major concern, the combination COL+RIF is also highly effective, as confirmed by the *in vitro* experiments showing a rapid and durable antibacterial killing.

In conclusion, the results of our study suggest that COL, VAN and RIF used in different combinations might confer a therapeutic benefit in the treatment of severe infections caused by MDR *A. baumannii*, even in the presence of COL-resistant strains. Furthermore, we highlight the importance of performing *in vitro* synergy analyses, in order to guide treatment decisions and predict the potential efficacy of the selected antimicrobial combination.

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