# EP0132 Efficacy of colistin, alone and in combination with rifampicin, in a murine sepsis model due to carbapenemase-producing *Klebsiella pneumoniae* clinical strains

**Contact person:** M Carmen Conejo. cconejo@us.es

**Pachón Ibáñez ME[1], Labrador Herrera G[1], Cebrero Cangueiro T[1], Diaz C[4], Smani Y[1], Pérez del Palacio J[4], Rodríguez Baño J[2,3], Pascual A[2,3], Pachón J[1,3], Conejo MC[3].**

[1]Biomedicine Institute of Seville (IBIS)-University Hospital Virgen del Rocío-Seville, [2]University Hospital Virgen Macarena-Seville, [3]University of Seville, [4]Fundacion Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, MEDINA Fundation, Granada.

## Introduction and Purpose.

Treatment of severe infections caused by carbapenemase-producing *Klebsiella pneumoniae* (CPKp) is very complicated because these organisms very often present resistance to other families of antimicrobial agents, and combined therapy is frequently used. This approach seems to be associated with a lower mortality. The purpose of this study was to evaluate the *in vivo* activity of colistin (COL) alone and in combination with rifampicin (RIF) against four clinical isolates of CPKp, in a murine sepsis model.

## Methods.

Strains: Four clonally unrelated clinical isolates of CPKp, producing VIM-1, VIM-1+DHA-1 (acquired AmpC β-lactamase), OXA-48+CTX-M-15 (extended spectrum β-lactamase) and KPC-3, respectively, were evaluated.

Animals: Immunocompetent C57BL/6 female mice were used for the animal model. Pharmacokinetic/pharmacodynamic (PK/PD) parameters of RIF and COL were calculated (HPLC-MS/MS). The Bacteria Minimal Lethal Dose (MLD) was characterized for each of the four strains.

A murine sepsis model was used to carry out the efficacy studies. Groups of 15 infected animals were randomly assigned to the following treatment groups (3 days):

- Control (untreated)
- RIF (25 mg/kg/6h intraperitoneally [ip])
- COL (20 mg/kg/8h, ip)
- RIF + COL.

All treatments were initiated 4 hours post-inoculation. Bacterial counts in spleen, bacteraemia and mortality were analysed (ANOVA, post-hoc tests, and chi-square test); a $p < 0.05$ was considered significant.

## Results.

PK parameters of RIF and COL, respectively, were: Cmax (mg/L), 72.58 and 2.87; $AUC_{0-24}$ (mg*h/L), 1103.58 and 14.41; $T_{1/2}$ (h), 19.33 and 1.1. PD parameters were: RIF (VIM-1, VIM-1+DHA-1, OXA-48+CTX-M-15 and KPC-3): $AUC_{0-24}$/MIC: 34.5, 8.60, 34.5 and 17.2, respectively; COL (VIM-1, VIM-1+DHA-1, OXA-48+CTX-M-15 and KPC-3): *f*AUC0-24/MIC: 9.4, <0.15, 9.4 and 0.15. The MLD ($Log_{10}$ UFC/ml) of the four CPKp were: 9.18 (VIM-1), 9.41 (VIM-1+DHA-1), 9.24 (OXA-48+CTX-M-15), and 8.64 (KPC-3).

Bacterial counts in spleen of mice infected with VIM-1- and OXA-48+CTX-M-15-producing strains and treated with RIF alone were lower than controls, and the RIF+COL combination was better than controls and COL alone in mice infected with these CPKp, and better than the controls in those infected with the KPC-3-producing strain.

The RIF+COL combination decreased the bacteraemia compared to controls monotherapies in mice infected with the VIM-1 producer. Finally, the mortality was lower in the RIF+COL group than in controls in mice infected with the KPC-3 producer, and than COL alone in those infected with OXA-48+CTX-M-15 and KPC-3 producers (Table). No treatment was effective in relation to bacterial count, bacteraemia and mortality for the mice infected with the VIM-1+DHA-1 producer.

| | VIM-1 | | | | VIM-1+DHA-1 | | | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **RIF 100 mg/kg** | **COL 60 mg/kg** | **RIF+COL** | **Control** | **RIF 100 mg/kg** | **COL 60 mg/kg** | **RIF+COL** |
| **Spleens Log CUF/g (Mean ± SD)** | 8.92 ± 0.46 | **7.14 ± 0.48[a]** | 8.60 ± 0.30 | **6.01 ± 1.77[a]** | 9.46 ± 0.32 | 9.68 ± 0.09 | 9.54 ± 0.34 | 7.64 ± 3.02 |
| **Bacteremia (%)** | 100 (9/9) | 100 (8/8) | 100 (15/15) | **53.33 (8/15)[b]** | 100 (10/10) | 100 (8/8) | 100 (6/6) | **73.33 (11/15)[b]** |
| **Mortality (%)** | 100 (9/9) | 100 (8/8) | 93.33 (14/15) | 93.33 (14/15) | 100 (10/10) | 100 (8/8) | 100(6/6) | 73.33 (11/15) |
| | **OXA-48+CTX-M-15** | | | | **KPC-3** | | | |
| | **Control** | **RIF 100 mg/kg** | **COL 60 mg/kg** | **RIF+COL** | **Control** | **RIF 100 mg/kg** | **COL 60 mg/kg** | **RIF+COL** |
| **Spleens Log CUF/g (Mean ± SD)** | 9.56 ± 0.47 | **8.24 ± 0.76[a]** | 9.52 ± 0.55 | **7.06 ± 2.50[a]** | 10.19 ± 0.29 | 7.20 ± 2.96 | 9.19 ± 1.85 | **6.76 ± 2.48[d]** |
| **Bacteremia (%)** | 100 (10/10) | 100 (10/10) | 100 (15/15) | 87.67 (13/15) | 100 (10/10) | 88.88 (8/9) | 100 (8/8) | 77 (13/15) |
| **Mortality (%)** | 100 (10/10) | 100 (10/10) | 100 (15/15) | **73.33 (11/15)[c]** | 100 (10/10) | **66.67 (6/9)[d]** | **75 (6/8)[c]** | **40 (6/15)[d]** |

a: $p \leq 0.02$ compared with the control and the colistin groups.
b: $p < 0.05$ compared with the control, the rifampicin and the colistin groups.
c: $p \leq 0.03$ compared with the colistin group.
d: $p \leq 0.05$ compared with the control group.

## Conclusions.

**The combination RIF+COL is effective in the bacterial clearance in spleen, and moderately in decreasing mortality and bacteraemia in systemic experimental infection by VIM-1-, OXA-48+CTX-M-15- and KPC-3- CPKp clinical isolates. No treatment was effective against the infection caused by the VIM-1+DHA-producing isolate.**

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