Antibiotic combinations for serious infections caused by carbapenem-resistant *Acinetobacter baumannii* in a mouse pneumonia model

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**Objectives:** Successful therapy of carbapenem-resistant *Acinetobacter baumannii* strains has been reported with colistin, but recently we argued against its use as monotherapy because of the poor results obtained in a mouse pneumonia model. Our aim was to identify antibiotic combinations that were valid therapeutic alternatives in the same model.

**Methods:** We used two carbapenem-resistant *A. baumannii* strains (D and E; MICs of imipenem, 8 and 512 mg/L, respectively). MICs of tobramycin, rifampicin and colistin for both strains were 8, 8 and 0.5 mg/L, respectively.

**Results:** In infections caused by strain D, lung bacterial counts (log10 cfu/g, mean ± S.D.) were: controls (10.86 ± 0.25), imipenem (5.99 ± 0.59, *P* < 0.05 versus controls), and colistin (10.43 ± 1.09); imipenem + tobramycin was the most active combination (5.46 ± 0.62, *P* < 0.05 versus controls). In infections caused by strain E, results were: controls (10.82 ± 0.33), rifampicin (5.62 ± 0.26, *P* < 0.05 versus controls), colistin (8.38 ± 1.22, *P* < 0.05 versus controls), and imipenem (11.01 ± 0.2); rifampicin + imipenem (3.79 ± 0.99) and rifampicin + tobramycin (3.96 ± 0.30) were the most active combinations (*P* < 0.05); results with rifampicin + colistin (5.59 ± 1.17) were similar to those with rifampicin alone.

**Conclusions:** Our data indicate that imipenem can still be the best alternative for carbapenem-resistant *A. baumannii* infections with moderate levels of imipenem resistance, preferably combined with aminoglycosides. For strains highly resistant to imipenem, a combination of rifampicin with imipenem, tobramycin or colistin may be useful, if resistance to rifampicin is only moderate.

Keywords: multiresistant, *A. baumannii*, experimental, animals

**Introduction**

*Acinetobacter baumannii* is a major nosocomial pathogen worldwide.1–7 *A. baumannii* infections pose a serious clinical problem due to the ability of the microorganism to acquire resistance to almost all groups of commercially available antibiotics, including carbapenems.16–22

Since 1992, our hospital has suffered a sustained endemic of multiresistant *A. baumannii* infections. The pathogen became carbapenem-resistant in 1997 and since then, most of the strains have only been susceptible to colistin in *vitro*.6 Because of its toxicity, colistin has not been used to treat infections caused by Gram-negative bacilli for many years. This antibiotic has been classically considered less effective than others, and clinical experience in treating infections caused by *A. baumannii* with colistin remains limited.23–26

Effective mouse models of pneumonia due to *A. baumannii* have been described previously, using immunosuppressed7 and immunocompetent mice.28 In our recent paper comparing the efficacy of colistin versus β-lactams, aminoglycosides and rifampicin in a mouse model of pneumonia caused by multiresistant *A. baumannii*, colistin showed the poorest results of all the antibiotics tested. We therefore argued against its use in patients with pneumonia caused by carbapenem-resistant *A. baumannii*...
strains. This study aimed to assess the efficacy of several antibiotic combinations, including rifampicin plus β-lactams (imipenem and sulbactam), tobramycin or colistin in a mouse model of pneumonia caused by carbapenem-resistant \textit{A. baumannii}, and thus to identify valid therapeutic alternatives for patients infected with such strains.

**Materials and methods**

**Challenge strains**

We selected two multiresistant strains of \textit{A. baumannii} that belonged to two major clones (named D and E) endemic in our hospital. MICs, MBCs and criteria of susceptibility or resistance were established according to NCCLS recommendations, except for rifampicin, for which we used the interpretative breakpoint recommended for \textit{Staphylococcus aureus} (resistance indicated by MIC ≥ 4 mg/L), and for colistin, for which the criterion of resistance was established according to MENSURA guidelines.

Genes encoding known carbapenemases of classes B and D were sought by PCR using specific primers as previously reported.

**In vitro procedures**

We evaluated the \textit{in vitro} bactericidal activity by time–kill curves. The methodology was described in detail in our previous work. The experiments were carried out with a final inoculum of 10^5 cfu/mL. To investigate possible synergy between combinations of two antibiotics, we selected subinhibitory concentrations of drugs ranging from 1/2 to 1/32 × MIC according to the MICs for the strains and concentrations achievable in serum in humans. Bactericidal activity of combinations was defined as ≥3-log_{10} decrease in the initial inoculum in cfu/mL at 6 and 24 h. The results were interpreted by the effect of the combination in comparison with the most active single drug alone. Synergism was defined as a ≥2-log_{10} increase in killing with the combination, in comparison with the most active single drug. Indifference was defined by <1-log_{10} change (increase or decrease) in killing, in comparison with the most active single antimicrobial alone. Antagonism was defined as a ≥2-log_{10} decrease in killing with the combination, compared with the most active single drug alone.

**In vivo experiments**

**Mouse pneumonia model.** As we described elsewhere, we used immunocompetent specific pathogen-free C57BL/6N young female mice, weighing 14–16 g, supplied by Harlan (Gannat, France). We used the model described by Esposito & Pennington, and modified by Rodriguez-Hernández \textit{et al.} For the intratracheal instillation, we used 50 μL of an inoculum of 5 × 10^6 cfu/mL in exponential growth phase diluted to 50% with porcine mucin.

All antibiotics used were obtained from laboratory standard powders and diluted in sterile saline solution immediately before intraperitoneal administration. Total daily doses of imipenem (Merck Sharp & Dohme, Madrid, Spain) (200 mg/kg), sulbactam (Pfizer, Madrid, Spain) (120 mg/kg), tobramycin (Braun, Barcelona, Spain) (60 mg/kg) and colistin-methanesulphonate (Pharmax Limited, Bexley, UK) (500 000 U/kg) were divided into four doses and administered every 6 h. Rifampicin (Aventis, Barcelona, Spain) (25 mg/kg) was administered in a single daily dose. The determination of pharmacokinetic and pharmacodynamic parameters of each antibiotic was described previously.

The antibiotic combinations studied in the pneumonia model differed for the two strains of \textit{A. baumannii}, due to differences in their susceptibility patterns and their therapeutic applicability in humans.

Therapies for strain D included a combination of two β-lactams (imipenem with sulbactam), one β-lactam (imipenem or sulbactam) with an aminoglycoside (tobramycin), and combinations of rifampicin with one β-lactam (the ‘gold standard therapy’ such as imipenem) or tobramycin. In pneumonia caused by strain E with OXA-24-mediated high-level imipenem resistance, imipenem and sulbactam showed no activity, and this combination was not tested. In contrast, combinations of rifampicin with sulbactam or colistin were included for this strain. The combination of tobramycin and colistin was not tested, since the high risk of nephrotoxicity precludes consideration in routine clinical practice.

Therapy was initiated 4 h after induction of the pneumonia, when histological features of pneumonia had already appeared and lung bacterial counts had reached a peak, which remained stable for at least 48 h. We randomized the infected animals to the control or treatment group. Controls were 15 and 18 mice at each time point of 24 and 48 h after inoculation in strains D and E, respectively. Furthermore, four mice were used in the treatment group at each combination therapy at each 24 and 48 h time points. Lungs were processed to obtain quantitative counts and results were expressed as mean ± S.D. of log_{10} of cfu per g of lung in the control and treated groups at the two time points, and the difference between the two groups was calculated (Δlog = mean_{treated} group – mean_{control} group).

Moreover, bacteraemia was investigated by collecting blood via cardiac puncture, and the survival of all animals was recorded at each 24 and 48 h time points.

To eliminate the antibiotic carry-over effect, we killed the treated animals more than 3 h after the last dose of antibiotic and homogenates of lungs were cultured in undiluted form in plates to analyse this effect. To investigate the development of rifampicin resistance, we determined the MIC by Etest at the end of therapy in a sample of isolates from lung homogenates of treated mice.

All control animals had positive blood cultures at 24 and 48 h after induction of pneumonia, reflecting the virulence of the infection model. At 24 h, all the control animals survived. Mortality at 48 h in controls infected with the two strains was not significantly different: six out of 15 animals (40%) (strain D) and eight out of 18 animals (44.4%) (strain E).

The studies described in this manuscript were reviewed and approved by the Ethics Committee for Animal Experiments at the University of Barcelona (Bellvitge Campus). Animal experimentation guidelines were followed.

**Statistical analysis**

The investigations were done in two sets of experiments, for monotherapies and combinations. Results of experiments regarding monotherapies were published previously. In the present set of combination experiments, the lung bacterial counts were calculated in the control and treated groups and presented as mean ± S.D. The Kolmogorov–Smirnov test showed that the data for lung bacterial counts were normally distributed. To evaluate the therapeutic efficiency of each antibiotic combination, we compared the lung counts and the percentages of bacteraemia and mortality in each group of treated mice for each therapy, strain and time point with the same data from the controls infected with the same strain in this set of experiments.

We compared the lung bacterial counts in the two groups of control animals (the previous set of monotherapies versus the present set of combinations) with the Student’s \textit{t}-test. As we did not find significant differences, we assumed that the two groups of control animals were equal and that we could also compare the counts obtained in the therapy groups between combinations.
Experimental therapies against multiresistant *A. baumannii* infections

and monotherapies. Student’s *t*-test and analysis of variance (ANOVA) with Scheffé’s correction test were used when appropriate to compare differences in bacterial counts between groups. In a similar way, we have shown that there were no significant differences in data for bacteraemia and mortality between the control groups of the two sets of experiments (monotherapies and combinations). To compare bacteraemia or mortality between groups, the two-tailed Fisher’s exact test was used. In all tests, differences were considered statistically significant when *P* < 0.05.

**Results**

**Susceptibilities of challenge strains and carbapenemase production**

*A. baumannii* strains D and E differed in their level of resistance to imipenem (MIC/MBC: 8/16 and 512/512 mg/L, respectively) and sulbactam (MIC/MBC: 4/64 and 128/>128 mg/L, respectively). Both strains were resistant to rifampicin (MIC/MBC 8/8 mg/L), showed intermediate resistance to tobramycin (MIC/MBC 8/32 mg/L), and were susceptible to colistin (MIC/MBC 0.5/1 and 0.5/2 mg/L for strains D and E, respectively).

A gene encoding an OXA-24-like carbapenemase was detected by PCR in strain E, but not in strain D. Neither of the strains contained genes encoding OXA-23-like enzymes or IMP- and VIM-type metallo-β-lactamases.

**In vitro time–kill studies**

Among the combinations tested at concentrations that were potentially achievable in serum in humans, a synergic effect was shown against strain D with imipenem and sulbactam, tobramycin or rifampicin; and with sulbactam and tobramycin (Table 1). For strain E, a synergic effect was found with rifampicin and imipenem, sulbactam or tobramycin; and with sulbactam and tobramycin (Table 2). Antagonism was not found for any combination.

**In vivo therapeutic efficacy**

Lung bacterial counts in treated and control animals are shown in Table 3. No significant differences in lung bacterial counts were found between the control animals for the two strains. Differences between means for treated and control animals are expressed in Figure 1. The efficacy of the antibiotics was more

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Table 1. Results of *in vitro* time–kill curves of *A. baumannii* strain D for combinations of antibiotics used in the model expressed as synergic (S) or indifferent (I) activity in relation to monotherapies

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentrations × MIC (mg/L)</th>
<th>Sulbactam</th>
<th>Tobramycin</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2 (2 mg/L)</td>
<td>1/4 (1 mg/L)</td>
<td>1/2 (4 mg/L)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1/4 (2 mg/L)</td>
<td>I</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>1/8 (1 mg/L)</td>
<td>I</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>Sulbactam</td>
<td>1/2 (2 mg/L)</td>
<td>S</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>1/4 (1 mg/L)</td>
<td>S</td>
<td>S</td>
<td>ND</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1/2 (4 mg/L)</td>
<td>S</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/4 (2 mg/L)</td>
<td>S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not done.

*Bactericidal activity.*

Table 2. Results of *in vitro* time–kill curves of *A. baumannii* strain E (OXA-24 producer) for combinations of antibiotics used in the model expressed as synergic (S) or indifferent (I) activity in relation to monotherapies

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentrations × MIC (mg/L)</th>
<th>Sulbactam</th>
<th>Tobramycin</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/4 (32 mg/L)</td>
<td>1/8 (16 mg/L)</td>
<td>1/2 (4 mg/L)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1/16 (32 mg/L)</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>1/32 (16 mg/L)</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Sulbactam</td>
<td>1/4 (32 mg/L)</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>1/8 (16 mg/L)</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1/2 (4 mg/L)</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>1/4 (2 mg/L)</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>1/2 (0.25 mg/L)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1/4 (0.12 mg/L)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not done.

*Bactericidal activity.*
evident at 48 h of therapy, so we show data only at this time point. Interestingly, all combinations tested showed some degree of antibacterial activity, reducing lung counts and bacteraemia significantly compared with controls (P < 0.05). In addition, survival was 100% in mice treated with any of the combinations used (P < 0.05 versus controls). For monotherapies versus strain D, survival was 100% for imipenem or rifampicin, 75% for sulbactam or tobramycin and similar to control animals for colistin alone; versus strain E, survival was similar to controls for imipenem or sulbactam, 100% for tobramycin or rifampicin and 87.5% for colistin alone.

Strain D (moderately carbapenem-resistant, MIC 8 mg/L). The addition of tobramycin to imipenem or sulbactam improved lung bacterial clearance by about 0.5–1 log [P = not significant (NS)], though this strain showed moderate tobramycin resistance (MIC, 8 mg/L). Imipenem plus tobramycin was the most active combination of those tested, reducing lung counts by 5.4 logs (P < 0.05). The possible additive effect of the imipenem–tobramycin combination was also observed in blood clearance, but it was not significant (bacteraemia 0% for the combination versus 37.5% for colistin alone).

The imipenem–sulbactam combination did not provide any additive effect in decreasing lung bacterial counts and there was even a degree of antagonism. No differences in bacteraemia were found (sulbactam 62.5%, the combination 37.5%, P = NS). Addition of rifampicin to imipenem or tobramycin did not result in any significant improvement versus monotherapies either in lung counts or in blood clearance (bacteraemia with rifampicin or imipenem–rifampicin 50%, tobramycin–rifampicin 37.5%).

Strain E (highly carbapenem-resistant, MIC 512 mg/L). Though monotherapies with imipenem or sulbactam were totally ineffective against this pneumonia, the addition of either to tobramycin significantly decreased lung bacterial counts in comparison with tobramycin alone (P < 0.05) (Table 3). This additive effect was not significant in the bacteraemia results (imipenem 100%, sulbactam 87.5%, tobramycin 25%, imipenem–tobramycin 25%, sulbactam–tobramycin 0%, P = NS).

While the addition of sulbactam to rifampicin did not have a significant effect, imipenem added to rifampicin provided better lung clearance than rifampicin alone (P < 0.05). In fact, the imipenem–rifampicin combination was the most active of all those tested, decreasing lung bacterial counts by 7 logs compared with control animals. As for bacteraemia, neither sulbactam–rifampicin (bacteraemia 12.5%) nor imipenem–rifampicin (bacteraemia 25%) combinations showed a significant additive effect versus rifampicin alone (bacteraemia 37.5%).

### Table 3. Lung bacterial counts \(^{(a)}\) (log\(_{10}\) cfu/g of lung tissue, expressed as mean counts ± S.D.) after 48 h of therapy according to therapy and infecting strain of *A. baumannii*

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Strain D</th>
<th>Strain E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.86 ± 0.25</td>
<td>10.82 ± 0.33</td>
</tr>
<tr>
<td>Monotherapies(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>imipenem</td>
<td>5.99 ± 0.59(^{c})</td>
<td>11.01 ± 0.2</td>
</tr>
<tr>
<td>sulbactam</td>
<td>7.16 ± 1.95(^{c})</td>
<td>10.73 ± 0.20</td>
</tr>
<tr>
<td>tobramycin</td>
<td>7.38 ± 0.94(^{c})</td>
<td>6.61 ± 1.16(^{c})</td>
</tr>
<tr>
<td>rifampicin</td>
<td>7.21 ± 0.29(^{c})</td>
<td>5.62 ± 0.26(^{c})</td>
</tr>
<tr>
<td>colistin</td>
<td>10.43 ± 1.09</td>
<td>8.38 ± 1.22</td>
</tr>
<tr>
<td>Combinations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPM + SUL</td>
<td>7.49 ± 0.29(^{c})</td>
<td>ND</td>
</tr>
<tr>
<td>IPM + TOB</td>
<td>5.46 ± 0.62(^{d})</td>
<td>5.18 ± 0.64(^{d})</td>
</tr>
<tr>
<td>IPM + RIF</td>
<td>7.01 ± 1.01(^{c})</td>
<td>3.79 ± 0.99(^{d})</td>
</tr>
<tr>
<td>SUL + TOB</td>
<td>6.21 ± 0.44(^{d})</td>
<td>5.82 ± 1.14(^{d})</td>
</tr>
<tr>
<td>SUL + RIF</td>
<td>ND</td>
<td>5.95 ± 0.67</td>
</tr>
<tr>
<td>RIF + TOB</td>
<td>6.86 ± 0.15(^{c})</td>
<td>3.96 ± 0.30(^{d})</td>
</tr>
<tr>
<td>RIF + COL</td>
<td>ND</td>
<td>5.59 ± 1.17</td>
</tr>
</tbody>
</table>

\(^{a}\)15 and 18 animals were used as controls for strains D and E, respectively. Furthermore, four mice were used in each therapy.

\(^{b}\)Data for monotherapies were published previously.\(^{29}\)

\(^{c}\)Differences were statistically significant compared with the control group (P < 0.05).

\(^{d}\)Differences were statistically significant compared with the more active antibiotic alone (P < 0.05).
Experimental therapies against multiresistant *A. baumannii* infections

The addition of tobramycin to rifampicin significantly improved the efficacy of rifampicin alone (*P* < 0.05), decreasing lung bacterial counts by 6.8 logs in comparison with control animals. However, this additive effect did not reach statistical significance in blood clearance (bacteraemia 0%, *P* = NS versus monotherapies). In contrast, the addition of colistin to rifampicin did not provide any significant difference in lung bacterial counts and bacteraemia (0%) in comparison with rifampicin alone.

With regard to the possible development of resistance to rifampicin, we did not find any changes in the MICs of rifampicin at the end of therapy in the samples examined.

**Discussion**

Among the nosocomial infections caused by *A. baumannii*, ventilation-associated pneumonia has been reported to be particularly severe. Since imipenem has been widely accepted in recent years as the gold standard therapy for these infections, the appearance of carbapenem resistance in many hospitals, including our own, is a matter of great concern.

For this study, we selected the *A. baumannii* mouse pneumonia model described by Rodríguez-Hernández et al., because it does not require immunosuppression to facilitate the development of pneumonia and thus reproduces more faithfully the usual condition of patients suffering from *A. baumannii* nosocomial pneumonia in an ICU setting. We used carbapenem-resistant isolates representing two clinical strains that are responsible for the current outbreak in our hospital: one was moderately resistant (strain D, imipenem MIC, 8 mg/L) and the other highly resistant (strain E, imipenem MIC, 512 mg/L). While the mechanism of low-level carbapenem resistance in strain D remains undefined, an OXA-24-like carbapenemase was identified in strain E. These enzymes have been reported previously in Spain. A combination of β-lactamase activity, loss of outer membrane proteins and penicillin-binding protein changes have been proposed to account for high-level carbapenem resistance in similar strains.

The present paper reports the results of our investigations with several antibiotic combinations, in search of potential therapeutic alternatives to treat patients with carbapenem-resistant *A. baumannii* life-threatening infections.

Our model systematically caused histological findings of pneumonia, with bacterial counts of 10–11 log10 cfu/g of lung, 100% bacteraemia at 24 and 48 h and mortality at 48 h varying between 40% and 44.4% according to strains. The results in control animals for lung bacterial counts, bacteraemia or survival were strongly homogenous, even between the two strains with different susceptibilities, demonstrating that the model was able to compare the efficacy of therapies. Overall, the results of *in vivo* efficacy of the antibiotics used alone were in close agreement with the pharmacokinetics and pharmacodynamics obtained in mice, with the exception of colistin. However, as we noted in our first report, the pharmacodynamics of tobramycin in this model may well have been overestimated, since the peak levels achieved are not usually found in humans at the recommended doses. Lung bacterial count at 48 h was the most useful comparative parameter for evaluation of antibiotic efficacy in this experimental model, since all antibiotic combinations provided 100% survival and the differences found in bacteraemia and lung bacterial counts were usually in the narrow range. While we had to use a reduced number of animals in each therapeutic group, according to the current and strict guidelines for animal experimentation, this number was enough to make evident the significant differences obtained in the variable ‘lung bacterial counts’ at 48 h between the majority of therapies.

In infections caused by the moderately resistant strain D, imipenem and sulbactam maintained good bactericidal efficacy. Tobramycin conferred a possible greater efficacy on these β-lactams, in the light of the synergy found with these combinations in time–kill curves. The imipenem–tobramycin combination was the most active therapy against pneumonia caused by this moderately carbapenem-resistant strain. Interestingly, the combination also showed a higher effect on the highly resistant strain E, against which monotherapy with imipenem or sulbactam was totally ineffective. The higher antibacterial effect of imipenem–tobramycin versus sulbactam–tobramycin was probably due to the greater *in vitro* bactericidal activity exhibited by imipenem than sulbactam in killing curves and the post-antibiotic effect reported with imipenem in treating *A. baumannii*. To our knowledge, no clinical data are currently available to evaluate the systematic use of the β-lactam–aminoglycoside combination to treat serious *A. baumannii* infections, but probably this practice is not routinely required. However, the additive effect of the combination of tobramycin and imipenem or sulbactam may be particularly important in the treatment of *A. baumannii* pneumonia with moderate carbapenem resistance.

In contrast, the combination of imipenem and sulbactam did not show any additive effect on the pneumonia caused by strain D, which was in disagreement with that reported by Wolff et al. in a pneumonia model in immunosuppressed mice.

Rifampicin presented high bactericidal activity when used alone against pneumonia caused by the highly carbapenem-resistant strain E. This efficacy was also observed with strain D, though to a lesser degree. The two strains had previously been considered resistant to rifampicin *in vitro* (MIC, 8 mg/L), but at high doses *in vivo* the pharmacodynamic parameters of rifampicin in this animal model were favourable. A similar pharmacokinetic behaviour may be achievable in humans using tolerable high doses about 20 mg/kg per day. In their model, Wolff et al. using *A. baumannii* strains with rifampicin MICs of 4–8 mg/L, reported similar results. Furthermore, among the combinations including rifampicin, rifampicin–imipenem and rifampicin–tobramycin were particularly active against the pneumonia due to strain E, providing a significantly better antibacterial effect than rifampicin alone. This effect was correlated to the microbiological results shown by time–kill curves. In contrast, the rifampicin–sulbactam combination had no impact on pneumonia caused by strain E, despite the synergy found *in vitro*. It is unclear why we observed different efficacy of rifampicin alone or in combination against strains D and E, while they showed similar MICs. We may speculate that the increase in antibiotic resistance in *A. baumannii* could lead these strains to a higher vulnerability, as reported for other multiresistant microorganisms. Finally, although *in vitro* synergic activity has been reported with the combination of colistin and rifampicin, it was not observed in the *in vivo* model, in a similar way to our results from the time–kill curves.

It is generally accepted that rifampicin should not be used as monotherapy against Gram-positive infections because of the early development of resistance. However, Gram-negative
infections caused by carbapenem-resistant
humans should be made with great caution, 41 we believe that our
While any extrapolation of results of experimental infections to
lyse this phenomenon. 29 Wolff
resistance to rifampicin after 48 h of therapy, but this time
We are indebted to: J. Pachón, M. J. Rodríguez-Herna´ndez and
Acknowledgements
bacteria are also probably able to develop early rifampicin resist-
against recommending a single therapy for these infections. Accordingly, from our data, we conclude that imipenem is ineffective against infections caused by strains with high-level carbapenem resistance, but that it can still be the best alternative for some strains with moderate carbapenem resistance, preferably used in combination with aminoglycosides. Monotherapy with colistin may not be the best option for infections caused by highly carbapenem-resistant strains susceptible only to this antibiotic in vitro; a combination of rifampicin–imipenem, rifampicin–aminoglycoside or even rifampicin–colistin may be more advisable, if resistance to rifampicin is only moderate. Further studies on the use of rifampicin in clinical practice should aim to identify which of these combinations is the most efficacious and which best prevents the emergence of resistance to this drug.

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References
Experimental therapies against multiresistant A. baumannii infections


