Efficacy of rifampin and its combinations with imipenem, sulbactam, and colistin in experimental models caused by imipenem-resistant *Acinetobacter baumannii*.

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Footnote

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ABSTRACT

Background

There are currently no defined optimal therapies available for multidrug-resistant (MDR) Acinetobacter baumannii infections. We evaluated the efficacy of rifampin, imipenem, sulbactam, colistin, and their combinations against MDR A. baumannii in experimental pneumonia and meningitis models.

Methods

The bactericidal in vitro activity of rifampin, imipenem, sulbactam, colistin, and their combinations were tested using time-kill curves. Murine pneumonia and rabbit meningitis models were performed using the A. baumannii strain Ab1327 (with MICs for rifampin, imipenem, sulbactam, and colistin of 4, 32, 32, and 0.5 mg/l, respectively). Mice were treated with the four antimicrobials and their combinations. For the meningitis model, the efficacy of colistin, rifampin and its combinations with imipenem, sulbactam, or colistin, and of imipenem plus sulbactam were assayed.

Results

In the pneumonia model, compared to the control group, rifampin alone or plus imipenem, sulbactam or colistin, colistin, and imipenem plus sulbactam significantly reduced lung bacterial concentrations (10.6±0.27 vs. 3.05±1.91, 2.07±1.82, 2.41±1.37, 3.4±3.07, 6.82±3.4, and 4.22±2.72 log_{10} CFU/g, respectively), increased sterile blood cultures (0% vs. 78.6%, 100%, 93.3%, 93.8%, 73.3%, and 50%), and improved survival (0% vs. 71.4%, 60%, 46.7%, 43.8%, 40%, and 85.7%). In the meningitis model rifampin alone or plus colistin reduced cerebrospinal fluid (CSF) bacterial counts (-2.6 and -4.4 log_{10} CFU/ml).

Conclusions
Rifampin in monotherapy or plus imipenem, sulbactam or colistin showed efficacy against MDR *A. baumannii* in experimental models of pneumonia and meningitis. Imipenem or sulbactam may be appropriate for combined treatment when using rifampin.
Acinetobacter baumannii is an important nosocomial pathogen worldwide (5, 36), with pneumonia, bacteremia, surgical site and urinary tract infections being the most important infections caused by this organism (16). A Spanish study showed A. baumannii as the cause of nearly 9% of cases of ventilator-associated pneumonia (VAP) (2), with a crude mortality of 40%-70% (14). A. baumannii may also cause meningitis and ventriculitis, especially in patients undergoing neurosurgical procedures or with head trauma (17), with mortality rates between 20%-27% (5).

The well-known ability of A. baumannii to acquire resistance to almost all groups of available antibiotics leads to serious problems in the management of infections caused by multidrug-resistant (MDR) A. baumannii infections (5, 16). In these cases, carbapenems have been considered the treatment of choice. However, increasing numbers of carbapenem-resistant A. baumannii isolates have been reported worldwide (1, 28), prompting the search for other therapeutic options.

Sulbactam has been used successfully in cases of meningitis and pneumonia caused by A. baumannii (17, 21, 40). Colistin, has good in vitro activity (38), but has showed contradictory results in clinical practice (12) and experimental models (23). Rifampin has demonstrated in vitro and in vivo bactericidal activity against MDR A. baumannii in an experimental pneumonia model (23), but rifampin-resistant mutants appear shortly after treatment initiation with rifampin alone (23, 27). The combination of rifampin plus imipenem has been evaluated in clinical infections caused by highly imipenem-resistant A. baumannii strains, with inconclusive results (34). Two clinical studies have shown efficacy rates of 76%-100% for colistin plus rifampin in VAP, bacteremia and meningitis (4, 25).
The aims of this study were to evaluate the efficacy of rifampin, and its combinations with imipenem, sulbactam and colistin, in experimental pneumonia and meningitis models caused by MDR *A. baumannii* strains.

**MATERIAL AND METHODS**

**Bacterial strains.** Four MDR *A. baumannii* strains (Ab506, Ab940, Ab1327, and Ab1417) isolated from patients with bacteremia and representing the most frequent clones isolated in our hospital (22) were studied. They were resistant to cefotaxime, imipenem, sulbactam, amoxicillin/clavulanate, and tetracycline, and susceptible to colistin and rifampin. *A. baumannii* Ab1327 was used for the *in vivo* studies.

**Antibiotics.** For *in vitro* assays, antimicrobials were used as standard laboratory powders. Imipenem was supplied from Merck Sharp and Dohme (Madrid, Spain), sulbactam from PharmaSierra (Madrid, Spain), and rifampin and colistin sulfate from Sigma-Aldrich (Madrid, Spain). For *in vivo* experiments, commercial vials were used: imipenem-cilastatin (Merck Sharp and Dohme), sulbactam (PharmaSierra), rifampin (Aventis, Madrid, Spain), and colistin methanosulfonate (1 mg equivalent to 12,500 IU) (Aventis, Bellon, France). The anesthetic used in the mouse pneumonia model was 5% w/v sodium thiopental intraperitoneally (ip) (B. Braun Medical S.A. Rubi, Barcelona, Spain), and ketamine (Ketolar, Parke-Davis, Madrid, Spain) and xylazine (Rompun, Bayer Healthcare, Kiel, Germany) intramuscularly (im) in the rabbit meningitis model.

**In vitro studies.** MICs of imipenem, sulbactam, rifampin, and colistin were determined according standard methods (8, 9). *Escherichia coli* ATCC 25922 was used as a control strain. The breakpoints for resistance were those defined by the Clinical and Laboratory Standards Institute (8), except for rifampin for which breakpoints from the French Society for Microbiology were used (9).
Bactericidal activity of antimicrobials was evaluated by the time-kill method (7). Time-kill curves were performed using concentrations of 1x MIC and bacterial growth was quantified 0, 2, 4, 8 and 24 h after incubation at 37°C by plating ten-fold dilutions on sheep blood agar (SBA). The limit of detection was 10 CFU/ml corresponding to 1 log CFU/ml. *In vitro* synergy studies (imipenem plus sulbactam or rifampin or colistin, sulbactam plus rifampin or colistin, and rifampin plus colistin) against the four strains were performed. Concentrations of 1xMIC of each antimicrobial in the combinations were used. Bacterial growth was measured at 0, 2, 4, 8 and 24 h after incubation at 37°C. An antimicrobial was considered bactericidal when a 3 log$_{10}$ decrease in CFU/ml was reached compared with the initial inoculum. Synergy was defined as 2 log$_{10}$ decreases in colony count of the combination compared with the most active antibiotic (11).

**Animals.** (i) **Pneumonia model.** C57BL/6 female mice, weighing 16 to 20 g (Universidad de Sevilla’s Facility, Sevilla, Spain) were used. (ii) **Meningitis model.** New Zealand female rabbits weighing 2.5 to 3 kg. (Charles River Laboratories, Barcelona, Spain) were employed. Animals were housed in regulation cages and given free access to food and water. The studies were approved by the Ethics Committee of the University Hospitals Virgen del Rocío (Sevilla, Spain).

**Pharmacokinetic/pharmacodynamic analysis.** (i) **Pneumonia model.** Serum antibiotic concentrations were determined in groups of 21 healthy mice after a single administration of imipenem 30 mg/kg/im, sulbactam 60 mg/kg/im, colistin 20 mg/kg/im or rifampin 25 mg/kg/ip. In sets of three animals and at 10, 15, 30, 60, 90, 120, and 150 minutes after administration, blood samples were obtained from anesthetized mice from the periorbital plexus. (ii) **Meningitis model.** Serum antibiotic concentrations were determined in groups of 4 healthy rabbits after a single administration of imipenem 120 mg/kg/im, sulbactam 30 mg/kg/im, colistin 12 mg/kg/im, or rifampin 25 mg/kg/intravenously (iv). Blood samples
were drawn from the marginal vein of the ear at 5, 10, 15, 30, 60, 90, 120, 240, and 480 minutes. Also, in infected rabbits, serum and CSF rifampin and colistin concentrations were measured at 2, 4, and 6 hours after colistin 12 mg/kg/im and rifampin 25 mg/kg/iv.

Concentrations of imipenem, sulbactam, colistin, and rifampin were determined by using an agar-diffusion bioassay with Micrococcus luteus ATCC 9341, A. baumannii ATCC 19606, Bordetella bronchiseptica ATCC 4617, and Bacillus subtilis ATCC 6633, as control strains, respectively (19). Antibiotic assays were performed in triplicate. Maximum serum concentration ($C_{max}$; mg/l), area under the concentration time-curve (AUC; mg h/l), elimination half-life ($t_{1/2}$; h), AUC/MIC, and $C_{max}$/MIC were calculated by using the linear trapezoid method and the PKCALC program (35). Time during which the serum concentration remained above the MIC ($T_{MIC}$; h) was extrapolated from the regression line of the serum concentrations (13).

The doses of imipenem and sulbactam used in the pneumonia model were those that, administered in combination with rifampin, prevented the appearance of rifampin-resistant mutants (27), and are in the ranges of plasma human concentrations after standard dosing for severe infections (sulbactam 1 g IV and imipenem 500 mg IV); in the meningitis model, doses of imipenem and sulbactam were chosen to reach a $C_{max}$ similar to those in the pneumonia model. For rifampin and colistin, because there are no defined pharmacodynamic parameters that predict efficacy, doses of antimicrobials were chosen to obtain a AUC similar to that found in humans (3, 18, 33).

**Experimental models.**

(i) **Pneumonia model.** A previously characterized pneumonia model (32) was used as follows: anesthetized C57BL/6 mice (thiopental 5% w/v ip) were infected by intratracheal instillation, using 50 µl of a final inoculum of $10^8$ CFU/ml mixed 1:1 with 10% porcine mucin (Sigma-Aldrich). Therapy was initiated 4 h after the inoculation.
Fifteen mice were randomly included as controls (no treatment) and the following treatment groups: imipenem, sulbactam, rifampin, colistin, imipenem plus sulbactam, rifampin plus imipenem, rifampin plus sulbactam, and rifampin plus colistin. The total daily doses of antimicrobials were: imipenem 120 mg/kg/im, sulbactam 240 mg/kg/im, rifampin 100 mg/kg/ip, and colistin 60 mg/kg/im. Total daily doses of the drugs therapies were divided in four doses for imipenem, sulbactam, and rifampin, and colistin was administered thrice daily. Mice were observed for 72 h for mortality and the surviving animals were sacrificed 6 h after the last dose by ip administration of a lethal dose of sodium thiopental. Immediately after death, thoracotomy was carried out. Through a cardiac puncture, blood samples were taken and 100 µl plated on SBA for qualitative cultures. Lungs were homogenized in 2 ml of sterile saline solution (Stomacher 80 Tekmar Co., Cincinnati, Ohio, USA) and ten-fold dilutions were plated on SBA for quantitative cultures.

In five randomly selected mice, lung samples were processed for histopathological studies. The lungs were fixed with 10% formaldehyde, embedded in paraffin and cut into 4 µm thick sections. The slices included all pulmonary lobes to be studied by optical microscopy. They were processed according to standard methods for haematoxylin-eosin, PAS, Gram, Masson’s Trichromatic, and silver reticulin stains.

(ii) Meningitis model. The experimental meningitis model described by Dacey and Sande (10) was used. The day before the inoculation, an acrylic helmet was affixed to the rabbit skull to provide stable fixing to the stereotactic frame. The day after, animals were anaesthetized with 35 mg/kg/im of ketamine (Parke-Davis) and 5 mg/kg/im of xylazine (Bayer Healthcare). Prior to bacterial challenge, a sample of cerebrospinal fluid (CSF) was taken, by an intracisternally extraction of 200 µL, to prove its sterility, and ensure the site of inoculation. Meningitis was induced by
inoculating the same volume of a saline suspension containing $10^7$ CFU/ml of Ab1327. A CSF sample was drawn 12 h after bacterial inoculation (time 0 h), when CSF features of meningitis developed. Treatment groups were those that reached efficacy in the previous experimental pneumonia model. Eight rabbits were randomly included in one of five groups: control group (no treatment), rifampin (25 mg/kg/iv), colistin (12 mg/kg/im), imipenem (120 mg/kg/im) plus sulbactam (30 mg/kg/im), rifampin plus imipenem (25 mg/kg/iv and 120 mg/kg/im, respectively), rifampin plus sulbactam (25 mg/kg/iv and 30 mg/kg/im, respectively), and rifampin plus colistin (25 mg/kg/iv and 12 mg/kg/im, respectively). A single dose of the antimicrobial alone or the combinations were administered. Sequential CSF samples were drawn through cisternal puncture at 2, 4, and 6 h after antimicrobial administration.

Studies of indices of meningeal inflammation (white blood cells [WBC] counts and lactic acid concentration) were determined at times 0 h and 6 h. WBC counts were performed using a Neubauer chamber. CSF lactic acid concentrations were determined by an enzymatic and colorimetric method (LOX/PAP) (Roche Diagnostics, Mannheim, Germany). Quantitative bacterial cultures were performed at times 0, 2, 4, and 6 h, through ten-fold dilutions of CSF and plating 100 µL on SBA. Immediately after the 6 h extraction of CSF, rabbits were sacrificed with the administration of sodium thiopental. After the sacrifice a craniotomy was performed and the brain was extracted, weighed and then desiccated in a heater for seven days at 100º C to measure brain water. Brain edema was defined as when more than 400% in the weight brain water-content was found (37).

**Statistical analysis. (i) Pneumonia model.** The variables analyzed were: survival (%), bacterial lung concentration (mean ± SD $\log_{10}$ CFU/g of lung tissue), and blood sterility (%). The two-tailed Fisher’s test, the analysis of variances (ANOVA), and the post-hoc
tests Dunnet and Tukey were used. (ii) **Meningitis model.** The analyzed variables were: bacterial CSF concentration (log_{10} CFU/ml of CSF), WBC concentration in CSF (cells/µL), lactate concentration in CSF (mmol/l) and brain edema (grams of water/100 grams of brain dry weight). Results are expressed as median (P_{25}/P_{75}). The nonparametric Mann–Whitney and Wilcoxon tests were used. Differences were considered significant with a P value < 0.05.

**RESULTS**

**In vitro studies.** All 4 isolates were resistant to imipenem and sulbactam but susceptible to rifampin and colistin. The MIC and MBC results of the antimicrobials are shown in Table 1. Time-kill experiments with the antimicrobials alone or in combination are presented in Figure 1. Rifampin exhibited bactericidal activity for the four strains, showing a transient bactericidal effect with regrowth in the cases of the strains Ab1327 (between 8 and 24 h) and Ab1417 (between 4, 8, and 24 h), while strains Ab506 and Ab940 appear to sterilize with rifampin exposure *in vitro*. Imipenem showed bactericidal activity at some time-points against the strains Ab940, Ab1327, and Ab1417. Sulbactam did not show bactericidal activity against any strain. Colistin only showed bactericidal activity against the strain Ab940.

In the synergy studies, the combinations with rifampin were those that exhibited best activity, especially rifampin plus colistin which presented synergistic activity against the four strains at 2 h, and sterilized all 4 strains within 2 or 4 hours. The synergistic effect of rifampin plus imipenem or sulbactam was not able to be demonstrated in some cases because of the high bactericidal activity of rifampin alone. Except for rifampin plus sulbactam with the strain Ab1327, all the antimicrobial combinations with rifampin...
sterilized the bacterial cultures. The combinations with the least synergistic effect were imipenem plus sulbactam (only against Ab506).

**Pharmacokinetics and pharmacodynamics.** The serum pharmacokinetic and pharmacodynamic parameters of each antimicrobial in mice and rabbits are shown in Table 2. The $C_{\text{max}}$ of rifampin, imipenem, and sulbactam were similar in mice and rabbits; in the case of colistin the $C_{\text{max}}$ was less than one third in rabbits compared to mice, but reached a higher AUC/MIC value. The serum and CSF levels of rifampin and colistin in infected rabbits are detailed in Figure 2. The ratios of CSF to serum levels at 2 h were 12.9% and 27.5% for colistin and rifampin, respectively.

**In vivo results**

**Pneumonia model.** The efficacy of the antimicrobials, expressed as survival, bacterial lung concentration, and sterility of blood cultures, is shown in Table 3.

**i. Survival.** All treatments, alone or in combination, increased survival compared with the control group; survival with rifampin (71.4%) was higher than that observed with imipenem, sulbactam, or colistin (28.6%, 40%, and 40% respectively; NS). With respect to the combinations, imipenem plus sulbactam (85.7% vs. 0%, $p<0.05$), rifampin plus sulbactam (46.7% vs. 0%, $p<0.05$), and rifampin plus colistin (43.8% vs. 0%, $p<0.05$) improved survival in comparison with the control.

**ii. Bacterial clearance from lungs.** Monotherapy with rifampin cleared bacteria from lung tissue compared with the controls (3.05±1.9 vs. 10.6±0.27, $p<0.05$), and also compared with imipenem alone (3.05±1.9 vs. 7.87±3.43, $p<0.05$). Colistin also decreased the bacterial lung concentration compared to controls (6.82±3.4 vs. 10.6±0.27, $p<0.05$). Rifampin plus imipenem, sulbactam or colistin reduced the bacterial concentration compared with the controls (2.07±1.82; 2.41±1.37; 3.4±3.07 vs. 10.6±0.27, respectively, $p<0.05$). It must be noted that the combination of rifampin plus
imipenem or sulbactam improve the results in terms of bacterial lung concentration compared with colistin in monotherapy. Finally, the combination of imipenem plus sulbactam also reduced the bacterial lung concentration respect the control group (4.22±2.72 vs. 10.6±0.27, p<0.05).

iii. Bacterial clearance from blood. Rifampin and colistin showed the best results among the monotherapies in sterilizing blood cultures (78.6% and 73.3%, respectively) compared with controls (0%, p<0.05) and the other monotherapies (p<0.05). Of the combinations, rifampin plus imipenem, sulbactam or colistin cleared bacteria from blood compared with imipenem plus sulbactam (100%, 93.3%, and 93.8% respectively, vs. 50%; p<0.05).

iv. Histopathological studies. Lungs showed acute inflammation, characterized by diffuse and/or focal affection of all lobes, with mild to severe infiltration of polymorphonuclear cells, sometimes forming segmentary abscesses, and mild to moderate infiltration of alveolar macrophages. Gram-negative bacterial colonies and alveolar haemorrhagic areas were also observed.

Meningitis model. Table 4 summarizes the results in bacterial CSF concentration, WBC CSF levels, and brain edema.

i. Bacterial clearance from CSF. All treatments reduced bacterial concentrations at 6 h with respect to those immediately before their administration (0 h time-point) (p<0.05), with the exception of colistin which reached only a reduction of 0.8 log_{10} CFU/ml. Rifampin in monotherapy or combined to colistin showed the maximal reduction in bacterial concentration (-2.6 and -4.4 log_{10} CFU/ml, respectively).

ii. Lactate and WBC CSF levels and brain edema. Lactate CSF levels were elevated at 12 h after inoculation (p<0.05) (data not shown). WBCs were absent before
inoculation, and there was an increase in their levels during the experiments in all groups. All treatment groups showed brain edema.

DISCUSSION

The present study shows that rifampin is efficacious in the treatment of both experimental pneumonia and meningitis caused by imipenem-resistant *A. baumannii*. It is worth noting the efficacy observed with rifampin in the pneumonia model, in terms of improving survival and decreasing bacterial burden from lungs and blood. These results are in accordance with the in vitro bactericidal activity found against the four strains tested. However, it must be noted that for Ab1327 (used in the in vivo experiments) and Ab1417, there was regrowth after the 8 and 4 h, respectively, which may be due to the higher MBC of rifampin against these strains compared with the other two strains. Another possible explanation is the development of rifampin-resistance during the experiments, but with the strain Ab1327 we have reported that the induction of rifampin-resistance appear between 24 and 48 h of incubation (27).

Imipenem and sulbactam were not efficacious, as expected, because of the MIC of 32 mg/l of both antimicrobials for the *A. baumannii* strain used, which prompted a $T_{MIC}$ of 0 hours in the case of imipenem and below the value necessary to obtain efficacy in this experimental model (30). Colistin, in spite of its *in vitro* activity against the *A. baumannii* strain used in the *in vivo* experiments, showed a similar activity compared with imipenem or sulbactam in terms of survival or bacterial clearance in the pneumonia model.

The pneumonia model was chosen because pneumonia is the most common nosocomial infection among those caused by *A. baumannii* (29), and it is a well characterized and reproducible model (31). Meningitis is not a frequent nosocomial
infection, but it also has a high mortality rate and the information about the treatment in cases produced by imipenem-resistant \textit{A. baumannii} includes only a small series evaluating sulbactam (17) or cases treated with colistin (18, 26). The model described by Dacey and Sande (10) was followed in order to characterize experimental meningitis by \textit{A. baumannii} for the first time. In the meningitis model, we used only the treatments that show efficacy in the pneumonia model, to spare the use of animals. Also, the meningitis model has the limitation of using only a single dose of antimicrobials; thus, it may be that a more extended therapy produces better results. However, the bacterial clearances observed with monotherapies in the meningitis model are in accordance with those in the pneumonia model.

The efficacy of rifampin in \textit{A. baumannii} infections had been shown in experimental pneumonia models in mice (23, 39). Montero \textit{et al.} (23), using three \textit{A. baumannii} strains with a MIC of rifampin of 8 mg/l, obtained a 100\% of survival with rifampin and a significant decrease in the counts of bacteria from lungs; as in the results of the present study, imipenem and sulbactam were not efficacious in increasing survival or reducing the bacterial lung concentration when using a strain with MIC of these antimicrobials of 512 and 128 mg/l, respectively (23). In another experimental pneumonia study in neutropenic mice (39), using two strains of \textit{A. baumannii} with rifampin MICs of 8 and 4 mg/l, respectively, rifampin significantly reduced the bacterial lung concentration, to the same degree as imipenem with both strains (39); in terms of survival, rifampin was better than imipenem in the experiments with an \textit{A. baumannii} strain with intermediate susceptibility to imipenem (8 mg/l). A recent study (36) in neutropenic mice also shows efficacy of rifampin in the pneumonia model by \textit{A. baumannii}, using strains with MIC of 4 and 8 mg/l, in reducing bacterial lung concentration and bacteremia. Although their results are difficult to compare, because of
the small size of the groups (3 mice each) and the short duration of the treatment (24 or 48 h), monotherapy with colistin was less efficacious than rifampin, and the addition of colistin to rifampin did not improve the results with rifampin alone.

Colistin was used in the present study as methanosulfonate because this is the parenteral form used in humans (12). However, in spite of showing a bacterial lung reduction of approximately 3 log_{10} CFU/g with respect to the control group, colistin only improves the activity of imipenem or sulbactam in monotherapy approximately 1 log_{10} CFU/g, correlating with the absence of bactericidal activity in the time-kill experiments. These results are not in accordance with those found by Montero et al. (23) using three strains of *A. baumannii*, also with a MIC of colistin of 0.5 mg/l; in this study, the efficacy of colistin was scarce or absent in reducing the bacterial lung concentration and it did not improve the survival in any case. This discrepancy may be explained by the lower colistin AUC (11.96 mg·h/l) obtained with the dose employed in contrast with the AUC of 26.42 mg·h/l obtained in the present study, which is similar to that in humans (23.43 mg·h/l) using 1,000,000 IU (18). The moderate activity of colistin in the pneumonia model could be explained by its lack of bactericidal activity *in vitro* and poor distribution in tissues (20). The results in experimental studies are in accordance with clinical results. The mortality rate with colistin in cases of nosocomial pneumonia caused by colistin-susceptible *A. baumannii*, is high, reaching 38% (15); this mortality rate is not superior to that found with imipenem (35.7%), suggesting that neither drug is optimal treatment for severe infections caused by *A. baumannii*, in spite of their extensive clinical use.

The combination of rifampin with imipenem or sulbactam was evaluated due to previous data, showing that either imipenem or sulbactam prevented the appearance of rifampin-resistant mutants *in vitro* and *in vivo* when used concomitantly with rifampin.
However, imipenem or sulbactam (MIC of 32 mg/l in both cases) used in combination with rifampin did not improve the therapeutic efficacy of rifampin alone in the clearance of the infection or in survival, in both experimental models. In the study of Montero et al. (23) the addition of imipenem to rifampin, using a strain with MIC of 8 mg/l of both antimicrobials, did not improve the results of rifampin alone. In the neutropenic pneumonia model, imipenem or sulbactam (MIC of 0.5 mg/l in both cases) neither improved the results of rifampin alone (39). The usefulness of the combination of imipenem or sulbactam plus rifampin in the treatment of A. baumannii infections seems to be reduced to the prevention of rifampin resistance during the treatment, which has been shown both in vitro and in vivo when the MIC of imipenem or sulbactam is not higher than 32 mg/l (27). In a clinical study evaluating the efficacy of rifampin plus imipenem in nosocomial A. baumannii infections a 70% cure rate was achieved but with the development of high-level resistance to rifampin in seven out of 10 patients, probably due to the fact that the MIC of imipenem was ≥64 mg/l in all of the isolates (34).

In the pneumonia model, the combination of rifampin plus colistin is similar to that of rifampin in monotherapy. In the same way, in the experimental pneumonia model of Montero et al., rifampin maintained the same efficacy when colistin was added to the therapy (24). It is worth noting that in the meningitis model this combination was the best in reducing bacterial CSF concentration, resulting in a reduction of more than 4 log_{10} CFU/ml after 6 hours of treatment. This in vivo activity agrees with the synergy observed in vitro between both antimicrobials.

Two clinical studies have evaluated the efficacy of rifampin plus colistin in severe infections caused by A. baumannii (4, 25). Motaouakkil et al. (25) found a 100% clinical cure in nine cases of bacteremia, but these authors did not provide the MIC of
rifampin against the clinical isolates. Bassetti et al. (4, 25) obtained clinical and microbiologic cure in eight out of 10 (80%) cases of bacteremia and in 14 out of 19 (73%) cases of nosocomial pneumonia. These results are similar to those found in imipenem-susceptible A. baumannii bacteremia treated with imipenem (87.5%) (6) and slightly better than that found in nosocomial pneumonia caused by susceptible A. baumannii strains, treated with imipenem (survival 64.3%) or colistin (62%) (15). Taking into account the efficacy of rifampin alone in the pneumonia model in the present study, it is possible that the good clinical results obtained with the combination of rifampin plus colistin depends on the bactericidal activity of rifampin, and that colistin prevents the appearance of A. baumannii resistance to rifampin, as suggested by the results of Bassetti et al (4).

The combination of imipenem plus sulbactam showed a marginal therapeutic effect in these experimental models. It was efficacious regarding survival in the pneumonia model, and in the meningitis model this combination slightly reduced the bacterial CSF concentration. This antimicrobial combination has been tested by Song et al. (36) in the A. baumannii pneumonia model in neutropenic mice, also showing worse results than rifampin in the reduction of bacterial lung concentration and in the clearance of bacteremia, but with the limitations of this model detailed above.

In summary, the results in both models show that rifampin is efficacious in the treatment of severe infections, such as pneumonia or meningitis, caused by imipenem-resistant A. baumannii strains. However, rifampin must not be used alone because rifampin-resistance appears after 24 hours of monotherapy in the experimental murine pneumonia by A. baumannii (27). In this context, it is necessary to add other antimicrobial to rifampin, to prevent the development of resistance. The results of the present study suggest that imipenem or sulbactam may be an appropriate option, if their
MIC is not higher than 32 mg/l. Finally, the addition of colistin to rifampin needs to be further evaluated, in order to know if colistin also prevents the appearance of rifampin-resistant-mutants as occur with imipenem or sulbactam.
TABLE 1. MIC and MBC of imipenem, sulbactam, rifampin, and colistin for the four multidrug-resistant *A. baumannii* strains.

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<th>Strains</th>
<th>MIC/MBC (mg/l)</th>
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<td></td>
<td>Imipenem</td>
<td>Sulbactam</td>
<td>Rifampin</td>
<td>Colistin</td>
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<tr>
<td>Ab506</td>
<td>32/32</td>
<td>32/32</td>
<td>4/4</td>
<td>0.5/2</td>
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<tr>
<td>Ab940</td>
<td>32/32</td>
<td>32/32</td>
<td>4/4</td>
<td>0.5/8</td>
</tr>
<tr>
<td>Ab1327</td>
<td>32/32</td>
<td>32/32</td>
<td>4/8</td>
<td>0.5/2</td>
</tr>
<tr>
<td>Ab1417</td>
<td>32/32</td>
<td>32/32</td>
<td>8/8</td>
<td>0.5/1</td>
</tr>
</tbody>
</table>

S: susceptible; I: intermediate; R: resistant

IPM and SUL: S ≤ 4 mg/l, I = 8 mg/l, R ≥ 16 mg/l.

RIF: S ≤ 4 mg/l, R ≥ 16 mg/l.

CST: S ≤ 2 mg/l, R ≥ 4 mg/l.
Figure 1 legend.

For the monotherapies: Control, vertical ellipse; imipenem full diamond; sulbactam full circle; colistin, square; rifampin full triangle. For the combinations:
imipenem plus sulbactam, inverted triangle rifampin plus imipenem, cross; rifampin plus sulbactam, open diamond; rifampin plus colistin, horizontal line.
TABLE 2. Pharmacokinetics/pharmacodynamics of antimicrobial agents in mouse and rabbit serum.

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Antimicrobial</th>
<th>$C_{\text{max}}$ (mg/l)</th>
<th>$t_{\frac{1}{2}}$ (h)</th>
<th>AUC (mg·h/l)</th>
<th>$^a\frac{C_{\text{max}}}{\text{MIC}}$</th>
<th>$^a\frac{\text{AUC}}{\text{MIC}}$</th>
<th>$^aT_{\text{MIC}}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>IPM (30 mg/kg)</td>
<td>23.25</td>
<td>0.26</td>
<td>11.29</td>
<td>0.73</td>
<td>0.35</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SUL (60 mg/kg)</td>
<td>89.45</td>
<td>0.35</td>
<td>87.51</td>
<td>2.8</td>
<td>2.73</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>RIF (25 mg/kg)</td>
<td>15.75</td>
<td>2.86</td>
<td>24.76</td>
<td>3.94</td>
<td>6.19</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>CST (20 mg/kg)</td>
<td>27.39</td>
<td>0.54</td>
<td>26.42</td>
<td>54.78</td>
<td>52.84</td>
<td>3.72</td>
</tr>
<tr>
<td>Meningitis</td>
<td>IPM (120 mg/kg)</td>
<td>25.35</td>
<td>1.74</td>
<td>50.45</td>
<td>0.79</td>
<td>1.58</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SUL (30 mg/kg)</td>
<td>75.33</td>
<td>0.54</td>
<td>57.07</td>
<td>2.35</td>
<td>1.78</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>RIF (25 mg/kg)</td>
<td>16.62</td>
<td>3.38</td>
<td>40.28</td>
<td>4.16</td>
<td>10.07</td>
<td>6.54</td>
</tr>
<tr>
<td></td>
<td>CST (12 mg/kg)</td>
<td>9</td>
<td>3.08</td>
<td>41.72</td>
<td>18</td>
<td>83.44</td>
<td>11.37</td>
</tr>
</tbody>
</table>

IPM, imipenem; SUL, sulbactam; RIF, rifampin; CST, colistin.

$^a$ Parameter calculated for the strain Ab1327.
FIGURE 2. Concentration of rifampin and colistin in serum and CSF obtained in infected rabbits after the administration of a single dose of 25 mg/kg and 12 mg/kg, respectively.

Full triangle serum levels, full square CSF levels.

* MIC of the antimicrobials against the Ab1327 strain.
TABLE 3. Experimental pneumonia model. In vivo results of the treatments with: control (CON, no treatment), imipenem (IPM), sulbactam (SUL), rifampin (RIF), and colistin (CST), and the combinations.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>N</th>
<th>Survival %</th>
<th>Log_{10} CFU/g of lung mean ± SD</th>
<th>Sterile blood culture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>15</td>
<td>0</td>
<td>10.6 ± 0.27</td>
<td>0</td>
</tr>
<tr>
<td>IPM</td>
<td>14</td>
<td>28.6 ±f</td>
<td>7.87 ± 3.43 ±h.c.d</td>
<td>30.8 ±a,b,c,d,e</td>
</tr>
<tr>
<td>RIF</td>
<td>14</td>
<td>71.4 ±a</td>
<td>3.05 ± 1.91</td>
<td>78.6 ±a</td>
</tr>
<tr>
<td>SUL</td>
<td>15</td>
<td>40 ±f</td>
<td>7.23 ± 4.41 ±c,d</td>
<td>33.3 ±a,b,c</td>
</tr>
<tr>
<td>CST</td>
<td>15</td>
<td>40 ±f</td>
<td>6.82 ± 3.4 ±c,d</td>
<td>73.3 ±a</td>
</tr>
<tr>
<td>RIF + IPM</td>
<td>15</td>
<td>60 ±a</td>
<td>2.07 ± 1.82 ±a</td>
<td>100 ±a</td>
</tr>
<tr>
<td>RIF + SUL</td>
<td>15</td>
<td>46.7 ±a</td>
<td>2.41 ± 1.37 ±a</td>
<td>93.3 ±a</td>
</tr>
<tr>
<td>RIF + CST</td>
<td>16</td>
<td>43.8 ±a</td>
<td>3.4 ± 3.07 ±a</td>
<td>93.8 ±a</td>
</tr>
<tr>
<td>IPM + SUL</td>
<td>14</td>
<td>85.7 ±a,d,e</td>
<td>4.22 ± 2.72 ±a</td>
<td>50 ±a,c,d,e</td>
</tr>
</tbody>
</table>

*p<0.05 respect a CON group; b RIF group; c RIF+IPM group; d RIF+SUL group; e RIF+CST group; f IPM+SUL group.*
TABLE 4. Experimental meningitis model. In vivo results of the treatments with monotherapies (rifampin, and colistin) and the combinations.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Bacterial CSF concentration (Log₁₀ CFU/ml)</th>
<th>White blood cells (cells/µl)</th>
<th>% g of H₂O/g of brain dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>5.3 5.1 5.2 5.5 0 1 6.6*</td>
<td>391.5</td>
<td></td>
</tr>
<tr>
<td>RIF</td>
<td>6.1 5.1* 4.5* 3.5* 0 3 8.2*</td>
<td>411.7</td>
<td></td>
</tr>
<tr>
<td>CST</td>
<td>5.15 5.2 4.7 4.3 0 3.4 4</td>
<td>383.1</td>
<td></td>
</tr>
<tr>
<td>IPM+SUL</td>
<td>5.8 4.8 4.6 4.6 0 1.9 6.9</td>
<td>410</td>
<td></td>
</tr>
<tr>
<td>RIF+IPM</td>
<td>6 4 3.5 3.7 0 3 7.3</td>
<td>407.7</td>
<td></td>
</tr>
<tr>
<td>RIF+SUL</td>
<td>6.2 5 5.1 5.2 0 3.5 6.9</td>
<td>403.6</td>
<td></td>
</tr>
<tr>
<td>RIF+CST</td>
<td>5.7 4 2.1 1.3 0 2.7 4.3</td>
<td>395.2</td>
<td></td>
</tr>
</tbody>
</table>

Note: All values are averages of three experiments. *p < 0.05 compared to CON.
No treatment (CON), rifampin (RIF), colistin (CST), sulbactam (SUL), imipenem (IPM).

Data are expressed as median (P_{25}/P_{75}). * p<0.05 respect to 0 h.
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