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Efficacy of Colistin versus β-Lactams, Aminoglycosides, and Rifampin as Monotherapy in a Mouse Model of Pneumonia Caused by Multiresistant *Acinetobacter baumannii*

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The treatment of life-threatening infections due to carbapenem-resistant *Acinetobacter baumannii* has become a serious challenge for physicians worldwide. Often, only colistin shows in general good in vitro activity against these carbapenem-resistant strains, but its antibacterial efficacy in comparison with the antibiotics most used in clinical practice is not well known. We studied the efficacy of colistin versus those of imipenem, sulbactam, tobramycin, and rifampin in an experimental pneumonia model with immunocompetent mice. We used three strains of *A. baumannii* corresponding to the main clones (A, D, and E) involved in the outbreaks of our hospital, with different grades of resistance to imipenem (imipenem MICs of 1, 8, and 512 μg/ml, respectively) and to the other antibiotics. The MIC of colistin was 0.5 μg/ml for the three strains. Reduction of $\log_{10}$ CFU/g in lung bacterial counts, clearance of bacteremia, and survival versus results with controls were used as parameters of efficacy. Imipenem and sulbactam (Δlung counts: −5.38 and −4.64 $\log_{10}$ CFU/ml) showed the highest level of bactericidal efficacy in infections by susceptible and even intermediate strains. Tobramycin and rifampin (−4.16 and −5.15 $\log_{10}$ CFU/ml) provided good results against intermediate or moderately resistant strains, in agreement with killing curves and pharmacodynamics. On the contrary, colistin showed the weakest antibacterial effect among the antibiotics tested, both in killing curves and in the in vivo model (−2.39 $\log_{10}$ CFU/ml; $P < 0.05$). We conclude that colistin did not appear as a good option for treatment of patients with pneumonia due to carbapenem-resistant *A. baumannii* strains. Other alternatives, including combinations with rifampin, may offer better therapeutic profiles and thus should be studied.

Over the last 15 years, *Acinetobacter baumannii* has emerged as an important nosocomial pathogen, and hospital outbreaks caused by this organism have increased worldwide (3, 4, 15, 16, 19, 28, 31). Its extraordinary ability to acquire resistance to almost all groups of commercially available antibiotics presents a clinical problem of great concern. In fact, most *A. baumannii* strains isolated in hospitals today are highly resistant to modern noncarbapenem β-lactams, aminoglycosides, and fluoroquinolones (2, 15, 17, 38, 39, 43). Imipenem used to be considered the "gold standard" therapy for severe infections (39, 44), but many countries have reported growing resistance to carbapenems (1, 5, 14, 23, 28, 30, 31, 41, 42).

Since 1992, our hospital has suffered from sustained large outbreaks of multiresistant (resistant to two or more groups of antibiotics) *A. baumannii* infections. In 1997, intermediate and high-grade carbapenem-resistant isolates appeared, posing a serious challenge to the treatment of life-threatening infections due to these multiresistant microorganisms. Currently, only colistin shows in vitro activity against the majority of *A. baumannii* strains in our hospital (MIC, 0.5 μg/ml) and according to some reports of other authors (7, 17, 28, 38). Although we achieved good results using local intrathecal colistin for treatment of catheter-associated ventriculitis (13) and successful intravenous therapy has also been reported in a case of meningitis (20) and in a variety of nosocomial infections (27), clinical experience with colistin is still limited (6, 11), and relatively little is known of its efficacy in treating severe infections, especially in comparison with other antibiotics. Furthermore, very few experimental studies using colistin in animal models in protection tests using *Pseudomonas aeruginosa* have been published (9, 35).

Pneumonia is the most serious nosocomial infection due to multiresistant *A. baumannii* (7, 12, 16). Effective mouse models of pneumonia due to this microorganism have been described (21, 22, 36). For these reasons we decided to study this infection using the clinical strains responsible for the current outbreaks in our hospital. Our aim was to compare the efficacy of colistin with that of β-lactams (imipenem and sulbactam), an aminoglycoside (tobramycin), and rifampin using a mouse model of experimental pneumonia due to strains of *A. baumannii* which were susceptible, intermediate, and highly resistant to β-lactams, intermediate and highly resistant to tobramycin, and resistant to rifampin.

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MATERIALS AND METHODS

Challenge microorganisms. We selected three multiresistant strains of *A. baumannii* (A, D, and E), uniformly susceptible to colistin but with various

TABLE 1. MICs and MBCs for strains of *Acinetobacter baumannii* used in the model[a]

Antibiotic	MIC/MBC (μg/ml) for:		
	Strain A (Ipm^s)	Strain D (Ipm^i)	Strain E (Ipm^r)
Imipenem	1/1	8/16	512/512
Sulbactam	2/64	4/64	128/>128
Tobramycin	128/256	8/32	8/32
Rifampin	8/8	8/8	8/8
Colistin	0.5/0.5	0.5/1	0.5/2

[a] Ipm^s, imipenem susceptible; Ipm^i, imipenem intermediate resistant; Ipm^r, imipenem resistant. Ticarcillin, piperacillin, gentamicin, and amikacin showed MICs of >256 μg/ml for all the strains. Ceftazidime, cefepime, and ciprofloxacin showed MICs of >32 μg/ml. For tetracycline, MICs were >8 μg/ml for all the strains. MIC interpretative standards of resistance (μg/ml) obtained from NCCLS (34) were as follows: piperacillin, ≥128; ticarcillin, ≥128; sulbactam, ≥16 (in combination with ampicillin): ceftazidime, ≥32; cefepime, ≥32; imipenem, ≥16; gentamicin, ≥16; amikacin, ≥64; tobramycin, ≥16; tetracycline, ≥16; ciprofloxacin, ≥4. There are no data about rifampin for infections by gram-negative bacteria; this is the reason we used the same standard as for *Staphylococcus aureus*, ≥4. In the case of polymyxins, the NCCLS does not provide data of sensitivity (11); we used the concentration of ≥4 μg/ml to define resistance (6).

degree of susceptibility to carbapenems and other antibiotics. Strains corresponded to the three major clones, named A, D, and E, responsible for the outbreak noted in our hospital (7).

Determination of MICs and MBCs. The MICs and minimal bactericidal concentrations (MBCs) of different antibiotics were determined by the standard macrodilution method in Mueller-Hinton broth by geometric twofold serial dilutions (32, 33). The cutoff concentrations are showed in Table 1 according to NCCLS standards (34).

Time-kill curves. In vitro bactericidal activity was also evaluated with time-kill curves by determining the killing rate for a bacterial isolate by an antimicrobial agent. Aliquots of *A. baumannii* strains used in the experimental study were unfrozen and cultured in tubes containing brain heart infusion, the bacterial inoculum at an approximate size of 10^5 CFU/ml, and the antibiotics to be tested at chosen concentrations of 0.25 MIC, 0.5 MIC, 1× MIC, and 2× MIC according to strain. For colistin, we also used 4×, 8×, 16×, and 32× MIC. One tube without antibiotic was used as a growth control in all the experiments. At 0, 6, and 24 h of incubation at 37°C, aliquots of 100 μl were obtained from each tube and 10-fold dilutions were made and cultured in Trypticase soy agar (TSA) plates for 24 h at 37°C to obtain quantitative results. Bactericidal activity, defined as 99.9% killing of the final inoculum, was determined from time-kill curves by noting the presence or absence of a 3-$\log_{10}$ decrease in CFU/ml (32).

Preparation of inoculum. Aliquots of the three strains of *A. baumannii* selected for the model were performed and frozen with milk at −80° until use. For the preparation of inoculum, one aliquot of the selected strain was thawed at ambient temperature and cultured in TSA with 5% sheep blood plates at 37°C for 24 h. Once grown, three or four colonies were resuspended in a tube with Trypticase soy broth (TSB) and incubated in a continuous shaking bath until reaching the exponential growth phase after approximately 4 h. Finally, the tubes were centrifuged and the deposit was reconstituted with sterile saline serum (sodium chloride, 0.9%) until it achieved 1 McFarland turbidity measured by spectrophotometry, equivalent to a concentration of 5×10^8 CFU/ml.

Drugs used. The anesthetics used in the model, ketamin and xylazin, were supplied by Parke-Davis (Morris Plains, N.J.) and Bayer AG (Leverkusen, Germany), respectively. All antibiotics used were obtained from laboratory standard powders and diluted in sterile saline serum immediately prior to administration. We used imipenem (Merck Sharp & Dohme, Madrid, Spain), sulbactam (Pfizer, Madrid, Spain), tobramycin (Braun, Barcelona, Spain), rifampin (Aventis, Barcelona, Spain), and colistin (Pharmax Limited, Bexley, United Kingdom). Colistin was used in the form of methanesulfonate in all in vitro and in vivo experiments because it is the unique form that can be given to humans (27, 29).

Animal experiments. (i) Mice. Immunocompetent specific-pathogen-free C57BL/6N young female mice, weighing 14 to 16 g, were used. They were supplied by Harlan (Gannat, France). Animals were quarantined for 1 week immediately after reception. They were given standard laboratory food and water ad libitum. After induction of pneumonia, infected mice were isolated in a biologic storage chamber with light controlled in 12-h day-night periods and with room air purified by HEPA filters. Treated and control animals were kept in separate cages. The study was approved by the Ethical Committee for Animal Experiments at the University of Barcelona (Bellvitge Campus).

(ii) Pneumonia model. We used the model described by Esposito and Pennington (10), modified by Rodríguez-Hernández et al. (36). After the quarantine period, animals were anesthetized with a mixture of 100 mg of ketamin/kg of body weight and 10 mg of xylazin/kg administered intraperitoneally. When animals were completely asleep and in a vertical position, the trachea was cannulated via the mouth with a blunt needle. The correct location was indicated by palpation of the epiglottis with the tip. A 50% mixture of a bacterial suspension containing 5×10^8 CFU/ml and porcine mucin (Sigma-Aldrich Co., Madrid, Spain) diluted to 10%, to enhance the virulence of bacteria (35, 36), was prepared, and finally 50 μl was instilled with a microliter syringe (80601; Hamilton Co., Reno, Nev.).

Mice remained suspended in a vertical position for 4 min and then in a 30° decubitus position until awake. Individualized experiments were performed to test each antibiotic and each *A. baumannii* strain. In each experiment, 14 infected animals were randomized to two groups: the control group ($n = 6$) and the treatment group ($n = 8$). Therapy was initiated 4 h after induction of the pneumonia when histological features of pneumonia had started to appear (36). All antibiotics and the placebo were administered intraperitoneally. Total daily doses of imipenem, sulbactam, tobramycin, and colistin were divided in four doses and administered every 6 h, except for rifampin, which was administered in a once-daily dose regimen. Treatment or placebo was continued until 44 h after inoculation. At 24 and 48 h after inoculation, four treated animals and three control animals were killed. Thoracotomies were performed, and lungs were removed, weighed, and finally processed for quantitative cultures by manual homogenization in 1 ml of sterile saline serum. Tenfold dilutions were performed, and aliquots of 100 μl were plated on TSA with 5% sheep blood plates for 24 h at 37°C. Once grown, colonies were counted in each dilution and each animal. Results of cultures were expressed as $\log_{10}$ of CFU per gram of lung. The lower limit of detection was 1.5 log CFU/g. The mean counts ± the standard deviation of the $\log_{10}$ CFU/g were obtained for the treated and control groups at 24 and 48 h after induction of pneumonia, and the difference between the two groups was calculated ($\Delta\text{logs} = \text{mean}_{\text{treated group}} - \text{mean}_{\text{control group}}$). A minimum sample of 100 μl of blood was collected by cardiac puncture immediately after death and cultured in Trypticase soy broth for 24 h at 37°C. Then, 100 μl was plated on TSA sheep blood agar plates and incubated for another 24 h at 37°C to identify bacterial growth. Results of blood cultures were qualitative and were expressed as positive or negative.

To evaluate the therapeutic efficacy in each experiment, we compared the lung bacterial counts and the percentages of bacteremia and mortality in the group of treated mice with those of the control group.

Pharmacokinetics. Prior to the pneumonia experiments, the pharmacokinetics of each antibiotic were determined. One single individualized weight-adjusted dose of antibiotics was administered intraperitoneally to a group of 21 healthy animals. In sets of three animals and at different time points after administration of antibiotic, blood was obtained from anesthetized mice from an incision in the periorbital plexus and was centrifuged; finally, the serum was separated. Levels of imipenem and colistin in serum were determined immediately after obtaining the specimens. The rest of the samples were immediately frozen at −80°C until assayed. Pharmacokinetic and pharmacodynamic parameters were determined by a computer-assisted method. These parameters were the following: (i) the peak drug concentration in serum (C_{max}); (ii) the elimination half-life ($t_{1/2}$); (iii) the area under the concentration-time curve (AUC); (iv) the inhibitory quotient (IQ) (IQ = C_{max}/MIC); and (v) the time the serum concentration remained above the MIC ($\Delta t >$ MIC). Finally, based on previous studies (8), single doses of antibiotics administered to mice were selected to achieve serum levels and pharmacokinetic and pharmacodynamic parameters similar to human ones: imipenem, 50 mg/kg (equivalent to a daily dose of 200 mg/kg); sulbactam, 30 mg/kg (daily dose, 120 mg/kg); rifampin, 25 mg/kg (daily dose, 25 mg/kg); and tobramycin, 15 mg/kg (daily dose, 60 mg/kg). Since no published data are available regarding the most appropriate dose of colistin in mice, we tested the dosage required to achieve levels in serum similar to those obtained with humans at the recommended dosage for treatment in life-threatening infections, that is, 50,000 U/kg/day (24). In humans, after a single intramuscular injection of colistin methanesulfonate in a dose of 31,250 U per kg, a peak serum level of 5 to 7 μg/ml occurs, with a serum half-life of 1.6 to 2.7 h. Repeated administration yields higher serum levels, with concentrations of 11 to 12 μg/ml (24). We finally used the dosage of 125,000 U/kg (daily dose of 500,000 U/kg). In order to exclude some toxicity of colistin in mice, we tested this dosage with a group of 10 uninfected mice and compared survival, weight differences, and activity with those of another group of 5 mice treated with sterile saline serum as a placebo. Since no significant differences in survival or weight were found between the two

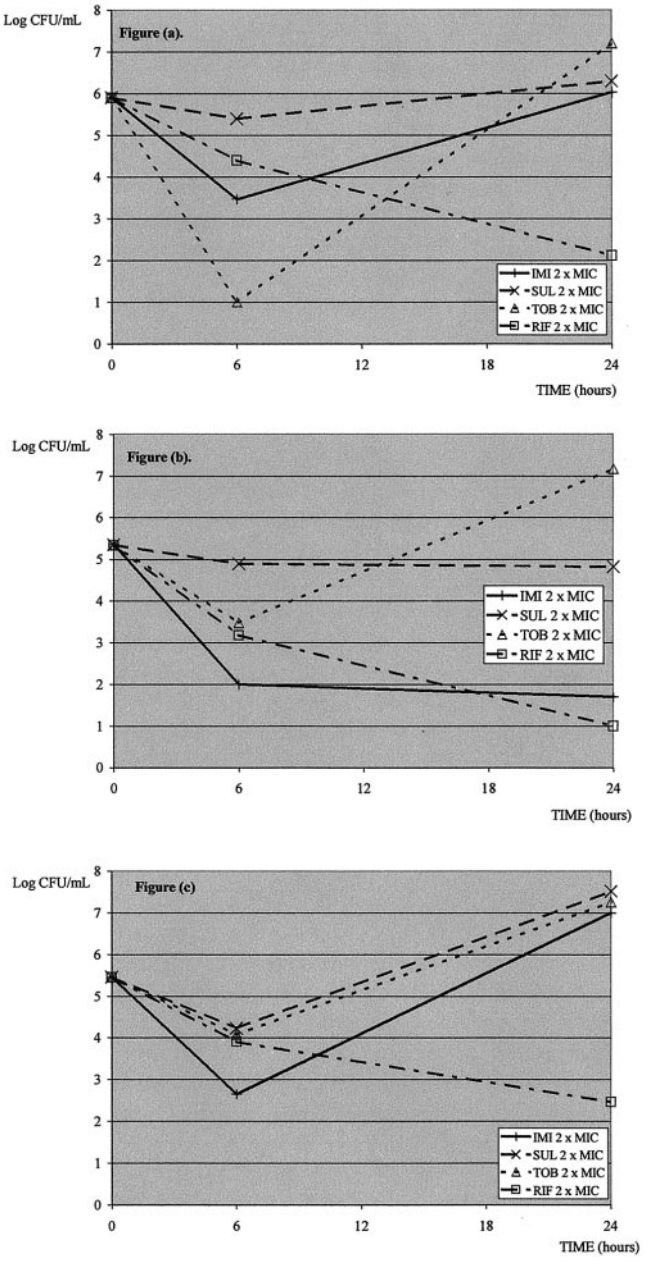

FIG. 1. Killing curves with imipenem (IMI), sulbactam (SUL), tobramycin (TOB), and rifampin (RIF) at 2× MIC for strains A (a), D (b), and E (c) of *A. baumannii*. See Table 1 for MICs of each strain.

groups (data not shown) and no differences in activity were observed, toxicity was excluded.

Drug assays. Two different aliquots from each sample were analyzed, each one in duplicate. (i) The imipenem concentration in serum was determined by a bioassay method using *Escherichia coli* ATCC 25922 as the reference standard. (ii) The method for measuring sulbactam concentrations was derived from the technique described by Haginaka (18). Serum concentrations were determined by high-performance liquid chromatography with UV detection at 326 nm. First, proteins were precipitated from the samples with acetonitrile and centrifuged. The supernatant was submitted to a precolumn derivatization with 1,2,4-triazole (2 M, pH 10) (volume 1:1) at ambient temperature for 24 h. Chromatographic separation was performed with an anionic exchange column of amine polyacrylamide (50 by 4.6 mm; IC Pak Anion). The mobile phase was a solution consisting of gluconate-borate buffer (25 ml), glycerin (3 ml), ultrapure Milli-Q water (1,000 ml), and acetonitrile (200 ml). The temperature was 30°, and the flow rate was 1 ml/min. The calibration curve was linear from 0.887 to 99.997 mg/liter. Variation within replicates was <5%. (iii) Tobramycin was measured by the fluorescence polarization immunoassay (FPIA) method. (iv) Rifampin concentrations were measured by a modification of the technique previously published by Swart and Papgis (40). Proteins were initially precipitated with acetonitrile and centrifuged. Chromatographic separation of the supernatant was performed with a Nova Pak C_{18} column (200 by 4.6 mm; Waters) and a solution of 60% KH_2PO_4–30% acetonitrile–10% methanol as a mobile phase. The flow rate was 1 ml/min. Determinations of rifampin concentrations were performed by UV detection at 342 nm. We used benzocaine as an internal standard and ascorbic acid as an antioxidant. The calibration curve was linear from 0.07 to 46.6 mg/liter. Variation within replicates was <5%. (v) Colistin concentrations were determined by microdilution using *E. coli* ATCC 25922 as the reference standard.

Statistical analysis. All bacterial counts and pharmacokinetic data are presented as mean ± standard deviation. Data of lung bacterial counts were found to be normally distributed in control and treated animals after applying the Kolmogorov-Smirnov test. After that, analysis of variance (ANOVA) and Scheffé's correction test were used to compare lung counts in control animals for each strain. Student's *t* test was used to compare differences between groups in bacterial counts. To compare bacteremia or mortality between groups, two-tailed Fisher's exact test was performed. For all tests, differences were considered statistically significant when *P* values were <0.05.

RESULTS

In vitro studies. (i) MICs and MBCs. MICs and MBCs of imipenem, sulbactam, tobramycin, rifampin, and colistin for strains A, D, and E are shown in Table 1. These microorganisms were tolerant to sulbactam.

(ii) Time-kill curves. At tested concentrations of 2× MIC, imipenem reached a decline of 2.44, 3.36, and 2.8 logs at 6 h for strains A, D, and E, respectively (concentrations of 2, 8, and 1,024 μg/ml). Similarly, tobramycin at 2× MIC (256 μg/ml) reached bactericidal activity at 6 h with strain A. Also, rifampin at 2× MIC (16 μg/ml) at 24 h reached bactericidal activity for all strains (Fig. 1).

At 1× MIC, only rifampin (8 μg/ml) (data not shown) was bactericidal for strains D and E. In contrast, sulbactam and colistin did not show any bactericidal activity even at the maximum concentration used of 2× MIC. Colistin reached bactericidal activity only at 24 h with concentrations of 16× MIC and 32× MIC (Fig. 2) in strains A and E.

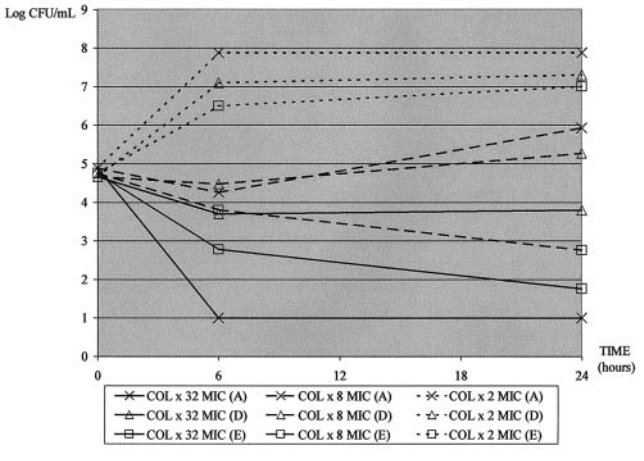

FIG. 2. Killing curves with colistin (COL) at 2×, 8×, and 32× MIC for strains A, D, and E of *A. baumannii*. See Table 1 for MICs of each strain.

TABLE 2. Pharmacokinetics and pharmacodynamics of antibiotics used in the experiments

Parameter	Value for drug[a]				
	Imipenem (50 mg/kg)	Sulbactam (30 mg/kg)	Tobramycin (15 mg/kg)	Rifampin (25 mg/kg)	Colistin (125,000 U/kg)
C_{max} (mg/liter)	37.49 ± 1.37	91.03 ± 0.04	32.87 ± 5.45	24.29 ± 10.26	13.33 ± 4.61
$t_{1/2}$ (h)	0.19	0.18	0.37	5.44	0.52
AUC (mg · h/liter)	11.99	24.57	11.54	196.22	11.96
IQ (C_{max}/MIC)					
Strain A	37.49	45.51	0.26	3.03	26.66
Strain D	4.69	22.76	4.11	3.03	26.66
Strain E	0.07	0.71	4.11	3.03	26.66
$t >$ MIC (h)					
Strain A	1.15	1.1	0	9.71	3.05
Strain D	0.58	0.91	0.69	9.71	3.05
Strain E	0	0	0.69	9.71	3.05

[a] Dosage is given in parentheses after drug name.

(iii) Pharmacokinetics. Serum pharmacokinetic and pharmacodynamic parameters obtained are shown in Table 2. $\Delta t >$ MICs of imipenem and sulbactam were low (below 20% of the interdose time) even for the most susceptible strain (strain A), due to the very short $t_{1/2}$ shown by these β-lactams in mice: 0.19 and 0.17 h, respectively. The C_{max} value of sulbactam was more than twice that of imipenem. In the case of tobramycin, we achieved a high serum peak, which determined a C_{max}/MIC ratio (IQ) of >4 for strains D and E with MICs of 8 μg/ml. The half-life time of rifampin was very long (5.43 h), showing a very high AUC of 196.224 mg · h/liter and $\Delta t >$ MIC values of greater than 40% for all strains with intermediate resistance. Colistin showed an IQ of 26.666 and $\Delta t >$ MICs of more than 50% for all the strains.

Therapeutic efficacy by strains: Bacterial clearance from lungs and from blood and survival. Lung bacterial counts for treated and control animals are shown in Table 3. Differences between means for treated and control animals are expressed in Fig. 3. The efficacies of different antibiotics were more evident at 48 h of therapy. In control animals, 100% of mice had positive blood cultures at 24 and 48 h after induction of pneumonia, reflecting the virulence of the infection model. Mortality at 48 h in the control animals of the three strains showed small differences (46.6% [strain A], 60% [strain D], and 53.3% [strain E], respectively), but they did not reached statistical significance. To analyze survival in treated animals, we compared treated animals with control animals infected with the same strain.

(i) Strain A. Imipenem, sulbactam, and rifampin showed significant bactericidal activity in reducing lung bacterial counts compared with controls. Imipenem and sulbactam were the most active. On the other hand, tobramycin was totally ineffective, and colistin demonstrated only moderate activity. The antibiotics that showed some activity in lung bacterial clearance also reduced bacteremia, with imipenem being the most effective: (imipenem, 25% bacteremia; sulbactam, 50%; rifampin, 37.5%; and colistin, 75% [$P < 0.05$ versus controls]). All mice receiving therapy with imipenem, sulbactam, and rifampin survived. Survival rates with these antibiotics showed a trend towards significance ($P = 0.06$) compared with rates for the control group. Tobramycin and colistin showed some reduction in mortality (25 and 37.5% mortality, respectively, versus 46.6% in controls), but it was not significant.

(ii) Strain D. In lung bacterial counts, imipenem, sulbactam, tobramycin, and rifampin showed significant bactericidal activity compared with controls. The activity of imipenem and sulbactam was lower than that obtained with strain A, according to pharmacodynamic findings, but the differences were not statistically significant. Overall, the most effective therapy was imipenem. Again, all antibiotics active in the lung bacterial clearance showed some reduction in the percentage of positive blood cultures: imipenem, 37.5% bacteremia; sulbactam,

TABLE 3. Results of lung bacterial counts[a] of *A. baumannii*

Therapeutic group	Bacterial count (no. of mice) for:					
	Strain A		Strain D		Strain E	
	24 h	48 h	24 h	48 h	24 h	48 h
Control	10.55 ± 0.46[b] (15)	10.70 ± 0.37[b] (15)	10.58 ± 0.26[b] (15)	10.80 ± 0.38[b] (15)	10.51 ± 0.43[b] (15)	10.78 ± 0.30[b] (15)
Imipenem	6.49 ± 0.39[c] (4)	5.32 ± 0.11[c,d] (4)	8.72 ± 1.32[c] (4)	6.32 ± 0.59[c,d] (4)	10.3 ± 0.98 (4)	11.02 ± 0.2 (4)
Sulbactam	7.89 ± 1.08[c] (4)	6.06 ± 0.58[c,d] (4)	8.73 ± 1.07[c] (4)	7.13 ± 1.95[c] (4)	10.31 ± 0.2 (4)	10.74 ± 0.20 (4)
Tobramycin	10.69 ± 0.3 (4)	10.72 ± 0.71 (4)	10.37 ± 0.26 (4)	7.35 ± 0.94[c] (4)	7.88 ± 0.73[c] (4)	6.62 ± 1.16[c] (4)
Rifampin	7.77 ± 0.26[c] (4)	6.89 ± 0.28[c,e] (4)	8.8 ± 0.5[c] (4)	7.18 ± 0.29[c] (4)	7.64 ± 0.45[c] (4)	5.63 ± 0.26[c,e] (4)
Colistin	9.68 ± 0.99[c] (4)	8.64 ± 0.66[c] (4)	10.61 ± 0.26 (4)	10.4 ± 1.09 (4)	10.41 ± 0.41 (4)	8.39 ± 1.22[c] (4)

[a] Lung bacterial counts are expressed in $\log_{10}$ of CFU/gram of lung tissue (mean of counts ± standard deviation).
[b] No significant differences were found between the three strains at 24 and 48 h.
[c] Differences were statistically significant compared with the control group ($P < 0.05$).
[d] Lung bacterial counts were significantly lower than those obtained with tobramycin, rifampin, and colistin.
[e] Lung bacterial counts were significantly lower than those obtained with colistin.

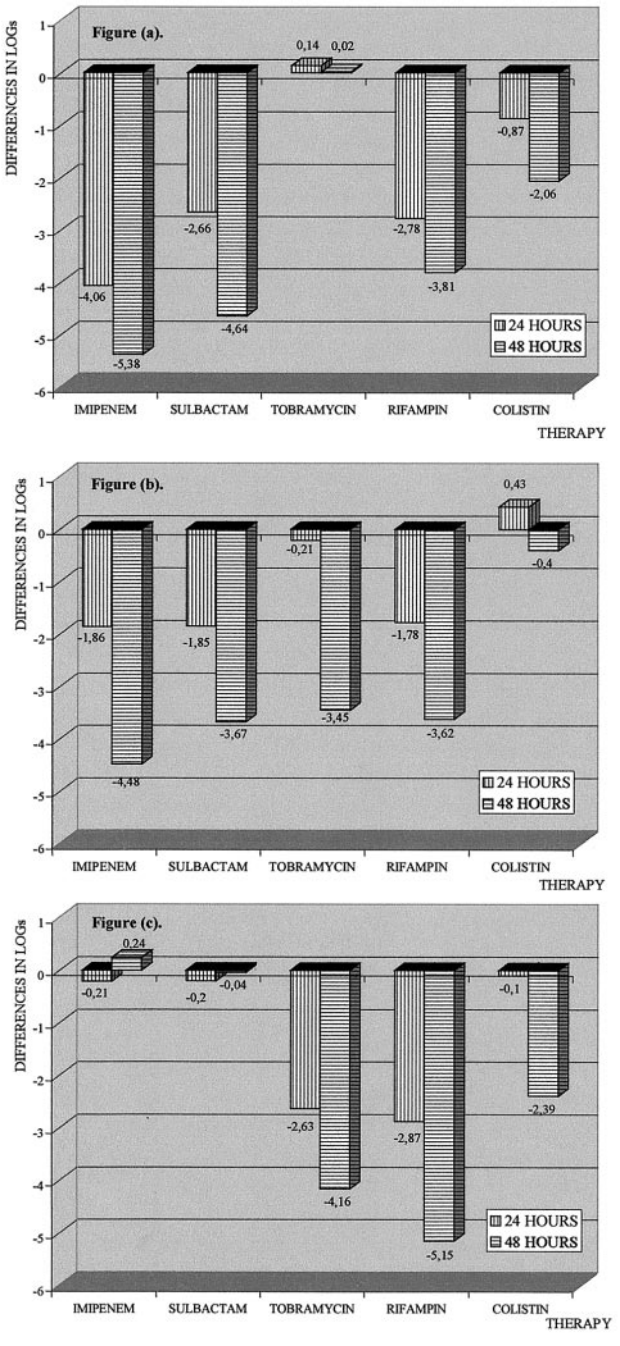

FIG. 3. In vivo therapeutic efficacy: results of lung counts in the pneumonia model for mice expressed as differences in $\log_{10}$ between the means of the treated and the control groups at 24 and 48 h of therapy by strains A (a), D (b), and E (c) of *A. baumannii*.

62.5%; tobramycin, 37.5%; and rifampin, 50% ($P < 0.05$ versus controls). All animals in the group with imipenem and that with rifampin survived, and only one in those treated with sulbactam or tobramycin died ($P < 0.05$ versus controls). Colistin did not show any effect according to lung counts and bacteremia reduction, and although some reduction in mortality was achieved, it was not significant.

(iii) Strain E. Imipenem and sulbactam did not reduce lung bacterial counts, and mortality was similar to that with control animals. Only sulbactam showed low efficacy in reducing bacteremia (87.5%). Tobramycin showed high lung bacterial clearance, as with strain D. Of note was the high bactericidal activity observed with rifampin in reducing the lung bacterial counts, significantly greater than that observed with tobramycin ($P = 0.06$) or colistin ($P < 0.05$). Tobramycin and rifampin were the most effective antibiotics in reducing bacteremia, 25 and 37.5%, respectively ($P < 0.05$ versus controls); all animals receiving these therapies survived ($P < 0.05$ versus controls). The behavior of colistin was similar to that observed with strain A, its results being poorer than those of the other effective therapies (Δlogs in Fig. 3; 62.5% bacteremia, $P < 0.05$ versus controls; and 12.5% mortality, $P = 0.06$ versus controls).

DISCUSSION

The emergence and further persistence of imipenem resistance among *A. baumannii* strains responsible for the outbreak in our hospital presented a serious therapeutic challenge (7). Our experimental study provides the first information regarding the efficacy of colistin for the treatment of experimental pneumonia due to multiresistant *A. baumannii*. Testing activity against the three major clones in our hospital, which were uniformly susceptible to colistin but presented different degrees of resistance to imipenem, sulbactam, tobramycin and rifampin, allowed us to examine in detail the comparative effects of these antibiotics at the time in order to choose the best alternative for use in clinical practice.

Of the previously described models of *A. baumannii* pneumonia in mice (21, 36), we selected the one described by Rodríguez-Hernández et al., since it does not require immunosuppression to facilitate the development of pneumonia and thus reproduces more faithfully the usual condition of ventilated patients suffering from nosocomial *A. baumannii* pneumonia in ICU wards (7, 12, 16). The mouse model of pneumonia used was reproducible, systematically causing histological findings of pneumonia with bacterial counts of 10 to 11 $\log_{10}$ CFU/g of lung tissue, 100% bacteremia, and mortality varying between 46 and 66% according to strain. The fact that the results for control animals did not differ significantly between strains meant that the model was suitable for comparing the efficacy of different antibiotics.

Pharmacodynamics in mice and in humans differ (8). Although we obtained standardized and reproducible results using doses which according to previous studies produce pharmacodynamics quite similar to those in humans, some aspects should be noted. In the case of β-lactams, despite the high C_{max} reached, the $\Delta t >$ MICs was only 20% of the interdose time due to the short $t_{1/2}$ of these antibiotics. In contrast, the C_{max} with tobramycin was higher than usual in humans. The dose we used in this study complied with the idea of using aminoglycosides in monodose in humans, but we repeated the dose in mice every 6 h, which means that the total dose administered was probably larger than estimated. Data for rifampin may be similar to those observed for humans using high doses. The colistin results appeared to be reasonable, but very little published data are available on the pharmacokinetics of the antibiotic.

Overall, the in vivo efficacies of the different antibiotics used were in accordance with the pharmacokinetics and pharmaco-

dynamics obtained in mice. We observed a good correlation between the three parameters used to evaluate clinical efficacy: bacterial clearance from lungs, bacterial clearance from the blood, and survival.

The unexpectedly weak effect shown by colistin was an exception to this observation. On the basis of MICs and MBCs, it might appear that colistin exhibited a good antibacterial activity for all the strains. However, the killing curves showed bactericidal activity only at high concentrations (greater than 8× MIC) and only for strains A and E. In the in vivo model, moderate bacterial clearance from lungs and blood and a small reduction in mortality rates were obtained, but, as in the in vitro studies, only in the infections due to strains A and E; no effect was detected against the infection by strain D. Overall, these results were the worst obtained for all the antibiotics used in this study and did not correlate with the good serum pharmacodynamic profile observed. The existent data are very limited, but it does not seem that binding to proteins in serum could interfere with the bactericidal effect of colistin (26). Reasons for these poor results may be the low level of bactericidal activity exhibited by this antibiotic and the fact that pharmacodynamics of colistin in serum may not be a good marker of antibiotic levels in lungs, since it is known that the large size of its molecule may cause poor distribution in tissues (24). The clinical experience with colistin nowadays is very sparse, since polymyxins were not used for many years to treat infections by gram-negative bacteria because other, less toxic antibiotics were available (11). Although it is true that some successful results have been reported, including with patients with cystic fibrosis or central nervous system *A. baumannii* infections (13, 20), other cases did not have a good outcome (27). In fact, our findings are in agreement with the fact that this antibiotic has been classically considered less effective than other groups of antibiotics, such as β-lactams, aminoglycosides, and quinolones.

Imipenem and sulbactam showed high bactericidal efficacy in therapy for pneumonia caused by susceptible and even intermediately resistant strains. These findings are in agreement with those reported in previous studies (21, 37, 45). These effects may even have been underestimated, taking into account the low $\Delta t >$ MICs achieved by these antibiotics in mice due to its short $t_{1/2}$. The pharmacokinetics of the two antibiotics were similar and were more or less equivalent to those obtained in humans using high daily doses (imipenem, 50 mg/kg, and sulbactam, 100 to 150 mg/kg). Surprisingly, the C_{max} of sulbactam was notably higher, a finding also found in other experimental studies (45). However, as a whole, imipenem demonstrated higher efficacy, even against strains that were intermediately resistant to imipenem and susceptible to sulbactam, although these differences did not reach statistical significance. This result was probably due to the greater in vitro bactericidal activity exhibited by imipenem than sulbactam in killing curves and the postantibiotic effect reported with imipenem in treating *A. baumannii* (8, 21). The pharmacodynamics suggested that these two antibiotics were totally ineffective against pneumonia caused by strain E.

Tobramycin was very effective for treating pneumonia caused by moderately tobramycin-resistant strains such as D and E (MIC, 8 μg/ml). However, as we noted above, the pharmacodynamics of tobramycin in this model may well have been overestimated, since the peak levels achieved are usually not found in humans at the recommended doses. Tobramycin had no effect on pneumonia caused by the highly resistant strain A (MIC, 128 μg/ml).

The excellent efficacy of rifampin against infections by strains A, D, and E, which were intermediately resistant to this antibiotic (MIC, 8 μg/ml) (33), was unexpected. However, these results were in agreement with the pharmacodynamics, which showed very high AUC and IQ, as well as the bactericidal activity observed in time-kill curves. Similar findings were reported by Jolly-Guillou (45). These pharmacokinetic data may be very similar for humans using doses of 20 mg/kg/day (25). The early development of resistance is well known and limits the use of monotherapy with rifampin. While no resistance developed in this in vivo model after 48 h of therapy, this phenomenon was reported in a previous study (45).

We conclude that this model is well suited to the comparison of antibiotic efficacy against multiresistant *A. baumannii* pneumonia in mice. Though the results of experimental infections require careful interpretation and any extrapolation to humans should be made with great caution, some preliminary conclusions can be drawn regarding antibiotic use in management care of these difficult-to-treat infections. Our results do not favor the use of colistin to treat *A. baumannii* pneumonia, even though in vitro studies using MICs have suggested that it is the most active alternative. β-lactams, aminoglycosides, and rifampin provided better therapeutic margins, including susceptible and intermediately resistant strains: imipenem was the most effective therapy, sulbactam used at high doses may be a secondary alternative to imipenem, and rifampin had very good efficacy against all the strains tested. Although the last drug cannot be recommended as monotherapy because of the development of resistance, it should definitely be considered in combination regimens. Studies of antibiotic combinations are now in progress in our laboratory in the search for better therapeutic alternatives for infections caused by multiresistant, carbapenem-resistant strains such as strain E.

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