Polymyxin B in Combination with Antimicrobials Lacking *In Vitro* Activity versus Polymyxin B in Monotherapy in Critically Ill Patients with *Acinetobacter baumannii* or *Pseudomonas aeruginosa* Infections

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There is no clinical evidence supporting the use of polymyxin B in combination with another antimicrobial for infections caused by extensively drug-resistant *Acinetobacter baumannii* or *Pseudomonas aeruginosa* isolates. We developed a cohort study of patients in two intensive care units from teaching hospitals to evaluate treatment with intravenous polymyxin B for ≥ 48 h for severe *A. baumannii* or *P. aeruginosa* infections. Covariates potentially associated with 30-day mortality were evaluated in a Cox proportional hazards model. A total of 101 patients were included; 33 (32.7%) were treated with polymyxin B in combination with an antimicrobial lacking *in vitro* activity and 68 (67.3%) with polymyxin B in monotherapy. The overall 30-day mortality was 59.4% (60 patients), comprising 42.4% (14 of 33) and 67.6% (46 of 68) in combination and monotherapy groups, respectively ($P = 0.03$). The mortality rates were 18.5/1,000 patient days and 36.4/1,000 patient days in the combination and monotherapy groups, respectively ($P = 0.02$). Combination therapy was independently associated with lower 30-day mortality (hazard ratio, 0.33; 95% confidence interval, 0.17 to 0.64; $P = 0.001$). Creatinine clearance of ≥ 60 ml/min was also a protective factor, while a higher acute physiology and chronic health evaluation (APACHE II) score and polymicrobial infection were associated with increased mortality. The results did not change after adding a propensity score for prescribing combination therapy into the model. The protective effect remained when only combination with β -lactam or carbapenem was considered and in both subgroups of patients: those with *A. baumannii* infection and those with lower respiratory tract infections. To our knowledge, this is the first clinical study to show a benefit of combination over monotherapy with polymyxin B for severe extensively drug-resistant *A. baumannii* or *P. aeruginosa* infections.

The emergence of carbapenem-resistant Gram-negative bacteria (GNB) in the last decade, mostly driven by carbapenemase-producing strains, importantly changed the epidemiology of hospital-acquired infections (1). Carbapenem-resistant GNB frequently present an extensively drug-resistant (XDR) profile (2), and these isolates have been an increasing public health concern worldwide (1, 2). Among XDR bacteria, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are pathogens of major clinical and epidemiological importance, notably in intensive care unit (ICU) patients (3, 4).

Since the discovery of new anti-GNB agents has dried up, polymyxins, both B and E (colistin), have reemerged in clinical practice as agents of last resort against XDR *A. baumannii* and *P. aeruginosa* (5, 6). However, despite the *in vitro* activity of polymyxins, some factors, such as a possible limited clinical efficacy and the potential for the emergence of resistance during treatment, along with some support from preclinical studies, have led many physicians to prescribe combination therapy, mostly a polymyxin combined with a second agent, for patients infected by XDR GNB, including *A. baumannii* and *P. aeruginosa* (1, 7). Nonetheless, there is no clinical study supporting the use of combination schemes as the standard of care for the treatment of these infections. Indeed, two clinical trials failed to find distinct clinical outcomes in patients treated with colistin alone compared to colistin plus rifampin in patients with *A. baumannii* infections (22, 23).

So far, the strongest clinical evidence suggesting that combina-

tions of antibiotics may be superior to monotherapy comes from cohort studies assessing carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections (8, 9). In these studies, combination therapy was defined as treatment with two drugs that have activity *in vitro*, and nothing could be concluded about the role of combining a second agent to which there is documented resistance *in vitro*. Furthermore, these studies did not evaluate either *A. baumannii* or *P. aeruginosa*. Notably, although there are studies evaluating combination therapy against XDR *A. baumannii*, only one study included infections caused by XDR *P. aeruginosa* (10).

Additionally, several shortcomings have been identified in studies that evaluated combination therapy against these GNB, including the potential for indication bias, the use of multiple distinct drug combinations, and the possible suboptimal use of

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polymyxins (actually all studies were performed with colistin), considering recent developments in pharmacokinetic knowledge of these antibiotics (1, 7).

In this study, we aimed to compare polymyxin B in combination with an antimicrobial lacking *in vitro* activity to polymyxin B as monotherapy against *A. baumannii* or *P. aeruginosa* infections in critically ill patients, addressing the potential for indication bias.

MATERIALS AND METHODS

Study design, settings, and participants. We performed a retrospective analysis of a cohort study at two tertiary-care teaching hospitals in Porto Alegre, Brazil. All patients admitted to the intensive care unit from 1 February 2013 to 31 January 2014, aged ≥ 18 years, who received at least one dose of polymyxin B for the treatment of infection caused by *A. baumannii* or *P. aeruginosa*, were eligible for the cohort. Patients were excluded if they received polymyxin B for < 48 h or died within this period, had an infection *a priori* defined as nonsevere (skin and soft tissue or lower urinary tract infections), were treated with combination schemes that included a second drug that had activity *in vitro*, or had an infection caused by an isolate that was resistant to polymyxin B or did not have its susceptibility to polymyxin B evaluated. In patients who had more than one treatment with polymyxin B, only the first course was considered. Therapy was at the discretion of the attendant physician, and all data were retrieved from medical records. Institutional protocols recommended doses of 1.5 to 3.0 mg/kg of body weight/day in two divided administrations. Dose adjustment according to creatinine clearance was not recommended, in accordance with recent pharmacokinetic data on polymyxin B (11). Loading doses of polymyxin B were not yet part of the institutional protocols during the study period. The study was approved by the local ethics committee of each hospital.

Variables and definitions. The primary outcome was 30-day mortality. The onset of infection was considered the day on which the culture that yielded the growth of isolates was collected. Combination therapy was considered the use, within the first 48 h of polymyxin B initiation and for at least 48 h, of a second antimicrobial with anti-*Acinetobacter* or anti-*Pseudomonas* activity but to which these bacteria were not susceptible *in vitro*. Variables potentially related to mortality included age, gender, weight, body mass index, bacterial species, and site of infection, which was defined according to CDC criteria (12), except for pneumonia. Pneumonia was defined by the following criteria: recovery of positive *A. baumannii* or *P. aeruginosa* cultures from purulent respiratory secretions (purulence was considered the presence of > 25 neutrophils and < 10 epithelial cells per high-power microscopic field) either from endotracheal aspirate samples ($> 10^5$ CFU/ml) or from bronchoalveolar lavage fluid samples ($> 10^4$ CFU/ml) plus the presence of a radiographic infiltrate that was new or progressive, along with the presence of two or more of the following criteria: fever (temperature of $> 38^\circ\text{C}$) or hypothermia (temperature of $< 36^\circ\text{C}$), purulent sputum, leukocytosis ($> 10,000$ cells/mm³) or leukopenia ($< 4,000$ cells/mm³), or a decline in oxygenation. Ventilator-associated pneumonia was defined as pneumonia occurring in patients receiving mechanical ventilation for > 48 h, and ventilator-associated tracheobronchitis was defined by the same criteria but in the absence of a pulmonary infiltrate. Other variables included polymicrobial infections, Charlson comorbidity score (13) and the presence of specific comorbid conditions, creatinine clearance at the onset of therapy (estimated by the Cockcroft-Gault equation), acute physiology and chronic health evaluation (APACHE II) score (14) at the beginning of polymyxin B therapy, length of ICU stay prior to infection, use of vasoactive drugs and mechanical ventilation at the beginning of polymyxin B therapy, time of initiation of polymyxin B therapy, and polymyxin B dose. Dosages were evaluated as the average daily dose of polymyxin B (the sum of each day's total dose divided by the number of days until the end of therapy or death) and by mg/kg/day.

Microbiology. All isolates were identified by using the Vitek system (bioMérieux, Marcy l'Etoile, France). Susceptibility was determined by the disk diffusion method for all antimicrobials and interpreted according to CLSI criteria (15), with the exception of polymyxin B for *A. baumannii* and rifampin. The MICs of polymyxin B were determined by broth microdilution or Etest (bioMérieux), and susceptibility was considered if the MIC was ≤ 2 mg/liter. Etest was used to determine the MICs for carbapenems, and broth microdilution for rifampin (> 4 mg/liter indicated resistance).

Statistical analysis. All statistical analysis was carried out using SPSS for Windows, version 18.0. Bivariate analysis was performed separately for each of the variables. *P* values were calculated using the χ^2 or Fisher's exact test for categorical variables and the Student *t* test or the Wilcoxon rank-sum test for continuous variables. The Kolmogorov-Smirnov test was performed to assess for normal distribution. Covariates with a *P* value of ≤ 0.2 were included in a Cox proportional hazards model in a forward stepwise regression. Variables with a *P* value of ≤ 0.10 were maintained in the model.

In order to adjust for potential confounding by indication, we compared variables between combination and monotherapies. Variables with *P* values of ≤ 0.20 were further included in the Cox model for mortality, and those with *P* values of ≤ 0.10 were maintained in the model. A propensity score was also created in a logistic regression model including the latter variables and was tested in the final model.

The proportional hazards assumption was graphically checked by inspecting the log[-log(S)] plot, where S is survival. Collinearity was assessed. Tests for interactions were not performed. All tests were two tailed, and a *P* value of ≤ 0.05 was considered significant.

RESULTS

A total of 101 patients were included in the study (Fig. 1). The mean age of patients ($\pm$ standard deviation) was 65.2 ± 15.7 years, 55.4% were male, and the mean weight was 70.1 ± 18.2 kg. The sites of infection were respiratory tract (82 [81.2%]), primary bloodstream infections (9 [8.9%]), intra-abdominal (6 [5.9%]), and upper urinary tract (4 [4.0%]). Ten patients (9.9%) had bacteremia complicating a primary site of infection. Thus, for purposes of analysis, 19 (18.8%) patients were considered to have bacteremia. Most infections were caused by *A. baumannii* (83 [82.2%]), and the rest (18 [17.8%]) by *P. aeruginosa*. All isolates presented the XDR profile, and none was resistant to polymyxin B. The MICs of polymyxin B were determined in 88 isolates, and the MIC₅₀ and MIC₉₀ were 0.25 and 1 mg/liter, respectively, ranging from 0.03 to 2 mg/liter.

The mean polymyxin B daily dose by mg/kg was 2.3 ± 0.6 , and the median average daily dose was 150 mg (interquartile range [IQR], 150 to 160). Thirty patients (29.7%) received doses of < 2 mg/kg/day of polymyxin B, 63 (62.4%) received doses between 2 and 2.99 mg/kg/day, and 8 (7.9%) received doses of ≥ 3 mg/kg/day.

The overall 30-day mortality was 59.4% (60 patients). The median time to death was 12 days (IQR, 7 to 19). Thirty-three (32.7%) patients were treated with polymyxin B in combination therapy, and 68 (67.3%) with polymyxin B in monotherapy. The 30-day mortality rates in patients receiving combination and monotherapy were 42.4% (14 of 33) and 67.7% (46 of 68), respectively (*P* = 0.03). After the onset of infection, the mortality rates were 18.5/1,000 patient days and 36.4/1,000 patient days (Fig. 2) in the combination and monotherapy groups, respectively (*P* = 0.02, log rank).

The median MICs of polymyxin B were similar between the combination and monotherapy groups, being 0.25 mg/liter (IQR,

FIG 1 Flowchart with the number of eligible included and excluded patients. *, includes patients with infections caused by *Acinetobacter baumannii* or *Pseudomonas aeruginosa* isolates that were also susceptible to antimicrobials other than polymyxin B; **, patients with infections caused by *Acinetobacter baumannii* or *Pseudomonas aeruginosa* isolates that were also susceptible to antimicrobials other than polymyxin B whose treatment was changed to another agent after the results of antimicrobial susceptibility tests were available. ICU, intensive care unit; XDR, extensively drug resistant.

≤ 0.125 to 0.50) and 0.25 mg/liter (IQR, ≤ 0.125 to 1), respectively ($P = 0.75$). The characteristics of patients receiving combination and monotherapy are shown in Table 1. Polymicrobial infection ($P = 0.02$), *A. baumannii* infection ($P = 0.19$), connective tissue disease ($P = 0.17$), and polymyxin B daily dose (forced into both models) were selected for inclusion in the Cox regression model and for creation of propensity scores.

The bivariate analysis for mortality is shown in Table 2. In multivariate analysis, combination therapy was independently associated with lower rates of 30-day mortality. Creatinine clearance of ≥ 60 ml/min was also a protective factor, while higher APACHE II scores and polymicrobial infections were associated with increased rates of mortality (Table 3). Time to initiate polymyxin B therapy was also forced into the model in order to adjust for potential residual confounding, as was the propensity score. Both were neither significant nor modified by more than 10% the β coefficients of any variable in the model.

Thirty-one (93.9%) of the 33 patients from the combination group initiated the combined antibiotic at the same time as polymyxin B; of the other 2, 1 (2.6%) initiated the combined antibiotic within the first 24 h and 1 (2.6%) within 48 h of polymyxin B initiation. The latter two patients received meropenem and ampicillin-sulbactam, respectively. Descriptions of the combination schemes are given in Table 4.

FIG 2 Survival curves of patients receiving polymyxin B in combination with an antimicrobial lacking *in vitro* activity (gray line) or as monotherapy (black line) for severe infections by extensively drug-resistant *Acinetobacter baumannii* or *Pseudomonas aeruginosa* isolates. Mortality rates were 18.5/1,000 patient days and 36.4/1,000 patient days in combination and monotherapy groups, respectively ($P = 0.02$).

Subgroup analysis. Combination therapy was also beneficial if only combination with a β -lactam or a carbapenem was considered and in the subgroups of patients with *A. baumannii* infections and with lower respiratory tract infections (Table 5). There was no difference between combination and monotherapy in patients with bacteremia (Table 5). Three (16.5%) of 18 patients with *P. aeruginosa* infections received combination therapy and all survived, while the 30-day mortality was 93.3% (14 of 15) in those treated with monotherapy ($P = 0.005$). The absence of death in the combination group precluded a multivariate analysis.

DISCUSSION

Our study found that polymyxin B in combination with an antibiotic lacking *in vitro* activity had a highly protective effect with regard to 30-day mortality of patients with XDR *A. baumannii* and *P. aeruginosa* infections. To our knowledge, this was the first study to demonstrate a beneficial effect of a polymyxin in combination with a second drug for infections caused by these organisms and the first to find such an effect for combination with a second drug lacking *in vitro* activity.

Indeed, most patients in the combination group received a β -lactam; of these, most were treated with carbapenems (all except one, who received imipenem, were treated with meropenem). As expected, both analyses that included only β -lactam and only carbapenems showed very similar protective effects on mortality. Considering that almost all patients receiving combinations actually received a β -lactam, as well as the results of subgroup analysis, we believe that the results of this cohort study could be more properly generalized to polymyxin B schemes including an antibiotic of this class, mainly a carbapenem, in combination. Notably, with the exception of one isolate of *A. baumannii* with a meropenem MIC of 4 mg/liter (a 30-day nonsurvivor), all other isolates presented high-level resistance to carbapenems.

The demonstration that the effect of combination therapy did

TABLE 1 Characteristics of patients receiving combination and monotherapy antimicrobial regimens

Variable	Value [no. (%), (mean ± SD), or median (IQR ^a)] for patients receiving:		P value
	Combination therapy (n = 33)	Monotherapy (n = 68)	
Gender (male)	18 (54.5)	38 (55.9)	0.99
Age (yrs)	62.7 ± 15.6	66.5 ± 15.7	0.56
Weight (kg)	72.1 ± 20.6	69.1 ± 16.9	0.43
Body mass index	26.2 ± 6.5	25.3 ± 5.9	0.88
Estimated creatinine clearance of ≥60 ml/min	11 (33.3)	20 (29.4)	0.86
Treated in hospital A	32 (97.0)	62 (91.2)	0.51
<i>A. baumannii</i> infection	30 (90.9)	53 (77.9)	0.19
Primary infection site			
Bloodstream	4 (12.1)	5 (7.4)	0.47
Respiratory tract ^b	25 (75.8)	57 (83.8)	0.33
Abdominal	3 (9.1)	3 (4.4)	0.63
Upper urinary tract	1 (3.0)	3 (4.4)	0.99
Bacteremia	5 (15.2)	14 (20.6)	0.70
Polymicrobial infection	11 (33.3)	8 (11.8)	0.02
Comorbidity			
Cardiovascular disease	22 (66.7)	43 (63.2)	0.91
Pulmonary disease	5 (15.2)	19 (27.9)	0.24
Central nervous system disease	8 (24.2)	18 (26.5)	0.99
Liver disease	3 (9.1)	5 (7.4)	0.71
Dialysis	6 (18.2)	11 (16.2)	0.99
Gastrointestinal disease	1 (3.0)	4 (5.9)	0.90
Connective tissue disease	0 (0)	5 (7.4)	0.17
Diabetes	9 (27.3)	12 (17.6)	0.39
Neoplasia	5 (15.2)	8 (11.8)	0.87
Lymphoproliferative disease	1 (3.0)	3 (4.4)	0.99
HIV/AIDS	2 (6.1)	7 (10.3)	0.71
Charlson comorbidity score	2 (1–5)	2 (1–3.75)	0.76
APACHE II score at polymyxin B initiation	20.8 ± 7.4	19.5 ± 6.4	0.34
Length of ICU stay before polymyxin B initiation	11 (5.5–16)	8.5 (6–16.75)	0.93
Vasoactive drug	19 (57.6)	30 (44.1)	0.29
Mechanical ventilation	27 (81.8)	53 (77.9)	0.85
Time to start polymyxin B (days ^c)	2 (0–3)	2 (0–3)	0.72
Polymyxin B dose			
mg/kg/day	2.2 ± 0.5	2.3 ± 0.6	0.61
Total mg/day	153.5 ± 22.0	152.5 ± 34.3	0.86

^a 25th to 75th percentile.
^b Twenty-seven with pneumonia, 47 with ventilator-associated pneumonia, and 8 with ventilator-associated tracheobronchitis.
^c Time zero is time of polymyxin B empirical therapy.

not rely on the use of a second antimicrobial with *in vitro* activity is of paramount importance. The first reason for this is that in clinical practice, susceptibility of GNB (particularly *A. baumannii* and *P. aeruginosa*) only to polymyxins is a quite common, if not the most common, phenotype of these XDR organisms (1). The

TABLE 2 Potential risk factors for 30-day mortality in patients treated with polymyxin B

Variable	Value [no. (%), (mean ± SD), or median (IQR ^a)] for patients that died (Yes)/did not die (No) within 30 days		P value
	Yes (n = 60)	No (n = 41)	
Gender (male)	32 (53.3)	24 (58.5)	0.75
Age (yrs)	66.0 ± 16.4	64.1 ± 14.7	0.76
Weight (kg)	71.1 ± 19.7	68.6 ± 15.1	0.51
Body mass index	26.1 ± 6.6	24.8 ± 5.2	0.10
Estimated creatinine clearance of ≥60 ml/min	12 (20)	19 (46.3)	0.01
Treated in hospital A	56 (93.3)	38 (92.7)	0.99
<i>A. baumannii</i> infection	46 (76.7)	37 (90.2)	0.11
Primary infection site			
Bloodstream	7 (11.7)	2 (4.9)	0.31
Respiratory tract	48 (80.0)	34 (82.9)	0.91
Abdominal	2 (3.3)	4 (9.8)	0.22
Upper urinary tract	3 (5.0)	1 (2.4)	0.65
Bacteremia	14 (23.3)	5 (12.2)	0.25
Polymicrobial infection	11 (18.3)	8 (19.5)	0.99
Comorbidity			
Cardiovascular disease	40 (66.7)	25 (61.0)	0.71
Pulmonary disease	17 (28.3)	7 (17.1)	0.29
Central nervous system disease	16 (26.7)	10 (24.4)	0.98
Liver disease	5 (8.3)	3 (7.3)	0.99
Dialysis	9 (15.0)	8 (19.5)	0.75
Gastrointestinal disease	2 (3.3)	3 (7.3)	0.39
Connective tissue disease	3 (5.0)	2 (4.9)	0.99
Diabetes	12 (20.0)	9 (22.0)	0.99
Neoplasia	8 (13.3)	5 (12.2)	0.99
Lymphoproliferative disease	4 (6.7)	0 (0)	0.14
HIV/AIDS	5 (8.3)	4 (9.8)	0.99
Charlson comorbidity score	2 (1–4.75)	1 (1–4)	0.16
APACHE II score at polymyxin B initiation	21.3 ± 5.2	17.9 ± 8.1	0.01
Length of ICU stay before polymyxin B initiation	8 (5.25–17.75)	10 (6–16)	0.31
Vasoactive drug	32 (53.3)	17 (41.5)	0.33
Mechanical ventilation	50 (83.3)	30 (73.2)	0.32
Combination therapy	14 (23.3)	19 (46.3)	0.03
Time to start polymyxin B (days ^b)	2 (0–2.75)	2 (0–3)	0.81
Polymyxin B dose			
mg/kg/day	2.3 ± 0.6	2.3 ± 0.5	0.99
Total mg/day	153.7 ± 34.5	153.1 ± 23.6	0.67

^a 25th to 75th percentile.
^b Time zero is time of polymyxin B empirical therapy.

second reason is that it suggests that a synergic action might have occurred *in vivo*, possibly resulting in a more potent microbiological action (16), since with the high-level resistance to carbapenems (most MICs were >32 mg/liter) presented by the isolates, the pharmacokinetic/pharmacodynamic target was unlikely to be attained even with high-dose extended infusion schemes (17).
One strength of this study is that we aimed to control for the potential for indication bias, either by evaluating variables potentially associated with combination therapy use one by one in the

TABLE 3 Cox proportional hazards regression model for 30-day mortality^a

Variable	HR ^b	95% CI ^c	P value
Combination therapy	0.33	0.17–0.64	0.001
APACHE II score at polymyxin B initiation	1.06	1.02–1.10	0.005
Creatinine clearance of ≥ 60 ml/min	0.42	0.22–0.81	0.009
Polymicrobial infection	2.01	1.01–4.0	0.05

^a Inclusion of the time to initiate therapy ($P = 0.33$) and the propensity score ($P = 0.36$) in the model did not change the results of the other variables. The propensity score for prescribing combination therapy included polymicrobial infection, *Acinetobacter baumannii* infections, connective tissue disease, and polymyxin B daily dose in a logistic regression model using a parsimonious backward stepwise method (Hosmer-Lemeshow goodness-of-fit test: $\chi^2 = 5.182$; $P = 0.64$). Body mass index, *Acinetobacter baumannii* infections, lymphoproliferative disease, Charlson score, polymyxin B dose (Table 2), and connective tissue disease (Table 1) were also evaluated in the model but did not remain because their P values were greater than 0.10.

^b HR, hazard ratio.

^c CI, confidence interval.

multivariate model or by including a propensity score in the model, created with these same variables. As variables that differed between combination and monotherapy groups, with the exception of polymicrobial infection, did not remain in the final model, and the propensity score did not change any variable in the final model, we believe that our main results were not affected by indication bias.

Another difference from previous studies is that the polymyxin B dose and time to initiation of therapy (both were forced into the model) were considered in the analysis. Nonetheless, neither was associated with the outcome. Different from a previous study (18), we did not find an association between dose and outcome and it was most likely due to the fact that there was a low variability in polymyxin B dose regimes, with most patients receiving doses between 2 and 3 mg/kg/day. Ultimately, this indicates that our results may not be explained by polymyxin B dose regimes that differ between combination and monotherapy. It should also be emphasized that doses were prescribed according to recent recommendations (11), and no adjustment for renal dysfunction was performed. Finally, in our study, polymyxin B was compared with polymyxin B plus a second antimicrobial, while most previous studies have compared several combination schemes (with and without polymyxins) against other distinct antimicrobials alone (7).

The protective effect was also found in the subgroup of patients with *A. baumannii* infections and, despite the low number of these infections, there was a remarkable difference in mortality rates for

TABLE 5 Hazard ratio of combination therapy for 30-day mortality in the subgroup analysis

Subgroup ^a	No. of patients	HR ^d	95% CI ^c	P value
Combination with a β -lactam ^b	97	0.33	0.17–0.66	0.002
Combination with carbapenem ^c	92	0.40	0.20–0.81	0.01
<i>Acinetobacter baumannii</i> infection	83	0.44	0.22–0.87	0.018
Lower respiratory tract infection	82	0.31	0.14–0.66	0.003
Bacteremia	19	0.58	0.11–3.12	0.52

^a Adjusted for APACHE II score at polymyxin B initiation, creatinine clearance of ≥ 60 ml/min, and polymicrobial infection.

^b Twenty-four patients received a carbapenem, 3 ampicillin-sulbactam, and 2 piperacillin-tazobactam.

^c Twenty-three patients received meropenem and 1 imipenem.

^d HR, hazard ratio.

^e CI, confidence interval.

the few patients treated with combination therapy and those given monotherapy for *P. aeruginosa* infections. In the subgroup of patients with respiratory tract infections, combination therapy remained protective in the multivariate model. In fact, since most patients had infections at this site, we believe that our results may be most readily and safely generalized to patients with respiratory tract infections, especially because we did not find a significantly protective effect of combination therapy in the group of patients with nonrespiratory infections (data not shown). Finally, there was no difference between the therapeutic strategies in bacteremic patients. However, the low number of patients included in this subgroup precluded any definitive conclusion.

The study was limited by the small number of patients with *P. aeruginosa* infections, which ultimately hampered the evaluation of this subgroup in multivariate analysis. We also could not obtain polymyxin B MICs for all of the *P. aeruginosa* isolates; however, all proved to be susceptible according to the disk diffusion method, which is recommended by the CLSI. Additionally, some isolates were tested for polymyxin B susceptibility not by broth microdilution but by Etest. However, although Etest results may not precisely correlate with broth microdilution results, many studies have found excellent rates of categorical agreement between these methods (19–21). Therefore, we believe it unlikely that this biased our results. Finally, there were many exclusions of patients who were treated for <48 h or died within the first 48 h of therapy. However, we believe that these exclusions are also unlikely to have affected our results. The first reason is that patients receiving at least one dose of polymyxin B for *A. baumannii* or *P. aeruginosa*

TABLE 4 Antimicrobials used in combination with polymyxin B

Antimicrobial ^a	No. (%) of patients receiving drug ($n = 33$)	Daily dose(s)	Baseline creatinine clearance (liter/min)	No. (%) of patients with 30-day mortality ($n = 14$ [42.4%])
Meropenem	23 (69.7)	3 g (2–3) ^b	52.3 (27.5–80.5) ^b ; 7 in dialysis	11 (47.8)
Imipenem	1 (3.0)	500 mg	24.4	1 (100)
Ampicillin-sulbactam	3 (6.4)	6 g, 9 g, 12 g	14.1, 27.3, 103.4	0
Piperacillin-tazobactam	2 (4.3)	9 g, 13.5 g	33.4, 54.2	1 (50.0)
Rifampin	1 (3.0)	900 mg	35.0	1 (100)
Amikacin	3 (9.1)	1,000 mg	62.9, 81.9, 161.1	0 (0)

^a All except one isolate (MIC of 4 mg/liter for meropenem and classified as “intermediate”) were resistant (all MIC ≥ 32 mg/liter) to these antimicrobials *in vitro* according to CLSI guidelines (15); rifampin MIC of >16 mg/liter.

^b Median (IQR).

were initially selected for the study, regardless of the antimicrobial susceptibility profile of the isolates, and posteriorly, when the susceptibility profile was available to the clinician, treatment was changed to another agent in 56 patients. Additionally, the exclusion of patients dying before 48 h of therapy had been previously defined based on the rationale that deaths occurring within this period were very unlikely to be related to the treatment itself (either combination or monotherapy), among other reasons because the steady state of polymyxin B is reached after 48 h of therapy (11).

In summary, our study showed that the combination of polymyxin B with an antimicrobial lacking *in vitro* activity, mainly one of the carbapenems, was able to significantly and importantly decrease the risk of mortality in critically ill patients infected by XDR *A. baumannii* or *P. aeruginosa*. Nonetheless, although the magnitude of the protective effect of combination therapy found in our study was clinically important, while randomized clinical trials are not available, we believe that further cohort studies addressing important confounding factors are necessary before adopting combination therapy as the standard of care for all patients with such infections.

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