

Colistin versus Colistin Combined with Ampicillin-Sulbactam for Multiresistant *Acinetobacter baumannii* Ventilator-associated Pneumonia Treatment: An Open-label Prospective Study

Demosthenes Makris, Efi Petinaki¹, Vassiliki Tsolaki, Efstratios Manoulakas, Konstantinos Mantzarlis, Olimpia Apostolopoulou, Dimitrios Styra², Epaminondas Zakynthinos

Departments of Critical Care and ¹Microbiology, University Hospital of Larissa, Larissa, ²Intensive Care Unit, Lamia General Hospital, Lamia, Greece

Abstract

Background: Retrospective studies have reported good clinical success rates using colistin as monotherapy to treat *Acinetobacter baumannii* ventilator-associated pneumonia (VAP), comparable to that obtained with colistin combined with other antibiotics. However, inadequate penetration into the pulmonary parenchyma for colistin has been shown in animal models. **Aim:** The aim of the study was to study prospectively the outcome, measured as clinical response and survival, of intravenously administered colistin versus colistin combined with high-dose ampicillin-sulbactam in Intensive Care Unit (ICU) patients with multiresistant *A. baumannii* VAP. **Methods and Subjects:** This prospective, open-label, randomized study included consecutive patients who developed microbiologically documented VAP due to *A. baumannii* with carbapenem-resistant strains but susceptible to colistin and ampicillin-sulbactam. Seventy-four patients were screened, but finally, 39 participants were enrolled and finished the study. Patients received colistin (Group A – 19 patients) or colistin and ampicillin/sulbactam (Group B – 20 patients). The clinical response of VAP was assessed on day 4th to 5th of treatment (early response). If therapy was considered unsuccessful after this period, ampicillin/sulbactam was added in Group A or changed therapy in B. **Results:** Early cure rates in Group A and B were 15.8% and 70%, respectively ($P = 0.001$). Multiple regression analysis revealed that combination treatment (odds ratio [OR]: 43.6, 95% confidence interval [CI]: 3.594–530.9) and Sequential Organ Failure Assessment score <8 (OR: 0.022, 95% CI: 0.001–0.43) were independently associated with favorable clinical response. APACHE II score ≤ 15 (OR: 0.049, 95% CI: 0.003–0.0942) and an early favorable response to treatment (OR: 244.4, 95% CI: 2.151–27850.9) were associated with survival and discharge from ICU. **Conclusion:** Combination therapy with colistin and a high dose of ampicillin/sulbactam was associated with a more favorable clinical response to VAP due to carbapenem-resistant *A. baumannii* than colistin monotherapy.

Keywords: *Acinetobacter baumannii*, colistin, Intensive Care Unit, pneumonia, ventilator

INTRODUCTION

Morbidity and mortality is increased in critically ill patients who develop ventilator-associated pneumonia (VAP).^[1,2] In particular, VAP caused by *Acinetobacter baumannii* is associated with high crude mortality rates.^[2-5] In addition, mortality rate is lower when VAP is caused by a specific pathogen with antibiotic-sensitive strains than when a drug-resistant strain is involved.

A. baumannii is characterized by its capacity to spread, its ability to survive on most environmental surface, and its amazing easiness with which it acquires antimicrobial multiple

resistance. The past 15 years, many *A. baumannii* nosocomial strains isolated worldwide are highly resistant to almost all available families of modern antibiotics,^[2-7] making treatment of *A. baumannii* VAP, the most important among nosocomial

Address for correspondence: Prof. Epaminondas Zakynthinos, Department of Critical Care, University Hospital of Larissa, Biopolis, 41110 Larissa, Greece. E-mail: ezakynth@med.uth.gr

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Makris D, Petinaki E, Tsolaki V, Manoulakas E, Mantzarlis K, Apostolopoulou O, et al. Colistin versus colistin combined with ampicillin-sulbactam for multiresistant *Acinetobacter baumannii* ventilator-associated pneumonia treatment: An open-label prospective study. Indian J Crit Care Med 2018;22:67-77.

Access this article online

Quick Response Code:



Website:
www.ijccm.org

DOI:
10.4103/ijccm.IJCCM_302_17

infections by such organisms, a challenge for physicians. Importantly, *Acinetobacter* is the dominant isolate in VAP in Greece.^[2]

Carbapenems are considered the antimicrobials of choice; however, *A. baumannii*, which is usually involved in VAP in the South countries of Europe, like Greece, shows high resistance rates to carbapenems;^[3,6,7] as alternative options are scarce in multiresistant strains of *A. baumannii*, colistin seems to be a solution to the problem.^[2-13] Yet, sulbactam component of ampicillin-sulbactam seems to be clinically effective against some carbapenem-resistant isolates.^[5,14] Garnacho-Montero *et al.* reported good clinical and microbiological success rates using intravenous colistin to treat *A. baumannii* VAP caused by carbapenem-resistant strains.^[7] In that case, colistin efficacy was comparable to that obtained with imipenem at the time to treat VAP due to imipenem-susceptible strains. However, colistin as monotherapy led to poor results in mouse pneumonia by multiresistant *A. baumannii*, due to inadequate penetration into the pulmonary parenchyma;^[15] in contrast, combination therapy with two antibiotics, with or without colistin one of them, seemed to be a good option in animal models.^[16] However, these combinations have not been tested clinically prospectively, as yet.

The presence of multiresistant *A. baumannii* isolates only susceptible to colistin and ampicillin-sulbactam *in vitro*, during an outbreak, prompted us to try treatment with colistin as a last resort in patients with VAP by such strains. As our first results in VAP cases treated with colistin alone were not satisfactory, we decided to perform this study to compare (a) the outcome, measured as clinical response and survival, of intravenously administered colistin as monotherapy versus combination therapy of colistin combined with high-dose ampicillin-sulbactam in Intensive Care Unit (ICU) patients with VAP.

MATERIALS AND METHODS

This prospective, open-label, randomized study was performed at two Greek medical-surgical ICUs in University Hospital of Larissa (12-bed unit) and Lamia General Hospital (6-bed unit). These hospitals are in proximity and in their ICUs are admitted patients from the same Neurosurgery Department based on the University Hospital of Larissa. Hospital Ethics Committees approved the study, and informed consent was obtained by participants or next of kin.

Subjects-study design

The study included all consecutive patients which were admitted within a 3-year period if they were >18 years, intubated for more than 48 h, and had a microbiologically documented VAP, due to *A. baumannii* resistant to carbapenems but susceptible to colistin and at least intermediate susceptibility to ampicillin/sulbactam (minimum inhibitory concentration for ampicillin/sulbactam <16 mg/L). These patients were randomized to receive colistin only (Group A) or combination therapy with colistin (dose as Group A) and a high dose

of ampicillin/sulbactam (Group B), hypothesizing that this combination may have satisfactory synergy and may demonstrate effective *in vivo*.

Data collection

Data collected included age, sex, admission diagnosis, past medical history, vital signs, and dates of admission and discharge from the ICU. The severity of illness was evaluated by the APACHE II score on the basis of the worst data point of the first 24 h in the ICU.^[16] The severity of multiple organ dysfunction syndrome was evaluated using the Sequential Organ Failure Assessment (SOFA) scale at the time of revealing VAP due to multidrug (including carbapenems)-resistant *A. baumannii*.^[17] SOFA score was recorded daily. Patients were evaluated daily for VAP. In the suspicion of a clinical diagnosis of VAP, tracheal aspirates with a sterile sputum trap for semi-quantitative cultures or bronchoalveolar lavage (BAL) were collected for bacteriological culture. Blood cultures were also obtained. The presentation of VAP with clinical signs of septic shock (needing inotropes) was documented.

Administration of antibiotics

On isolation of strains of multiresistant *A. baumannii* (including carbapenems), patients were randomly assigned to colistin monotherapy or combination therapy. The dosage of colistin sulfomethate sodium (Colistin; Norma, Athens, Greece), administered intravenously, was 3 MU three times daily, adjusted for creatinine clearance according to formulas.^[18] Colistin (in the same dose as previously) was combined with ampicillin/sulbactam 6 (4 + 2) gr four times daily. Ampicillin/sulbactam was reduced in the half when the estimated creatinine clearance was below 20 ml/min.

Nearly all patients initiated antimicrobial treatment empirically when VAP was clinically diagnosed. However, the treatment was modified (i.e., previous empiric antibiotic regimen administered until then for Gram-negative bacteria were discontinued) when the pathogens were cultured, and their susceptibilities were identified, according to our protocol. Seven patients overall – three to A, four to B groups – were receiving colistin in the initial empirical antimicrobial scheme but <72 h, usually in combination with imipenem, when susceptibilities were available. In these patients, colistin was continued, and ampicillin/sulbactam was started as appropriate (Group B). No patient was receiving ampicillin/sulbactam as empiric therapy.

Participants were excluded from the study if empirical antimicrobial treatment against Gram-negative bacteria was longer than 72 h. Participants were randomized and included in the study if colistin was in the initial empirical antimicrobial scheme for VAP. Patients with a clinical diagnosis of VAP but without microbiological documentation, as well as polymicrobial VAP were excluded from the study.

Day 0 (D0) was considered the ICU day of initiating the study treatment. The therapy was scheduled to continue for at least 10 days; however, the exact length of treatment was decided

by agreement among the physicians of the department. If the physician in charge of the patient considered imperative to change or to add treatment (i.e., because of the isolation of a new microorganism other than *A. baumannii* in the face of a new sepsis), this case was included in the study, if there was clear evidence of the course of the therapy.

When monotherapy with colistin was considered unsuccessful after 4 days, ampicillin/sulbactam was added; again, when this scheme was considered unsuccessful, the physician in charge was free to modify treatment. Similarly, the combination therapy (Group B) could change after the 4th day, considering this case as unsuccessful treatment. The physician was free to continue or stop colistin thereafter [Figure 1].

Evaluation of outcome

Clinical response was considered when there was an improvement of symptoms (i.e., fever resolution) for at least 48 h, decrease of purulent bronchial secretions with parallel

improvement in vital signs, or apparent constant decrease of vasopressor dose in the following days, leading to the final end of vasopressor use. The decision of the clinical response was made by the physician in charge of the patient; however, when there was doubt, the decision was taken by agreement among the physicians of the department. Tracheal secretions were evaluated before extubation or at the end of antimicrobial therapy, if the patient was still intubated, to assess *A. baumannii* eradication (microbiological cure). Treatment was considered as a failure if the patient died during deteriorating clinical findings of VAP or septic shock or if the physician in charge of the patient changed therapy after deteriorating clinical findings; however, if the death occurred within 48 h of the initiation of therapy, the case was not considered as true antibiotic failure and was not included in the study.

Accordingly, VAP-related mortality was defined as death that occurred during the treatment period, when the signs of

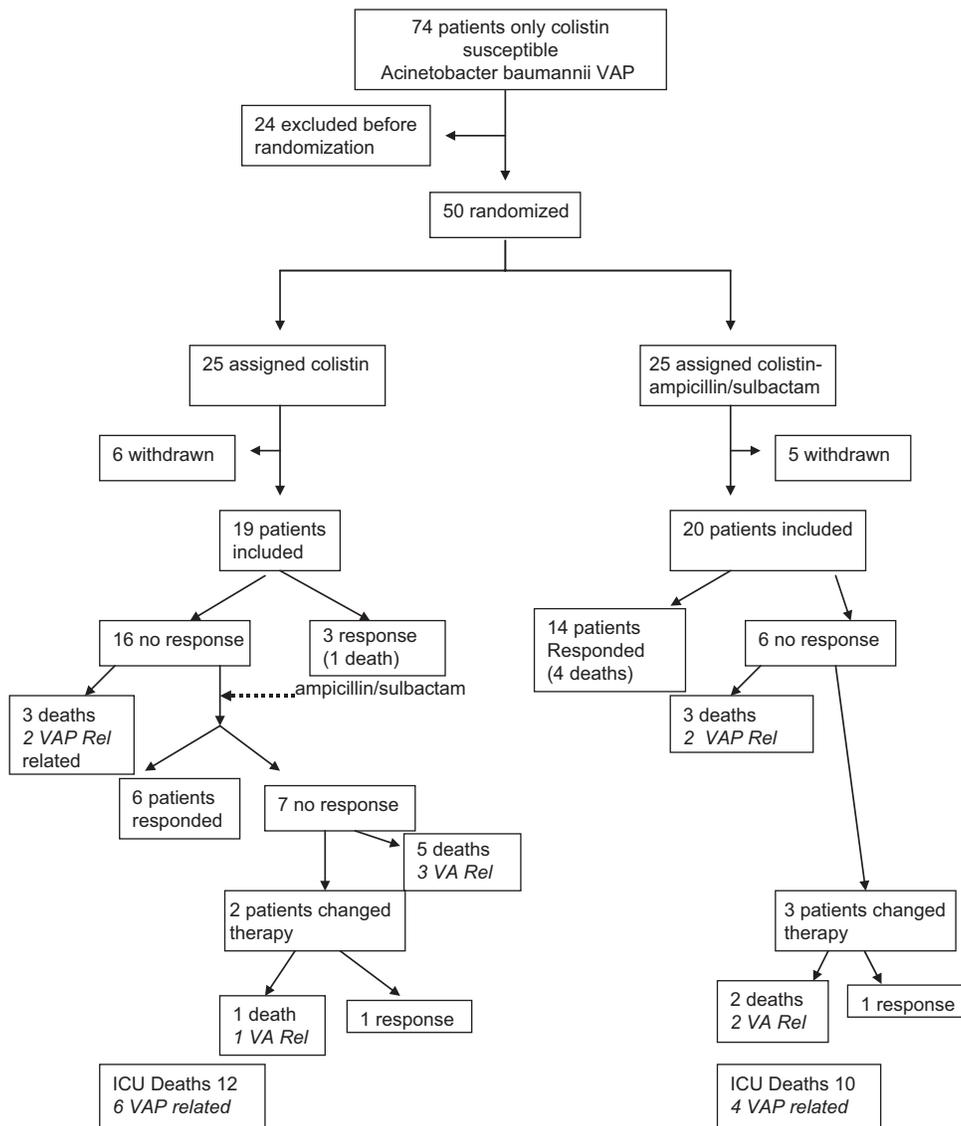


Figure 1: Trial profile

pneumonia remained; death due to septic shock was considered when the cause could not be attributed to other causes than VAP. Overall mortality was assessed at 28 days of VAP onset.

Definitions

The clinical prerequisite for the diagnosis of VAP was the radiographic appearance of a new and persistent pulmonary infiltrate and at least two of the following criteria: temperature of $>38.0^{\circ}\text{C}$ or $<35.5^{\circ}\text{C}$, leukocytosis (leukocyte count $>12,000$ cells/ mm^3) or leukopenia (leukocyte count <4000 cells/ mm^3) or 10% premature leukocytes/ mm^3 , and purulent bronchial secretions. Microbiological documentation of VAP required bacterial growth of $>10^4$ colony-forming units (CFU)/ml from a diagnostic BAL or $>10^5$ CFUs/mL from endotracheal aspirates. Endotracheal aspirates were required to have >25 neutrophils present on Gram's stain, with ten epithelial cells or fewer per high-power field to be accepted for culture of potential pathogens. Only episodes of VAP caused by *A. baumannii* alone were considered. Pneumonia was considered to be ventilator associated when onset occurred 48 h after the initiation of mechanical ventilation (MV) and was judged not to have been incubating before the initiation of MV.

Treatment period for VAP was defined as the period where patients received treatment for VAP (treatment could be the same during the whole period of treatment or different regimes could be used according to the attending physician's decision. This period is not the same in different patients [Figure 1]).

Blood examinations (white blood cell and platelet counts and biochemistry for renal and liver function) were closely monitored. CRP on D0 was defined as the CRP value before the initiation of the study treatment, whereas CRP on D5 was defined as the CRP value on the 4th–5th of treatment. When the patient died before 4th–5th day, D5 was considered as the last value available. The value of creatinine of the last day of colistin administration (in alive or succumbed patients) was compared to creatinine at treatment initiation with colistin. Any decline in renal function that resulted in the need for renal replacement therapy (continuous venovenous hemofiltration [CVVH]) was also recorded.

Statistical analysis

Quantitative variables were expressed as mean value \pm standard deviation. In comparing the two groups, continuous parameters were compared using Mann–Whitney U-test (Wilcoxon Signed-Rank Test) and categorical parameters were compared using Fisher's exact test. The associations between response outcome (or survival outcome) and treatment or other confounding parameters were tested using univariate and multivariate logistic regression. Associations were expressed in terms of unadjusted and adjusted odds ratio (OR) with 95% confidence intervals (CIs). The first of VAP was considered in analysis for each patient. The scale parameters APACHE II and SOFA were also transformed to categorical ones, by splitting in the median value, and sepsis parameter was recorded in two groups: No septic shock/Yes septic

shock. The probability of being alive in the two treatments was compared using survival curve (Kaplan–Meier) analysis and the log-rank test. An effect is considered significant when $P < 0.05$. The statistical analysis was performed using SPSS v. 13.0 (Chicago, IL, USA).

RESULTS

During the study period, 74 mechanically ventilated patients presented VAP with *A. baumannii* susceptible to colistin but resistant to carbapenems. Twenty-four patients were excluded from the study either because they were still requiring antibiotics against Gram-negative bacteria for infections other than VAP or because they had received empirical antibiotic treatment for VAP more than 72 h when results from tracheal aspirates or BAL were available or, due to multimicrobial VAP (more than one Gram-negative pathogen was isolated), or due to favorable clinical response to the initial empirical treatment (that was not colistin) according to the attending physician. Eleven other patients that were initially included in the study were excluded in the course of the study because they died <48 h (2 patients) following recruitment, or due to the presence of other infections necessitating additional antibiotics against Gram-negative bacteria, or due to unclear clinical status, i.e., improvement or deterioration [Figure 1].

Clinical data of participants are shown in Table 1. Age, race, severity of illness on admission to ICU (APACHE II), and admission diagnosis were similar in both groups. SOFA score at VAP diagnosis was 7.6 ± 2 in the first group and 7.9 ± 3.1 in the second group, respectively ($P = \text{NS}$). Hospital stay and MV before VAP were similar in both groups.

Clinical response of ventilator-associated pneumonia to treatment

A considerably high percentage of VAP patients presented septic shock (47.4% and 40% in Groups A and B, respectively). Bacteremia (with *A. baumannii*) presented in 47.4% and 45% in the two groups, respectively [Table 1]. The effect of APACHE score and SOFA score was evaluated on VAP outcome as consecutive measurements, and categorical parameters (lower or higher values of the median) [Tables 2 and 3]. In univariate analysis, treatment (colistin alone or combination therapy) and SOFA score at recruitment were significantly associated with the clinical response of VAP in the 4th–5th day. Colistin alone resulted in effective response in 15.8% of patients (SOFA score 6.7 ± 1.5), whereas Colistin with a high dose of ampicillin-sulbactam resulted in effective response in 70% of patients (SOFA score 7.0 ± 3.0) (OR: 12.4, 95% CI: 2.6–59.3, $P = 0.001$). The clinical severity at the initiation of VAP treatment was a major determinant of a favorable response. A lower SOFA score was present in cases with favorable response [$P = 0.044$, Table 3]. SOFA score >8 was associated with no response in 80% of cases, and with favorable response in 20% of cases, whereas SOFA score ≤ 8 was associated with no response in 41.7% and 58.3%, respectively [OR: 0.18, 95% CI: 0.04–0.8, $P = 0.024$, Table 2].

Table 1: Demographic characteristics

	Patient data, by treatment group		P
	Group A (n=19)	Group B (n=20)	
Age (years)	56.6±14.3	56.9±18.7	0.53
Male sex, n (%)	12 (63)	15 (75)	0.50
Admission diagnosis (ICU)			
Medical	8 (42)	12 (60)	
Surgical	11	8	
Trauma	9	6	
Nontrauma	2	2	
APACHE II	14.5±3.1	16.5±4.7	0.11
APACHE II ^a >15 (%)	33.3	66.7	0.111
APACHE II ≤15 (%)	61.9	38.1	
Characteristics on initiation of VAP treatment			
SOFA	7.6±2.0	7.9±3.1	0.75
SOFA ^a >8 (%)	50	50	1.0
SOFA ≤8	46.7	53.3	
Septic shock, n (%)	9 (47.4)	8 (40)	
Septic shock ^a (yes) (%)	52.9	47.1	0.75
Septic shock (no) (%)	45.5	54.5	
Days MV before VAP	9.1±3.8	11.8±7.9	0.5
Hospital days before VAP	10±3.8	14±7.8	0.11
CRP D0 ^a	21.0±6.2	22.3±5.8	0.41
Characteristics during VAP			
Bacteremia (acinetobacter in blood)	9 (47.4)	9 (45)	
Bacteremia ^a (yes) (%)	50	50	1.0
Bacteremia (no) (%)	47.6	52.4	
CRP D5	22.2±8.1	13.9±6.6	0.002
Eradication (initial therapy)	1/3	10/14	0.191

^aYes or no (Fisher's exact test) of the total amount of APACHE II >15 or APACHE II ≤15, SOFA >8 or SOFA ≤8, septic shock, or bacteremia in both groups; General characteristics of patients in colistin as monotherapy (Group A) and combination therapy with colistin-ampicillin-sulbactam (Group B). APACHE: Acute Physiologic Assessment and Chronic Health Evaluation; VAP: Ventilator-associated pneumonia; CRP: C-reactive protein; MV: Mechanical ventilation; SOFA: Sequential Organ Failure Assessment; ICU: Intensive Care Unit

Multiple regression analysis revealed that combination treatment [OR 43.6, 95% CI: 3.594–530.9, Table 2] and a milder severity of patient's situation on recruitment, i.e., SOFA score <8 [OR 0.022, 95% CI: 0.001–0.43, Table 2] were independent predictors for good clinical response.

CRP at D0 did not differ between patients in the two groups; CRP at D0 did not differ between patients that responded to treatment on the 4th–5th day ($n = 17$) or not ($n = 22$) (22.88 ± 5.04 vs. 20.68 ± 6.52 , $P = 0.22$). The difference between CRP D5 and CRP D0 was 10 ± 1.73 ($n = 3$) and 11.71 ± 6.63 ($n = 14$) when VAP responded in A and B groups, respectively, and -3.31 ± 8.28 ($n = 16$) and 0.67 ± 9.35 ($n = 6$) when there was no response to

treatment. Overall, CRP D5 decreased 10.9 ± 2.4 in patients who responded ($n = 17$) ($P = 0.001$) and -3.31 ± 8.28 in patients who did not respond ($n = 22$) to antibiotic treatment ($P = \text{NS}$). Therefore, CRP decreased when VAP improved independently of the treatment (colistin alone or colistin with ampicillin-sulbactam).

When ampicillin-sulbactam was added to colistin after treatment failure in Group A, 46.2% responded to this therapy [Figure 1] (in contrast to 70% response in Group B, when ampicillin-sulbactam was given early). However, this was not statistically significant ($P = 0.28$).

Therapy duration with colistin in patients that VAP responded positively to treatment was 18.1 ± 2.4 and 15.3 ± 4.3 days, in Group A and Group B, respectively ($P = \text{NS}$).

Mortality

In total, 22 (56%) patients died in ICU after VAP onset. Mortality rates in Group A and B were 12/19 (63%) and 10/20 (50%), respectively ($P = \text{NS}$). APACHE II score on admission was lower in patients finally survived ICU [13.8 ± 3.6 vs. 16.9 ± 4.0 , $P = 0.024$, Table 4]. SOFA score in the initiation of VAP treatment was, also, lower among patients who dismissed ICU, than among those who died in ICU [6.41 ± 2.64 vs. 8.77 ± 1.97 , $P = 0.007$, Table 4]. Yet, an initial response to treatment on the 4th–5th day of VAP treatment was fundamental to ICU discharge [$P = 0.004$, Tables 4 and 5]. Ten patients from Group B and 2 patients from Group A that initially responded to treatment and survived at 28 days of infection onset [Figure 1]. There was a trend for younger people to have a better prognosis than older patients [$P = 0.059$, Tables 4 and 5].

In multiple regression analysis, APACHE II score ≤15 [OR: 0.049, 95% CI: 0.003–0.0942, Table 5] and an initial positive response to treatment on the 4th–5th day of VAP treatment [OR: 244.4, 95% CI: 2.151–27850.9, Tables 4 and 5] were associated with survival and discharge from ICU. The selection of therapy, i.e., Group A or Group B (although the addition of ampicillin-sulbactam after treatment failure for VAP in Group A confuses the result) did not affect mortality as is shown in Tables 4 and 5 and in Kaplan–Meier analysis [Figure 2], $P = 0.21$.

During VAP treatment period [Figure 1], nine out of the 19 (47%) and 5 out of the 20 (25%) patients died ($P = 0.191$, OR = 0.96–1.44); death in six patients of Group A (32%) and four patients of Group B (20%) was attributed by the attending physician (or by agreement among the physicians of the department, when there was a doubt) to VAP [VAP-related death, Figure 1]. Five patients of Group A, whose death was attributed to *A. baumannii* pneumonia, died in septic shock. However, one patient in this group (who was included in VAP-related deaths by agreement among the physicians of the department) died with brain edema and brain death. In that case, there was no evidence of response to VAP treatment until his death (although ampicillin-sulbactam had been added). One

Table 2: Response to treatment at day 4th-5th (I)

Results	Response		Unadjusted OR (95% CI)	P	Adjusted OR (95% CI)
	No	Yes			
Treatment (%)					
Colistin	84.2	15.8	12.444 (2.614-59.251)	0.001	43.608 (3.582-530.917)
Beg and Col	30.0	70.0			
Age	58.18±12.31 ^a 58.50 (34-77) ^b	54.82±21.00 64 (18-78)	NA	0.899	0.960 (0.901-1.023)
APACHE category (%)					
≤15	61.9	38.1	1.625 (0.453-5.824)	0.528	3.117 (0.283-34.348)
>15	50.0	50.0			
SOFA category (%)					
≤8	41.7	58.3	0.179 (0.040-0.803)	0.024	0.022 (0.001-0.428)
>8	80.0	20.0			
Bacteremia (%)					
No	52.4	47.6	0.700 (0.195-2.511)	0.748	1.289 (0.195-8.511)
Yes	61.1	38.9			

^aMean±SD; ^bMedian (minimum–maximum). APACHE: Acute Physiologic Assessment and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; OR: Odds ratio; CI: Confidence interval; SD: Standard deviation; NA: Not available

Table 3: Response to treatment at day 4th-5th (II)

Results	Response		Unadjusted OR (95% CI)	P	Adjusted OR (95% CI)
	No	Yes			
Treatment (%)					
Colistin	84.2	15.8	12.444 (2.614-59.251)	0.001	25.505 (3.183-204.361)
Beg and Col	30.0	70.0			
Age	58.18±12.31 ^a 58.50 (34-77) ^b	54.82±21.00 64 (18-78)	NA	0.899	0.999 (0.951-1.050)
APACHE	15.64±3.63 14.5 (8-23)	15.41±4.69 16 (7-28)	NA	0.887	0.941 (0.741-1.195)
SOFA	8.36±2.24 9 (3-14)	6.94±2.77 7 (2-13)	NA	0.044	0.704 (0.476-1.041)
Bacteremia (%)					
No	52.4	47.6	0.700 (0.195-2.511)	0.748	0.776 (0.139-4.348)
Yes	61.1	38.9			

^aMean±SD; ^bMedian (minimum–maximum). SD: Standard deviation; APACHE: Acute Physiologic Assessment and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; NA: Not available; OR: Odds ratio; CI: Confidence interval

patient in Group B died suddenly from abdominal hemorrhage. Finally, three patients in Group A and one patient in Group B died during the treatment period (VAP period), but their death was not associated with deterioration of VAP [Figure 1]. Finally, three patients in Group A and five patients in Group B, who had responded positively to treatment, died later in ICU due to other causes.

Microbial eradication

Four patients (29%) in Group B considered to be clinically cured, but *Acinetobacter* was still present in tracheal aspirates after the end of treatment. Culture results were negative for the rest ten patients (71%). Two out of three patients who presented favorable response to treatment in the Group A, tracheal aspirates were still positive after the end of treatment (66%) ($P = \text{NS}$). Interestingly, one out of four patients in Group B, who presented microbiological

failure, was extubated at the 8th day following VAP, having an obvious clinically successful treatment; tracheal cultures were still positive for resistant *A. baumannii* at that point. Although that case was considered as microbiological failure, probably it was rather early to be definite (initial therapy for VAP was continued for four more days). Yet, in four patients out of six in Group A, who responded with the addition of ampicillin-sulbactam to treatment, *A. baumannii* was eradicated from tracheal aspirates.

Renal toxicity

Serum creatinine levels were not different between groups at the initiation of study treatment. However, creatinine was different between patients that responded positively to therapy with colistin (colistin with ampicillin-sulbactam, colistin alone, or colistin adding ampicillin-sulbactam later on Group A) compared to those with an unfavorable outcome (nonresponders).

Table 4: Mortality at 28 days of ventilator-associated pneumonia onset (II)

Results	Survival		Unadjusted OR (95% CI)	P	Adjusted OR (95% CI)
	Not alive	Yes, alive			
Treatment (%)					
Colistin	63.2	36.8	1.714 (0.477-6.163)	0.523	0.749 (0.050-11.248)
Beg and Col	50.0	50.0			
Age	61.77±12.17 ^a	50.18±19.28	NA	0.059	0.933 (0.851-1.024)
	63 (34-78) ^b	52 (18-73)			
APACHE II	16.91±3.98	13.76±3.56	NA	0.024	0.667 (0.407-1.094)
	16 (11-28)	14 (7-22)			
SOFA	8.77±1.97	6.41±2.65	NA	0.007	0.972 (0.574-1.664)
	8.5 (6-14)	6 (2-11)			
Bacteremia (%)					
No	47.6	52.4	0.455 (0.124-1.670)	0.334	0.223 (0.025-1.955)
Yes	66.7	33.3			
Response (%)					
No	77.3	22.7	8.160 (1.927-34.549)	0.004	27.579 (1.077-706.358)
Yes	29.4	70.6			

APACHE: Acute Physiologic Assessment and Chronic Health Evaluation; SOFA: Sequential organ failure assessment; NA: Not available; OR: Odds ratio; CI: Confidence interval; a: mean +/- SD; b: median (25-75 quartiles)

Table 5: Mortality at 28 days of ventilator-associated pneumonia onset (I)

Results	Survival		Unadjusted OR (95% CI)	P	Adjusted OR (95% CI)
	Not alive ("0")	Yes alive ("1")			
Treatment (at final survival) (%)					
Colistin Group A	63.2	36.8	1.714 (0.477-6.163)	0.523	0.326 (0.018-5.893)
Beg and Col Group B	50.0	50.0			
Age	61.77±12.17 ^a	50.18±19.28	NA	0.059	0.910 (0.816-1.015)
	63 (34-78) ^b	52 (18-73)			
APACHE category (%)					
≤15	42.9	57.1	0.288 (0.075-1.108)	0.106	0.049 (0.003-0.942)
>15	72.2	27.8			
SOFA category (%)					
≤8	45.8	54.2	0.308 (0.076-1.245)	0.112	2.908 (0.281-30.112)
>8	73.3	26.7			
Bacteremia (%)					
No	47.6	52.4	0.455 (0.124-1.670)	0.334	0.200 (0.020-1.974)
Yes	66.7	33.3			
Response (at any time, in both groups) (%)					
No	77.3	22.7	8.160 (1.927-34.549)	0.004	244.424 (2.145-27,850.937)
Yes	29.4	70.6			

APACHE: Acute Physiologic Assessment and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; NA: Not available; OR: Odds ratio; CI: Confidence interval; a: mean +/- SD; b: median (25-75 quartiles)

Creatinine levels in responders ($n = 23$) were 1.48 ± 0.65 at recruitment that decreased to 1.32 ± 0.56 whereas creatinine was 1.84 ± 1.1 in nonresponders ($n = 16$) at baseline and increased to 2.15 ± 0.64 at the last day of colistin treatment. One patient in responders and two patients in nonresponders were on CVVH at baseline. At the end of colistin administration, four patients in responders and seven patients in nonresponders ($P = 0.01$) needed CVVH. However, all patients with renal impairment that needed renal replacement therapy with CVVH were on septic shock needing noradrenaline (responders and

nonresponders) or developed septic shock in the course of VAP therapy (nonresponders). Interestingly, in all patients with a good outcome of VAP that finally discharged from ICU (2 patients), renal failure was mild, the creatinine level was decreasing, and CVVH was not necessary.

Four isolates, randomly selected, from blood cultures from individual patients with VAP and bacteremia (with an at least 3-month difference in collection time) were examined for their clonality [Figure 3]. All isolates were from the same clone showing an outbreak.

DISCUSSION

To the best of our knowledge, this is the first prospective study to provide detailed data on the efficacy of colistin as monotherapy versus combination of colistin with high-dose sulbactam (as part of ampicillin-sulbactam regime) in adult patients with multiresistant *A. baumannii* VAP.

The main finding of our study was that colistin as monotherapy proved insufficient to treat VAP caused by multidrug-resistant *A. baumannii* resistant to carbapenems. On the contrary, the combination of colistin with a high dose of ampicillin-sulbactam resulted in 70% clinical response (vs. 16%) when started early. Second, although the selection of the arm treatment (A or B) did not influence mortality, the initial response to treatment on 4th–5th day (consigned mainly to group with combination therapy from VAP onset) was critical for 28-day outcome. Third, the severity of patients in the initiation of VAP treatment was essential for the infection cure rate, whereas the severity on admission to ICU was crucial for mortality. Fourth, renal toxicity seemed to be associated with the clinical status of the patients (septic shock needing noradrenaline, not responding to therapy) and not to the use of colistin.

Over the past years, colistin has been extensively used for the treatment of VAP caused by resistant *A. baumannii*. The clinical cure rate of VAP patients receiving colistin monotherapy or combination therapy with other antibiotics varies largely between 15% and 87%.^[2,6-11,13]

It is controversial whether colistin combined therapy is more effective than colistin monotherapy or not. Older studies, as well as recent ones, have reported that combination therapy did not provide any significant advantage in the treatment of VAP,^[2,7-13] although this was not universally accepted.^[4] However, all these studies were retrospective.

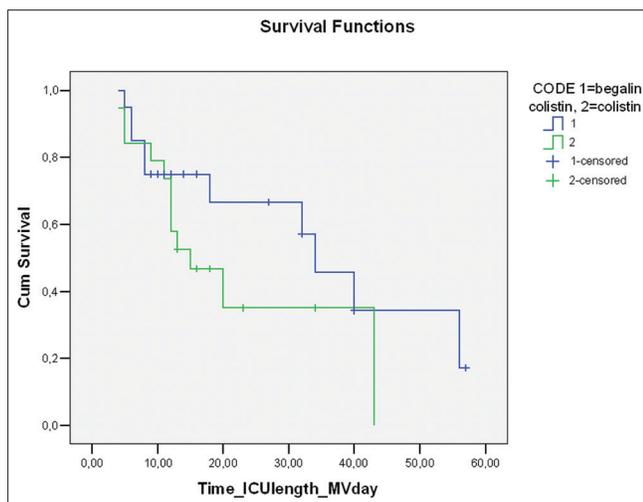


Figure 2: Kaplan–Meier survival analysis for death at 28 days after ventilator-associated pneumonia onset. Comparison between treatment regimens by log-rank test: Colistin alone versus colistin-sulbactam, $P = 0.210$

In most of these, the other drug or drugs than colistin – in combination treatment – were different even in the same study, doses varied, there was no report for the exact time of the initiation of treatment nor for its duration.^[2,8,9,11-13] Moreover, it may seem reasonable for the attending physician to administer combination therapy – and not only colistin – in VAP caused by *A. baumannii* in a critically ill patient, or to add suitable antibiotics in the course of the infection if the patient deteriorates or shows no improvement. Notably, in a recent report by Falagas *et al.*, various combinations were compared in *A. baumannii* infections, and the clinical cure with colistin monotherapy was found to be superior to colistin and meropenem and to colistin and piperacillin-tazobactam (87%, 83.9%, and 67.7%, respectively). Patients' severity is also difficult to be evaluated precisely, and therefore results are not adjusted convincingly for severity in many studies.^[2,8,9] In a recent retrospective analysis, comparing the clinical efficacy of colistin and colistin/sulbactam for the treatment of multidrug-resistant *A. baumannii* VAP in ICUs, the authors concluded that although the difference was not statistically significant, clinical cure rates were better in the combination group than colistin monotherapy. However, it should be noted that the median APACHE II score was higher and diabetes mellitus was more common in the colistin/sulbactam group ($P < 0.05$).^[4] In our study, APACHE II score on admission and more significantly, SOFA on the initiation of VAP was not different between groups. The rate of clinical response on the 4th–5th day with colistin as monotherapy was only 16%, while combination therapy resulted in 70% response when ampicillin-sulbactam was administered from the initiation of therapy (Group B). The combination treatment and the severity of the patient's status (SOFA score >8) were the only independent predictors of the early response (on the 4th–5th day). CRP decreased when VAP improved independently of the treatment, indicating that clinical assessment was accurate.^[19] Recently, a multicenter study assessing the combination therapy in patients who had bloodstream infections due

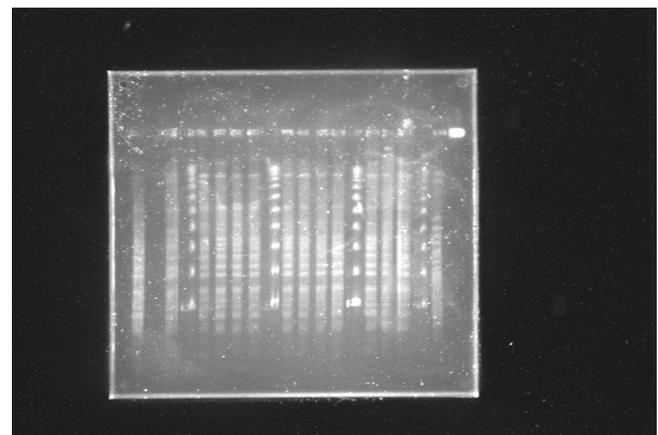


Figure 3: Clonality of four isolates from blood, analyzed by pulsed-field gel electrophoresis method

to extensively drug-resistant (XDR) *A. baumannii* found that colistin-based combination therapy resulted in relatively higher cure and 14-day survival rates, compared to colistin monotherapy.^[20] In our study, bacteremia with *Acinetobacter* was 47%, which is very high for VAP infection but indicates the severity of the patients and may justify the favorable effect of the combination treatment according to the previous study.^[20] Yet, a low penetration of colistin into the lungs has been reported in an animal model;^[15] sulbactam has been found to have synergistic effects with colistin *in vitro*.^[21,22] Since the penetration of sulbactam into the lung tissue is adequate, combination therapy (of colistin-sulbactam) may be indicated in severe VAP as sulbactam is one of the recommended antibiotics for carbapenem-resistant *A. baumannii* VAP infections.^[14,22,23] Finally, as colistin-heteroresistant strains are present among *A. baumannii* isolates,^[24] combining colistin with sulbactam may have prevented the selection and prevalence of colistin-heteroresistant strains.

When sulbactam (as ampicillin-sulbactam) was added to colistin after treatment failure in Group A on the 4th day, half of the patients (46.2%) responded to the therapy. In our opinion, the delay in starting the second (sulbactam) appropriate antibiotic regimen – with *in vitro* activity against *Acinetobacter* – led to the lower rate of response compared to B group when combination therapy with colistin and ampicillin-sulbactam was given early (although not significant). The American Thoracic Society and the Infectious Diseases Society of America focus on the clinical benefit of appropriate therapy for the management of VAP, defined as using initial (early as possible) antibiotics with *in vitro* activity against identified microorganisms causing infection.^[1] Inadequate empirical (initial) therapy was reported to be associated with poor prognosis in patients with *A. baumannii* infection.^[3] However, it is not reported clearly if one or combination therapy is better.

A. baumannii, is one of the most common pathogens causing VAP, associated with high ICU mortality varying among countries (20%–70%).^[2,4,8-15,18,22,25] In our study, 56% patients died before the 28th from VAP onset. This high-mortality rate may be justified by the high percentage of patients with VAP who presented septic shock and/or bacteremia (47.4% vs. 40% and 47.4% vs. 45% in Groups A and B, respectively), which is unusual in VAP.

In recent studies, APACHE II (on admission) and advanced age were identified as independent predictors of 28-day mortality, but no difference in mortality between colistin-based combination therapy and colistin alone groups was produced.^[2,3,13] Similarly, we found high APACHE II (>15) to be independent predictor of mortality. Yet, in our prospective study, an early positive VAP response to treatment (assigned mainly to Group B with combination therapy from the onset of VAP) was an independent predictor of mortality. In most retrospective studies, combination

therapy has been reported to provide a poor survival benefit, or none, among patients who were infected with *A. baumannii*.^[8-13,25] In a multicenter study from Turkey that evaluated the benefits of combination therapy (including colistin with sulbactam) versus colistin monotherapy in patients who had bloodstream infections due to XDR *A. baumannii*, the rates of 14-day survival were relatively higher, and in-hospital mortality was significantly lower in the combination therapy group.^[20] Possibly, the higher percentage of bacteremia in our study played a crucial role. Moreover, in meta-analysis of Kumar *et al.*, combination antibiotic therapy improved survival and clinical response of high-risk, life-threatening infections (not only VAP), particularly those associated with septic shock (as in our study). This benefit was >25% in these patients with high-risk, life-threatening infections.^[26] The selection of therapy, i.e., Group A or Group B in our study did not affect the mortality as is shown in Tables 4 and 5 and in Kaplan–Meier analysis. It is likely that the addition of ampicillin-sulbactam after the initial treatment failure for VAP in Group A obscured any significant difference. Nevertheless, it would be unethical not to add (or change) therapy in this group that did not respond to colistin treatment on the 4th to 5th day.

Microbiological eradication in our study was 71% in the combination group which is consistent with retrospective publications.^[4,11-14] Only one out of three patients of colistin group that were cured without adding or changing therapy and had negative tracheal aspirates after the end of treatment. However, this result is not indicative ($P = NS$). In a recent retrospective study that evaluated the effect of colistin and sulbactam combination therapy on VAP and hospital-acquired pneumonia, Kalin *et al.* reported 72.3% and 85.7% bacteriological cure rates, in colistin versus colistin/sulbactam groups, respectively, but the difference was not statistically significant.^[4]

Interestingly, creatinine level was decreased in patients who responded positively to colistin (colistin with ampicillin-sulbactam, colistin alone, or colistin adding ampicillin-sulbactam later on Group A) ($P = NS$). In contrast, creatinine was increased in those with an unfavorable outcome. In addition, all patients with renal impairment who needed renal replacement therapy with CVVH were on septic shock needing noradrenaline. It seems that infection was not responding, and septic shock was the main culprit of renal impairment and not colistin *per se*. Our findings are consistent with previous studies reporting less nephrotoxicity than previously described.^[11-14] Recently, Durante-Mangoni *et al.* reported acute kidney injury during colistin treatment occurs in a third to a half of colistin-treated patients and is more likely in elderly and kidney disease patients. However, there were no details about the patients on septic shock.^[27]

The main advantage of our study is that it is prospective. However, there are some limitations. The study was

performed in two hospitals, and results may not be generalizable to other settings. Clonality was examined in only a few isolates (randomly selected), showing to come from the same clone. Different clones may have a different reaction to treatment. Culture results with formal microbial identification and resistance patterns were reported to the treating physicians, 2 (but < 3) days after sampling. Therefore, there was a delay between clinical diagnosis of VAP and the administration of the antibiotic regimen (colistin or colistin/ampicillin-sulbactam). Designation of the study defined to add ampicillin-sulbactam or to change therapy in colistin group, having the potential to restrict the ability to detect differences in the mortality outcome. Finally, pharmacodynamics and pharmacokinetics of the colistin could not be evaluated, and more importantly, a loading dose was not used.

CONCLUSION

This is the first prospective study evaluating the impact of combination therapy of colistin versus colistin/ampicillin-sulbactam in *A. baumannii* VAP caused by carbapenem-resistant strains. Our findings suggest that combination therapy was more effective than monotherapy in clinical cure rates of VAP in severe patients. However, larger prospective randomized trials are needed to determine the most effective therapy for VAP with multiresistant *A. baumannii*.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;171:388-416.
- Tsioutis C, Kritsotakis EI, Karageorgos SA, Stratakou S, Psarologakis C, Kokkini S, et al. Clinical epidemiology, treatment and prognostic factors of extensively drug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia in critically ill patients. *Int J Antimicrob Agents* 2016;16:30202-3.
- Koulenti D, Tsigou E, Rello J. Nosocomial pneumonia in 27 ICUs in Europe: Perspectives from the EU-VAP/CAP study. *Eur J Clin Microbiol Infect Dis* 2017;36:1999-2006.
- Kalin G, Alp E, Akin A, Coskun R, Doganay M. Comparison of colistin and colistin/sulbactam for the treatment of multidrug resistant *Acinetobacter baumannii* ventilator-associated pneumonia. *Infection* 2014;42:37-42.
- Betrosian AP, Frantzeskaki F, Xanthaki A, Douzinas EE. Efficacy and safety of high-dose ampicillin/sulbactam vs. colistin as monotherapy for the treatment of multidrug resistant *Acinetobacter baumannii* ventilator-associated pneumonia. *J Infect* 2008;56:432-6.
- Kalle H, Hergafi L, Bahloul M, Hakim A, Dammak H, Chelly H, et al. Safety and efficacy of colistin compared with imipenem in the treatment of ventilator-associated pneumonia: A matched case-control study. *Intensive Care Med* 2007;33:1162-7.
- Garnacho-Montero J, Ortiz-Leyba C, Jiménez-Jiménez FJ, Barrero-Almodóvar AE, García-Garmendia JL, Bernabeu-Wittel M, et al. Treatment of multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia (VAP) with intravenous colistin: A comparison with imipenem-susceptible VAP. *Clin Infect Dis* 2003;36:1111-8.
- Michalopoulos A, Falagas ME. Colistin and polymyxin B in critical care. *Crit Care Clin* 2008;24:377-91, x.
- Falagas ME, Kasiakou SK, Michalopoulos A. Treatment of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* pneumonia. *J Cyst Fibros* 2005;4:149-50.
- Reina R, Estenssoro E, Sáenz G, Canales HS, Gonzalvo R, Vidal G, et al. Safety and efficacy of colistin in *Acinetobacter* and *Pseudomonas* infections: A prospective cohort study. *Intensive Care Med* 2005;31:1058-65.
- Falagas ME, Rafailidis PI, Kasiakou SK, Hatzopoulou P, Michalopoulos A. Effectiveness and nephrotoxicity of colistin monotherapy vs. colistin-meropenem combination therapy for multidrug-resistant gram-negative bacterial infections. *Clin Microbiol Infect* 2006;12:1227-30.
- Chan JD, Graves JA, Dellit TH. Antimicrobial treatment and clinical outcomes of carbapenem-resistant *Acinetobacter baumannii* ventilator-associated pneumonia. *J Intensive Care Med* 2010;25:343-8.
- Falagas ME, Rafailidis PI, Ioannidou E, Alexiou VG, Matthaiou DK, Karageorgopoulos DE, et al. Colistin therapy for microbiologically documented multidrug-resistant gram-negative bacterial infections: A retrospective cohort study of 258 patients. *Int J Antimicrob Agents* 2010;35:194-9.
- Levin AS, Levy CE, Manrique AE, Medeiros EA, Costa SF. Severe nosocomial infections with imipenem-resistant *Acinetobacter baumannii* treated with ampicillin/sulbactam. *Int J Antimicrob Agents* 2003;21:58-62.
- Montero A, Ariza J, Corbella X, Doménech A, Cabellos C, Ayats J, et al. Efficacy of colistin versus beta-lactams, aminoglycosides, and rifampin as monotherapy in a mouse model of pneumonia caused by multiresistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2002;46:1946-52.
- Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: A severity of disease classification system. *Crit Care Med* 1985;13:818-29.
- Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on sepsis-related problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;22:707-10.
- Levin AS, Barone AA, Penço J, Santos MV, Marinho IS, Arruda EA, et al. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin Infect Dis* 1999;28:1008-11.
- Póvoa P, Coelho L, Almeida E, Fernandes A, Mealha R, Moreira P, et al. C-reactive protein as a marker of ventilator-associated pneumonia resolution: A pilot study. *Eur Respir J* 2005;25:804-12.
- Batirel A, Balkan II, Karabay O, Agalar C, Akalin S, Alici O, et al. Comparison of colistin-carbapenem, colistin-sulbactam, and colistin plus other antibacterial agents for the treatment of extremely drug-resistant *Acinetobacter baumannii* bloodstream infections. *Eur J Clin Microbiol Infect Dis* 2014;33:1311-22.
- Rodríguez-Hernández MJ, Cuberos L, Pichardo C, Caballero FJ, Moreno I, Jiménez-Mejías ME, et al. Sulbactam efficacy in experimental models caused by susceptible and intermediate *Acinetobacter baumannii* strains. *J Antimicrob Chemother* 2001;47:479-82.
- Ko WC, Lee HC, Chiang SR, Yan JJ, Wu JJ, Lu CL, et al. *In vitro* and *in vivo* activity of meropenem and sulbactam against a multidrug-resistant *Acinetobacter baumannii* strain. *J Antimicrob Chemother* 2004;53:393-5.
- Kempf M, Djouhri-Bouktab L, Brunel JM, Raoult D, Rolain JM. Synergistic activity of sulbactam combined with colistin against colistin-resistant *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2012;39:180-1.
- Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2006;50:2946-50.
- Yilmaz GR, Guven T, Guner R, Kocak Tufan Z, Izdes S, Tasyaran MA,

- et al.* Colistin alone or combined with sulbactam or carbapenem against *A. baumannii* in ventilator-associated pneumonia. *J Infect Dev Ctries* 2015;9:476-85.
26. Kumar A, Safdar N, Kethireddy S, Chateau D. A survival benefit of combination antibiotic therapy for serious infections associated with sepsis and septic shock is contingent only on the risk of death: A meta-analytic/meta-regression study. *Crit Care Med* 2010;38:1651-64.
27. Durante-Mangoni E, Andini R, Signoriello S, Cavezza G, Murino P, Buono S, *et al.* Acute kidney injury during colistin therapy: A prospective study in patients with extensively-drug resistant *Acinetobacter baumannii* infections. *Clin Microbiol Infect* 2016;22:984-9.