Colistin *vs.* the combination of colistin and rifampicin for the treatment of carbapenem-resistant *Acinetobacter baumannii* ventilator-associated pneumonia

H. AYDEMIR[1]*, D. AKDUMAN[1], N. PISKIN[1], F. COMERT[2], E. HORUZ[1], A. TERZI[2], F. KOKTURK[3], T. ORNEK[4] AND G. CELEBI[1]

[1] *Bulent Ecevit University Medical Faculty, Department of Infectious Diseases and Clinical Microbiology, Zonguldak, Turkey*
[2] *Bulent Ecevit University, Medical Faculty, Department of Microbiology, Zonguldak, Turkey*
[3] *Bulent Ecevit University, Medical Faculty, Department of Biostatistics, Zonguldak, Turkey*
[4] *Bulent Ecevit University, Medical Faculty, Department of Pulmonary Disease, Zonguldak, Turkey*

SUMMARY

The aim of this study was to compare the responses of colistin treatment alone *vs.* a combination of colistin and rifampicin in the treatment of ventilator-associated pneumonia (VAP) caused by a carbapenem-resistant *A. baumannii* strain. Forty-three patients were randomly assigned to one of two treatment groups. Although clinical ($P=0.654$), laboratory ($P=0.645$), radiological ($P=0.290$) and microbiological ($P=0.597$) response rates were better in the combination group, these differences were not significant. However, time to microbiological clearance (3.1 ± 0.5 days, $P=0.029$) was significantly shorter in the combination group. The VAP-related mortality rates were 63.6% (14/22) and 38.1% (8/21) for the colistin and the combination groups ($P=0.171$), respectively. Our results suggest that the combination of colistin with rifampicin may improve clinical and microbiological outcomes of VAP patients infected with *A. baumannii*.

Key words: *Acinetobacter baumannii*, adverse drug reaction, colistin, ventilator-associated pneumonia.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative coccobacillus that has emerged over the past 15 years as a cause of infections acquired in hospitals, particularly in intensive care units (ICUs). In most institutions, the majority of *A. baumannii* isolates are recovered from the respiratory tracts of hospitalized patients and the proportion of ICU-acquired cases of ventilator-associated pneumonia (VAP) caused by *A. baumannii* increases significantly with prolonged ICU stay [1]. Outbreaks of multidrug-resistant and pan-drug-resistant strains of this microorganism have created major problems for infection control in hospitals, with limited treatment options and high morbidity and mortality rates [1, 2]. The high resistance barrier of *A. baumannii* to available antimicrobial agents has motivated researchers and clinicians to search for alternative antimicrobial agents or to use novel combinations of antibiotics for the treatment of infections. Colistin is an old antibiotic that was used until the early 1980s for the treatment of infections due to Gram-negative bacilli but as alternative treatment regimens became available, its use decreased

* Author for correspondence: H. Aydemir, M.D., Bulent Ecevit University Medical Faculty, Department of Infectious Diseases and Clinical Microbiology, 67600 Zonguldak, Turkey.
(Email: drhaydemir@yahoo.com)

because of its perceived adverse side-effects, which included neurotoxicity and nephrotoxicity [3, 4]. The emergence of *A. baumannii* strains resistant to all of the routinely tested antimicrobials has prompted the revival of interest in colistin as a treatment option [1] and several *in vitro* and animal studies have highlighted its synergistic activity with other agents particularly rifampicin [5–7]. However, data from prospective studies about *in vivo* efficacy and side-effects of colistin therapy are limited [8]. In this prospective study our aim was to evaluate the efficacy of colistin and the combination of colistin and rifampicin for treatment of patients with VAP due to carbapenem-resistant *A. baumannii* (CRAB).

MATERIALS AND METHODS

Patients and study design

This open, comparative, prospective, randomized, single-centre study was conducted between March 2011 and March 2012 in Bulent Ecevit University Teaching and Research Hospital, a 350-bed tertiary care centre. This study was approved by the ethics committee of the hospital and written informed consent was obtained from patients' legal representatives. A preliminary power analysis was performed, and to achieve a 5% type I error probability and 80% prior power with 0·60 effect size, the sample size was determined to be 88 patients. Forty-three patients met the inclusion criteria during the planned study period, which had been approved by the local ethics committee for 1 year. The eligibility criteria were: (1) patients aged ⩾18 years with a diagnosis of VAP whose culture and antimicrobial susceptibility results indicated infection with CRAB within 48 h after onset of VAP; and (2) patients whose legal representatives accepted and signed the informed consent form. Exclusion criteria included the following: (1) patients whose cultures were polymicrobial; (2) patients who had another infection site due to a different microorganism; (3) patients who had been diagnosed with VAP due to CRAB in another centre and were transferred to our centre while receiving colistin treatment; and (4) patients who died within 72 h of colistin or colistin + rifampicin treatment. Carbapenem resistance was defined as resistance to imipenem and meropenem [1].

The severity of the clinical condition of the patients was evaluated with the Acute Physiological and Chronic Health Evaluation (APACHE II) scoring system on the basis of the worst data point during the first 24 h in the ICU [9]. Organ failure and severity of multiple-organ dysfunction syndrome were evaluated using the Sequential Organ Failure Assessment (SOFA) score on the day of VAP diagnosis and during the subsequent clinical course [10]. Main characteristics of the patients, comorbidities, laboratory findings, microbiological and radiological results, type and duration of empirical and definitive antimicrobial therapies, and duration of hospitalization were also recorded. Renal function was monitored by daily measurement of the serum creatinine level. The creatinine clearance rate was calculated using the equation of Cockcroft & Gault [2]. Liver function was monitored by the daily measurement of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and total bilirubin levels. Daily platelet, haemoglobin, and white blood cell counts were also recorded. In patients with normal renal function (serum creatinine level <1·2 mg/dl), nephrotoxicity was defined as a serum creatinine value of >2 mg/dl, which represented a reduction in the calculated creatinine clearance of 50% relative to the value at the initiation of antibiotic therapy. In patients with pre-existing renal dysfunction, nephrotoxicity was defined as an increase of ⩾50% from the baseline creatinine level, which represented a reduction in the calculated creatinine clearance of 50% relative to the value before colistin therapy was initiated.

Administration of antibiotics

Patients were randomly assigned to one of the treatment groups according to a computer-generated random-number program. The groups were treated either with sodium colistimethate (Colimycin®, Kocak Farma, Turkey, each vial contained 150 mg colistin base activity which is approximately equivalent to 360 mg sodium colistimethate) intravenously (i.v.) only or with a combination of colistin i.v. and rifampicin (600 mg/day) nasogastrically. All patients in the ICU were monitored by the infectious diseases team by daily rounds. Colistin or the combination of colistin + rifampicin treatment was started as soon as a patient was suspected of having VAP and in whom cultures grew CRAB within 48 h of VAP diagnosis. In the combination therapy group, we planned to stop rifampicin when we did not observe synergy in the presence of rifampicin resistance. We adjusted the

colistin doses in patients with renal impairment according to the manufacturer's recommendations. For all of the patients with normal renal function, the dosage of colistin base activity was set at 300 mg/day, which was divided into three i.v. doses; for a serum creatinine level of 1·3–1·5 mg/dl, the dosage was set at 230 mg/day divided into two doses and for a serum creatinine level of 1·6–2·5 mg/dl, the dosage was set at 150 mg/day in one dose. If the serum creatinine level was >2·5 mg/dl, the dosage was set at 100 mg every 36 h. No other drugs were used in combination with colistin to treat *A. baumannii* infection during the study period.

Definitions

A diagnosis of pneumonia required a new or persistent infiltrate on chest radiography plus two or more of the following: abnormal temperature (>38 °C or <35·5 °C), leukocytosis (leukocyte count >12 000 cells/mm^3) or leukopenia (leukocyte count <4000 cells/mm^3), and purulent bronchial secretion. Pneumonia was considered to be ventilator-associated when the onset occurred 48 h after the initiation of mechanical ventilation [2].

Isolation of *A. baumannii* was conducted using a protected specimen brush (PSP) or a quantitative tracheal aspirate. *A. baumannii* was considered to be the aetiological agent of VAP if the PSP yielded >10^3 c.f.u./ml or if the tracheal aspirate culture yielded >10^6 c.f.u./ml of the organism [2, 11]. Tracheal aspirates having >25 neutrophils present and ⩽10 epithelial cells on the Gram stain were accepted for culture. Blood cultures were also taken from all patients included in the study. The infectious disease specialist who conducted daily follow-up evaluations of the patients decided the duration of the treatment. Empirical antimicrobial therapy was initiated in all patients as soon as the diagnosis of VAP was made and definitive therapy was eventually modified within 48 h when the susceptibilities of *A. baumannii* strains were available. The total length of ICU stay was defined as the number of days spent in the ICU until death, discharge or transfer to another unit. The primary outcome was the clinical response of VAP. The following criteria were considered indicative of a clinical response: (1) resolution of fever or hypothermia with the body temperature being between 36 °C and <38 °C; (2) disappearance of tracheal secretions or the absence of purulence in the tracheal secretion, with polymorphonuclear leukocyte counts <25 cells/mm^3 in Wright stain smears of the tracheal secretion; (3) a PaO$_2$/FiO$_2$ ratio (the ratio of partial pressure of arterial O$_2$ to the fraction of inspired O$_2$) >240, or mechanical ventilation no longer needed; and (4) the partial or total resolution of respiratory crackles, upon physical examination [2, 11, 12].

The secondary endpoints were microbiological, laboratory and radiological responses. All of the patients included in the study were followed until death or discharge. Cultures of bronchial secretions and blood were taken at the time of diagnosis and on days 3, 5, 7, and 10 after diagnosis during the follow-up period and at the end of the course of therapy. A microbiological response was considered to be achieved if subsequent cultures were negative for *A. baumannii*. If a patient exhibited a clinical response, but the cultures were still positive, treatment was stopped, and the case was considered to be colonized. A laboratory response was defined as the normalization of leukocyte counts (4000–10 000 cells/mm^3), a decrease in the sedimentation rate, or a 40% decrease of the CRP levels at the time of diagnosis. Partial resolution or absence of infiltration and absence of pleural effusion, which was detected at the time of diagnosis, and the absence of a new infiltration on chest X-rays were considered as a radiological response to therapy [12]. VAP-related mortality was defined as death during the treatment period, or death that occurred when the signs and symptoms of pneumonia were present, or death due to septic shock [2].

Isolates and antimicrobial susceptibility testing

A. baumannii clinical isolates were identified by conventional techniques and confirmed by the BBL Crystal enteric/non-fermenter identification system (Becton Dickinson, USA). Susceptibility to antibiotics was determined by a disk diffusion method, and results interpreted as recommended by the Clinical Laboratory Standards Institute (CLSI) [13]. Resistance to imipenem and meropenem was verified by determination of the minimal inhibitory concentrations (MICs) with E-tests (AB Biodisk, Sweden) [13]. The MICs of rifampicin and colistin were determined using the broth microdilution method according to CLSI recommendations. Standard powder forms of rifampicin (Sigma Chemical Co., USA) and colistin sulphate (Sigma Chemical Co.) were stored at 2–8 °C until use. The stock solutions and serial

twofold dilutions of each drug (to at least double the MIC) were prepared according to CLSI recommendations [13] and in-house prepared panels of concentrations of 0·125–512 μg/ml were used. The breakpoints for colistin resistance were defined by CLSI recommendations ($\leqslant 2$ μg/ml for susceptible and $\geqslant 4$ μg/ml, for resistant), while for rifampicin, breakpoints established by the French Society for Microbiology were used ($\leqslant 4$ μg/ml for susceptible and > 16 μg/ml for resistant) [14].

Synergy testing

Synergistic effects between colistin and rifampicin were determined for all *A. baumannii* isolates by the chequerboard microbroth dilution method. The stock solutions and serial twofold dilutions of each drug (to at least double the MIC) were prepared as above and 50 μl Mueller–Hinton broth was distributed into each well of sterile microdilution plates. The first antibiotic of the combination was serially diluted along the ordinate, while the second drug was diluted along the abscissa. An inoculum equal to a 0·5 McFarland turbidity standard was prepared for each *A. baumanni* isolate in Mueller–Hinton broth and 10 μl of this suspension (inoculum $\sim 5 \times 10^7$ c.f.u./ml) was added to each well. The plates were incubated at 35 °C for 18 h under aerobic conditions. The MIC was defined as the lowest concentration of an antibiotic that completely inhibited visible growth of the organism. A synergistic effect was indicated when the ratio of the concentration of each antibiotic to the MIC of that antibiotic was the same for all of the components of the mixture. The total fractional inhibitory concentration ($\sum$FIC) was calculated as follows: $\sum$FIC $= \sum$FIC A $+ \sum$FIC B, where FIC A was the MIC of drug A in the combination/MIC of drug A alone, and FIC B was the MIC of drug B in the combination/MIC of drug B alone. The combination was considered synergistic when the $\sum$FIC was $\leqslant 0·5$, indifferent when the $\sum$FIC was $> 0·5$ to < 4, and antagonistic when the $\sum$FIC was $\geqslant 4$ [14]. The $\sum$FIC was non-determined (ND) for some concentrations of the combination if the MICs of each agent were at the test range extremes (i.e. greater than the highest or less than or equal to the lowest concentration tested), and if the combination MICs were not at least fourfold different. If the MIC interaction ranges did not provide useful information, the test was repeated on a newly designed concentration panel with appropriate ranges [15].

Pulsed-field gel electrophoresis (PFGE) analysis

Analysis of the chromosomal DNA of the isolates recovered before treatment was performed by PFGE according to a published protocol [16]. The DNA profiles on each gel were normalized to external reference strains and analysed using GelCompar software (version 3.0; Applied Maths, Belgium) with a 1% band tolerance. Cluster analysis was performed using the unweighted pair-group method using arithmetic average (UPGMA) [17] and strains were categorized as indistinguishable, closely related, possibly related or different according to the Tenover criteria [18].

Statistical analysis

Statistical analysis was performed with SPSS v. 18.0 software (SPSS Inc., USA). The distribution of data was determined by the Shapiro–Wilk test. Continuous variables were expressed as the means $\pm$ standard deviation, categorical variables as the frequency and percent. Continuous variables were compared with the independent sample *t* test or Mann–Whitney *U* test, and categorical variables were compared using Pearson's χ^2 test or Fisher's exact χ^2 test for two groups. The correlation between two continuous variables was evaluated by Spearman's correlation analysis. *P* values of $< 0·05$ were considered to be statistically significant for all tests.

RESULTS

During the course of the study period, 69 patients were diagnosed with VAP due to CRAB. The cultures of six patients were polymicrobial and eight patients had another infection site due to a different microorganism. One patient transferred to our centre was already receiving colistin treatment and 11 patients died within 72 h of colistin or colistin and rifampicin treatment. These 26 patients were excluded from the study and the remaining 43 patients were entered into the study.

The demographic data, comorbidities and clinical characteristics of the patients are shown in Table 1. Twenty-two patients were treated with colistin alone, and 21 with a combination of colistin + rifampicin. The mean ICU stay to diagnosis of VAP was 20 days for the colistin group and 13 days for the combination group. Although there were no statistically significant differences between the APACHE II scores

Table 1. *Demographic data and clinical characteristics of ventilator-associated pneumonia patients treated with colistin alone and in combination with rifampicin*

Variables	Total ($n=43$)	Colistin group ($n=22$)	Colistin + rifampicin group ($n=21$)	P
Gender (male/female) (%)	30 (69·8)/13 (31·2)	16 (72·7)/6 (27·3)	14 (66·7)/7 (33·3)	0·920
Age, mean ± s.D.	61 ± 20	63 ± 17	58 ± 23	0·535
Comorbidity				
Diabetes mellitus (%)	12 (27·9)	7 (31·8)	5 (23·8)	0·806
Chronic renal failure (%)	4 (9·3)	2 (9·1)	2 (9·5)	1·000
Hypertension (%)	21 (48·8)	14 (63·6)	7 (33·3)	0·093
Congestive heart failure (%)	7 (16·3)	5 (22·7)	2 (9·5)	0·412
Malignancy (%)	5 (11·6)	2 (9·1)	3 (14·3)	0·664
Cerebrovascular disease (%)	4 (9·3)	3 (13·6)	1 (4·8)	0·607
Trauma (%)	6 (14·0)	4 (18·2)	2 (9·5)	0·664
Operation (%)	4 (9·3)	3 (13·6)	1 (4·8)	0·607
ICU stay before diagnosis, mean ± s.D.	16·3 ± 14·2	19·6 ± 18·2	12·8 ± 7·4	0·644
Total length of ICU stay, mean ± s.D.	37·6 ± 26·0	34·9 ± 26·0	40·4 ± 26·4	0·368
Duration of mechanical ventilation, before diagnosis (days), mean ± s.D.	15·4 ± 14·3	18·4 ± 18·2	12·2 ± 7·8	0·742
Duration of mechanical ventilation (days), mean ± s.D.	32·4 ± 22·6	33 ± 25·9	31·8 ± 19·1	0·752
Prior receipt of carbapenem (%)	22 (51·2)	11 (50·0)	11 (52·4)	1·000
Presence of bacteraemia (%)	8 (18·6)	3 (13·6)	5 (23·8)	0·457
APACHE II scores, mean ± s.D.	19·1 ± 6·0	18·0 ± 4·9	20·1 ± 6·8	0·379
SOFA scores, mean ± s.D.	7·4 ± 2·9	6·5 ± 2·6	8·2 ± 2·9	0·044
Duration of treatment (days), mean ± s.D.	9·3 ± 3·2	8·9 ± 3·5	9·8 ± 2·9	0·223

ICU, Intensive care unit; APACHE, Acute Physiological and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment.

Table 2. *Outcomes of patients treated with colistin alone and in combination with rifampicin*

Variables	Total ($n=43$)	Colistin group ($n=22$)	Colistin + rifampicin group ($n=21$)	P
Clinical response (%)	20 (46·5)	9 (40·9)	11 (52·4)	0·654
Laboratory response (%)	22 (51·2)	10 (45·5)	12 (57·1)	0·645
Radiological response (%)	18 (41·9)	7 (31·8)	11 (52·4)	0·290
Microbiological response (%)	28 (65·1)	13 (59·1)	15 (71·4)	0·597
Time to microbiological clearance (days), mean ± s.D.	3·8 ± 1·4	4·5 ± 1·7	3·1 ± 0·5	0·029
In-hospital mortality (%)	29 (67·4)	16 (72·7)	13 (61·9)	0·666
VAP-related mortality (%)	22 (51·2)	14 (63·6)	8 (38·1)	0·171

VAP, Ventilator-associated pneumonia.

($P=0·379$) at the time of ICU admission between the two groups, the difference between the SOFA scores ($P=0·044$) of the two groups on the day of VAP diagnosis reached statistical significance; SOFA scores were higher in the colistin + rifampicin combination group ($8·2 \pm 2·9$) than in the colistin group ($6·5 \pm 2·6$). No statistically significant difference was found between the duration of ICU stay for the two groups ($34·9 \pm 26·0$ days for the colistin group and $40·4 \pm 26·4$ for the combination group, $P=0·368$).

Microbiological cure was observed in 13 (59·1%) patients in the colistin group and in 15 patients (71·4%) in the colistin + rifampicin group. The time to microbiological clearance was significantly shorter in the colistin + rifampicin group ($3·1 \pm 0·5$ days, $P=0·029$). Although the clinical ($P=0·654$), laboratory ($P=0·645$), radiological ($P=0·290$) and microbiological ($P=0·597$) response rates were better in the combination group, these differences were not significant (Table 2). The crude in-hospital mortality

rates were 72·7% (16/22 patients) in the colistin group and 61·9% (13/21 patients) in the colistin + rifampicin group (*P* = 0·666). The VAP-related mortality rates were 63·6% (14/22 patients) and 38·1% (8/21 patients) for the colistin and the combination groups (*P* = 0·171), respectively. The time to start of definitive therapy was the same in both treatment groups.

As all patients were treated with colistin, treatment groups were not considered when evaluating the renal toxicity of colistin. At the beginning of antimicrobial therapy serum creatinine levels were 1·2 ± 1·0 mg/dl for all patients and the highest creatinine level recorded during therapy was 1·7 ± 1·2 mg/dl. Ten (23%) patients developed nephrotoxicity during colistin treatment and none had renal insufficiency before treatment. The development of renal toxicity did not result in the discontinuation of treatment or the need for dialysis, but required adjustment of the colistin dosage in all patients. None of the patients developed hepatotoxicity due to rifampicin. No neurotoxicity, such as dizziness, weakness, facial paraesthesia, vertigo, visual disturbances, confusion, ataxia, or an abnormal neurological physical examination finding was observed, but we were not able to evaluate the patients electrophysiologically for the development of polyneuropathy.

Nine different antibiotic resistance phenotypes of *A. baumannii* were identified. All isolates were carbapenem-resistant and susceptible to colistin. Fourteen isolates were resistant to all of the other antimicrobials tested and the remainder showed variable susceptibility to these agents. Susceptibility to rifampicin was not uniform. Eleven isolates (three from patients in the colistin + rifampicin group) showed elevated rifampicin MICs (256 µg/ml to ⩾ 512 µg/ml), Three isolates (one from the colistin + rifampicin group) showed intermediate MICs (8–16 µg/ml), and the remainder were fully susceptible (MIC ⩽ 4 µg/ml).

Synergy between colistin and rifampicin was demonstrated for all isolates. It was not necessary to repeat a new panel for the ND ∑FICs because all combination MICs were fourfold less than the single agent's MICs, which was interpreted as synergy. There was no correlation between ∑FICs and the time to bacterial clearance (*r* = 0·10, *P* = 0·079).

PFGE analysis of 40 isolates revealed closely related DNA profiles comprising 10 different genotype clusters. Genotype 1, which formed the largest cluster, contained 13 isolates which was indicative of an outbreak strain.

DISCUSSION

The increasing rate of resistance of *A. baumannii* strains to available antibiotics, particularly to carbapenems, has rekindled interest in using colistin for the treatment of these infections. Several *in vivo* studies have investigated the effect of the use of colistin alone or in combination with rifampicin for the treatment of infections due to CRAB [2, 11, 19–22] but to the best of our knowledge, this is the first prospective, randomized clinical trial to compare the response of colistin treatment alone with the combination of colistin + rifampicin to treat VAP patients infected with CRAB. In a study conducted by Petrosillo *et al.* [21] the clinical outcome of CRAB infections treated with a combination of i.v. colistin + rifampicin was evaluated, and 7/14 patients died from VAP due to *A. baumannii* with a microbiological response rate of 64·3% (9/14). Another observational study on the efficacy of colistin combined with rifampicin (i.v. and aerosolized routes) in nine patients with bacteraemia and in three patients with VAP due to multidrug-resistant *A. baumannii* reported favourable clinical outcomes for all patients [11]. Further, Bassetti *et al.* [20] observed high microbiological and clinical response rates (76%) in critically ill patients infected with multidrug-resistant *A. baumannii* who were treated with colistin + rifampicin and this rate was superior to the foregoing studies [11, 21] and the current investigation. However, the infection-related mortality rate of 21% was lower than our VAP-related mortality rate of 51·2% (22/43) for all patients. This rate was lower in the colistin + rifampicin group (38·1%) than in the colistin monotherapy group (63·6%).

In other published studies on the efficacy of i.v. colistin therapy for nosocomial infections caused by multidrug-resistant organisms, favourable clinical response rates ranged from 57% to 73% [2, 19, 22]. We compared the clinical, microbiological, radiological and laboratory response rates of the two treatment groups and all responses including the clinical (52·4% *vs.* 40·9), microbiological (71·4% *vs.* 59·1%), laboratory (57·1% *vs.* 45·5%), radiological (52·4% *vs.* 31·8%) and mortality (38·1% *vs.* 63·6%) rates were superior for the combination therapy group, despite the significantly higher initial SOFA scores. The time to microbiological clearance was significantly shorter (∼3 days *vs.* 5 days) in the colistin + rifampicin group.

In this study we used the oral form of rifampicin because the parenteral form is not available in

Turkey. Although the absorption and the distribution of orally administered rifampicin is good [23], it has been shown that a higher metabolic ratio results after oral dosing than by the i.v. route [24]. Further, the use of inhaled colistin was not approved by the Turkish Ministry of Health during the study period. The low systemic and high local concentrations of inhaled colistin support the use of this form in patients with cystic fibrosis (CF) infected with *Pseudomonas aeruginosa*, but whether this applies to non-CF patients with nosocomial pneumonia due to CRAB is not known [25]. Nevertheless, limited experience with aerosolized colistin therapy suggests that it may be an effective and safe adjunctive treatment for critically ill patients with VAP caused by CRAB [26]. In another retrospective study of 45 patients with VAP caused by CRAB, in whom inhaled colistin was used, the microbiological cure rate, clinical cure rate and mortality rate were reported to be 33%, 58%, and 42%, respectively, and no side-effects were observed [27]. It is therefore possible that the outcomes of our study could have been improved if the parenteral form of rifampicin or the inhaled form of colistin had been available for combination treatment.

Giannouli *et al.* [28] recently investigated the mechanisms of rifampicin resistance in *A. baumannii* isolates. They reported that no synergistic effect of rifampicin and colistin was observed in the isolates with elevated rifampicin MICs due to the mutations in the *rpo*B target gene. By contrast, we found a clear synergistic effect between rifampicin and colistin for all *A. baumannii* isolates including those with elevated rifampicin MICs. Oxa-58-producing strains became endemic in our institution after the outbreak seen in 2005 [29]. Current isolates examined here may have similar carbapenem resistance mechanisms to the earlier strains and we did not plan to look for synergy between colistin and carbapenems, but such an investigation may be informative in strains with carbapenem resistance due to membrane-based changes (porins and efflux pumps).

Renal toxicity developed in ten patients (10/43, 23%) during the treatment and all had normal baseline creatinine levels. Similar results were reported by Turkoglu *et al.* [30] who evaluated the effectiveness of colistin in critically ill patients with chronic renal failure and found 23% of them without renal impairment developed nephrotoxicity. By contrast, two earlier studies did not observe nephrotoxicity in patients with normal renal function

at the beginning of colistin therapy [19, 20]. However, in one of these studies renal function tests showed further deterioration in three patients (3/29, 10%) who already had renal failure at the beginning of colistin therapy [20]. Significant renal dysfunction was not reported by Rios *et al.* [31] for VAP patients treated with colistin. In our study, four patients had previous renal impairment and high baseline creatinine levels at the beginning of colistin treatment. Initial colistin dosage was adjusted in these patients according to their serum creatinine levels, but none of them experienced further deterioration of renal function during colistin therapy and none required dialysis. For patients with normal renal function, the dosage of colistin was 300 mg/day, and this was adjusted for patients with renal impairment according to the manufacturer's recommendations. By contrast, for patients with renal impairment, Garonzik *et al.* [32] did not recommend larger dosage intervals for colistin calculated according to the serum creatinine levels.

This study has several limitations, including a small sample size, the unavailability of i.v. rifampicin and inhaled colistin, the performance of the study at only one centre, and difficulty in the diagnosis of VAP and in the evaluation of the clinical, radiological, and laboratory responses in critically ill patients with other underlying comorbidities. Nevertheless its strengths are that it was a prospective, randomized clinical trial and the performance of synergy testing. Synergy between colistin and rifampicin was demonstrated for all isolates included in the study and all types of responses to treatment were evaluated in all patients; and patients were followed up to identify adverse reactions to the drugs.

Although not statistically significant, higher clinical, laboratory, and radiological response rates and a lower VAP-related mortality rate were observed in the colistin + rifampicin group. These results warrant further studies with a larger patient sample size to further investigate the potential benefits of this combination treatment. Although nephrotoxicity developed in 23% of the patients, a colistin dosage adjustment was sufficient to compensate for this toxicity, and discontinuation of treatment or dialysis was not required. Because high mortality rates have been reported for infections due to CRAB, future prospective *in vivo* studies evaluating the efficacy of different treatment regimens are needed to determine the appropriate treatments necessary for better clinical outcomes.

ACKNOWLEDGEMENTS

We thank Associate Professor Dr Barış Otlu (Department of Clinical Microbiology, Molecular Microbiology Section, Faculty of Medicine, Inonu University, Malatya, Turkey) for his kind contribution of the PFGE analysis of the isolates. We also thank the technical staff of the laboratory, and Eldan Subası for her help with the MIC and synergy testing.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Peleg AY, Seifert H, Paterson DL.** *Acinetobacter baumannii*: emergence of a successful pathogen. *Clinical Microbiology Reviews* 2008; **21**: 538–582.
2. **Garnacho-Montero J, et al.** Treatment of multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia (VAP) with intravenous colistin: a comparison with imipenem-susceptible VAP. *Clinical Infectious Diseases* 2003; **36**: 1111–1118.
3. **Olesen S, Madsen PO.** Intravenous administration of sodium colistimethate in urinary tract infections. *Current Therapeutic Research, Clinical and Experimental* 1967; **9**: 283–287.
4. **Devlieger H, et al.** Acute renal failure due to colistin intoxication. *Acta Paediatrica Belgica* 1977; **30**: 179–181.
5. **Tascini C, et al.** Evaluation of the activities of two-drug combinations of rifampicin, polymyxin B and ampicillin/sulbactam against *Acinetobacter baumannii*. *Journal of Antimicrobial Chemotherapy* 1998; **42**: 270–271.
6. **Song JY, et al.** In vitro activities of carbapenem/sulbactam combination, colistin, colistin/rifampicin combination and tigecycline against carbapenem-resistant *Acinetobacter baumannii*. *Journal of Antimicrobial Chemotherapy* 2007; **60**: 317–322.
7. **Timurkaynak F, et al.** In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from intensive care units. *International Journal of Antimicrobial Agents* 2006; **27**: 224–228.
8. **Zavascki AP, et al.** Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *Journal of Antimicrobial Chemotherapy* 2007; **60**: 1206–1215.
9. **Knaus WA, et al.** APACHE II: a severity of disease classification system. *Critical Care Medicine* 1985; **13**: 818–829.
10. **Vincent JL, 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 Medicine* 1996; **22**: 707–710.
11. **Motaouakkil S, et al.** Colistin and rifampicin in the treatment of nosocomial infections from multiresistant *Acinetobacter baumannii*. *Journal of Infection* 2006; **53**: 274–278.
12. **American Thoracic Society.** Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia: 2005, 15 February 2005. *American Journal of Respiratory and Critical Care Medicine* 2005; **171**: 388–416.
13. **Clinical and Laboratory Standards Institute.** Performance standards for antimicrobial susceptibility testing; 21st informational supplement. Document M100–S21. Wayne, PA: CLSI; 2011.
14. **Antibiogram Committee of the French Microbiology Society.** Report, 2010. Antibiogram Committee of the French Microbiology Society, Paris, France.
15. **Isenberg HD.** *Clinical Microbiology Procedures Handbook*, 2nd edn. Washington, DC: ASM Press, 2004.
16. **Durmaz R, et al.** The optimization of rapid pulse field electrophoresis for the typing of *Acinetobacter baumannii*. *Escherichia coli* and *Klebsiella* spp. *Japanese Journal of Infectious Diseases* 2009; **62**: 372–377.
17. **Rementeria A, et al.** Comparative evaluation of three commercial software packages for analysis of DNA polymorphism patterns. *Clinical Microbiology and Infection* 2001; **7**: 331–336.
18. **Tenover FC, Arbeit RD, Goering RV.** How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular typing working group of the society for healthcare epidemiology of America. *Infection Control and Hospital Epidemiology* 1997; **8**: 426–439.
19. **Kallel 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 Medicine* 2007; **33**: 1162–1167.
20. **Bassetti M, et al.** Colistin and rifampicin in the treatment of multidrug-resistant *Acinetobacter baumannii* infections. *Journal of Antimicrobial Chemotherapy* 2008; **61**: 417–420.
21. **Petrosillo N, et al.** Combined colistin and rifampicin therapy for carbapenem-resistant *Acinetobacter baumannii* infections: clinical outcome and adverse events. *Clinical Microbiology and Infection* 2005; **11**: 682–683.
22. **Levin AS, et al.** Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clinical Infectious Diseases* 1999; **28**: 1008–1011.
23. **Acocella G.** Clinical pharmacokinetics of rifampicin. *Clinical Pharmacokinetics* 1978; **3**: 108–127.
24. **Loos U, et al.** Pharmacokinetics of oral and intravenous rifampicin during chronic administration. *Wiener klinische Wochenschrift* 1985; **63**: 1205–1211.
25. **Ratjen F, et al.** Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. *Journal of Antimicrobial Chemotherapy* 2006; **57**: 306–311.
26. **Linden PK, Paterson DL.** Parenteral and inhaled colistin for treatment of ventilator-associated pneumonia. *Clinical Infectious Diseases* 2006; **43**: S89–94.

27. **Lin CC, *et al.*** Aerosolized colistin for the treatment of multidrug-resistant *Acinetobacter baumannii* pneumonia : experience in a tertiary care hospital in northern Taiwan. *Journal of Microbiology, Immunology and Infection* 2010 ; **43** : 323–331.

28. **Giannouli M, *et al.*** Molecular epidemiology and mechanisms of rifampicin resistance in *Acinetobacter baumannii* isolates from Italy. *International Journal of Antimicrobial Agents* 2012 ; **39** : 58–63.

29. **Kulah C, *et al.*** Characterisation of carbapenem-resistant Acinetobacter baumannii outbreak strains producing OXA-58 in Turkey. *International Journal of Antimicrobial Agents* 2010 ; **36** : 114–118.

30. **Turkoglu M, *et al.*** Colistin therapy in critically ill patients with chronic renal failure and its effect on development of renal dysfunction. *International Journal of Antimicrobial Agents* 2012 ; **39** : 142–145.

31. **Rios FG, *et al.*** Ventilator-associated pneumonia due to colistin susceptible-only microorganisms. *European Respiratory Journal* 2007 ; **30** : 307–313.

32. **Garonzik SM, *et al.*** Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrobial Agents and Chemotherapy* 2011 ; **55** : 3284–3294.