Journal of Global Antimicrobial Resistance 15 (2018) 127–135

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Prospective, comparative clinical study between high-dose colistin monotherapy and colistin–meropenem combination therapy for treatment of hospital-acquired pneumonia and ventilator-associated pneumonia caused by multidrug-resistant *Klebsiella pneumoniae*

Mohamed Farouk Ahmed Abdelsalam[a,*], Maged Salah Abdalla[b,1], Hanan Salah El-Din El-Abhar[c]

[a] *Clinical Pharmacy Department, The Teachers' Hospital, Al-Jazeera, Cairo, Egypt*
[b] *Anesthesia Department, Faculty of Medicine, Cairo University, Cairo, Egypt*
[c] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 11 May 2018
Received in revised form 4 July 2018
Accepted 6 July 2018
Available online xxx

Keywords:
Multidrug-resistant
Klebsiella pneumoniae
Hospital-acquired pneumonia
Ventilator-associated pneumonia
Hospital mortality
Procalcitonin

ABSTRACT

Objectives: In clinical practice, colistin is used as combination therapy to improve its antibacterial activity, despite the consequent increase in toxicity. This prospective, comparative study evaluated the effectiveness and adverse effects of using colistin alone at a loading dose of 9 million international units (MIU) followed by 3 MIU every 8 h (q8 h) versus colistin + meropenem 1 g q8 h in treating multidrug-resistant (MDR) *Klebsiella pneumoniae*-induced hospital-acquired pneumonia (HAP) or ventilator-associated pneumonia (VAP). The primary outcome measure was in-hospital mortality. The secondary measure was the occurrence of colistin toxicity.

Methods: A total of 60 patients were divided into two groups (30 patients each); the first group received intravenous colistin at a mean daily dose of 8.304 MIU and the second group received colistin 8.58 MIU combined with meropenem (mean daily dose of 2.88 g for 15 days).

Results: The colistin–meropenem combination group showed a significant decrease in mortality versus colistin alone [16.7% (5/30) vs. 43.3% (13/30); $P = 0.047$]. The improved clinical response mediated by combination therapy was not associated with any significant nephrotoxicity, hepatotoxicity or neurotoxicity. Moreover, the 42 surviving patients showed normal procalcitonin values associated with a decrease in SOFA score, whilst 12 of them showed significantly elevated C-reactive protein (CRP) ($P = 0.0002$).

Conclusions: This study revealed the superiority of colistin–meropenem combination therapy over colistin monotherapy in the treatment of MDR *K. pneumoniae*-induced HAP or VAP and highlights the advantage of procalcitonin over CRP as a marker for eradication of sepsis and suspension of therapy.

1. Introduction

Infections caused by multidrug-resistant (MDR) Gram-negative bacteria, which are resistant to three or more antimicrobial categories, represent an important clinical problem associated with a significant increase in morbidity and mortality worldwide [1,2]. The ability of Gram-negative bacteria to develop resistance to most available antibiotics has encouraged the medical community to reconsider the use of colistin (polymyxin E). Colistin is one of the polymyxins, a group of polypeptide antibiotics consisting of five different chemical compounds, namely polymyxin A, B, C, D and E, among which only polymyxins B and E are of clinical value [3]. Colistin is known to act by binding to lipopolysaccharide and phospholipid molecules in the cell membrane of Gram-negative bacteria, producing a disruptive physiochemical effect that leads to an alteration in cell membrane permeability and eventually leading to cell death [4]. From the 1960s until the 1990s, owing to a high incidence of fetal toxicity, especially nephrotoxicity, colistin use was restricted to the treatment of patients with cystic fibrosis having acute exacerbations of lung infection due to MDR *Pseudomonas aeruginosa* strains [5,6]. However, the revival of

* Corresponding author.
E-mail address: farouq.mfa@gmail.com (M.F.A. Abdelsalam).
[1] Principle investigator.

https://doi.org/10.1016/j.jgar.2018.07.003
2213-7165/

colistin use started lately because of its high effectiveness against most Gram-negative bacteria, including MDR *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *P. aeruginosa* strains [7].

Although the minimum inhibitory concentration (MIC) is used as a parameter to assess the therapeutic concentration of antimicrobial agents, several in vitro [8,9] and in vivo [10] studies have reported that the area under the unbound (free) concentration–time curve to MIC ratio (*f*AUC/MIC) or AUC/MIC ratio are more predictive parameters than MIC alone as a measure of colistin activity.

It has been shown that intravenous (i.v.) colistin administration at the standard dose level of 2 million international units (MIU) every 8 h (q8 h) results in a steady-state plasma trough concentration ($C_{trough,ss}$) of 1.03 ± 0.69 mg/L [11]. This concentration is below the required MIC for Enterobacteriaceae (2 mg/L) and *Pseudomonas* spp. (4 mg/L) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12]. In contrast, the Clinical and Laboratory Standards Institute (CLSI) recommends 2 mg/L as a breakpoint both for Enterobacteriaceae and *Pseudomonas* spp. [13]. Furthermore, Imberti et al. reported an AUC_{0-24}/MIC ratio of 17.3 ± 9.3 using a colistin standard dose [11], which is again below the recommended range (25–35) required to achieve optimum bacterial killing both of *A. baumannii* and *P. aeruginosa* in lung infection [9,14].

Accordingly, other studies started to use a higher colistin dose. Markou et al. used a colistin dose of 2.8 MIU q8 h, which showed a higher maximum serum concentration (C_{max}) (2.93 ± 1.24 mg/L vs. 2.21 ± 1.08 mg/L at the standard dose of 2 MIU q8 h), but with the same $C_{trough,ss}$ and adverse effects [15]. In another prospective study, Plachouras et al. highlighted the advantage of using loading and maintenance doses of colistin to achieve an effective therapeutic level faster than the standard regimen without the loading dose [16]. The authors used a loading dose of colistin (9–12 MIU) in intensive care unit (ICU) patients with subsequent administration of 9 MIU i.v. in two or three divided doses daily. After several studies [3,16,17], such a protocol was approved and updated in 2017 by the University of California, Los Angeles (UCLA) Health System Pharmaceutical Services [17].

Apart from using colistin as monotherapy against MDR Gram-negative bacterial infections, many in vitro and in vivo studies have documented the synergistic effect obtained from combining it with other antibiotics [1,18], e.g. colistin combined with ceftazidime [19], ciprofloxacin [19] or piperacillin [20] against *P. aeruginosa* strains and with meropenem against carbapenem-resistant *K. pneumoniae* [21]. In an in vitro study, Timurkaynak et al. stated that the effect of the colistin–meropenem combination is superior to that of colistin alone [22]. This conclusion was further emphasised by a retrospective study revealing a synergistic effect when colistin was co-administered with meropenem against carbapenem-resistant *K. pneumoniae* infection in ICU patients [23].

Although a recent study was published comparing colistin alone versus colistin plus meropenem for the treatment of carbapenem-resistant Gram-negative bacterial infections including hospital-acquired pneumonia (HAP), ventilator-associated pneumonia (VAP), bloodstream infection and urosepsis [24], prospective data are still lacking regarding their possible synergistic effect when both antibiotics are used specifically against MDR *K. pneumoniae*-induced HAP or VAP. The latter has recently been recognised as a spreading MDR micro-organism in ICU patients with either HAP or VAP [25].

Referring to the aforementioned data, the current study is the first prospective study to evaluate the efficacy and safety of the new high doses of colistin as monotherapy versus combination therapy with meropenem in non-cystic fibrosis HAP and VAP patients caused by MDR *K. pneumoniae*.

2. Subjects and methods

2.1. Study design

This study was a prospective, comparative, single-blind, randomised study conducted on 60 adult patients (age ≥18 years) divided into two equal groups. The first group (n = 30) received colistin as monotherapy and the second group (n = 30) received colistin–meropenem combination therapy. The study was approved by the Ethics Committee of the Faculty of Pharmacy, Cairo University (Cairo, Egypt).

2.2. Inclusion criteria

Patients with HAP or VAP caused by MDR *K. pneumoniae*, as defined by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) [26], who were hospitalised in the general ICU during the period from the start of May 2016 to the end of October 2016 and confirmed with carbapenem-resistant *K. pneumoniae*-positive culture results from sputum within the previous 4 days were included in the study.

2.3. Exclusion criteria

All patients without a MDR carbapenem-resistant *K. pneumoniae*-positive culture isolated from the sputum were excluded from the study. In addition, the following patients were excluded: patients with a Glasgow Coma Scale (GCS) score of <9 in non-ventilated patients or <6 in ventilated patients; patients with end-stage metastatic malignant cancer; and all terminal patients with Acute Physiology and Chronic Health Evaluation (APACHE) II or Sequential Organ Failure Assessment (SOFA) scores of >34 or >15, respectively, and risk of mortality >85% or >80% on the first day of colistin administration, respectively [27,28]. Moreover, patients who received i.v. colistin therapy for <72 h were excluded from further analysis.

2.4. Microbiological testing

Sputum specimens were isolated from all patients before the start and after the end of treatment, and identification of all causative micro-organisms was performed using routine microbiological methods. Antimicrobial susceptibility testing was performed by the disk diffusion method for all antimicrobial agents except colistin. Colistin susceptibility was determined by the broth microdilution method according to the CLSI reference method [13,29]. The breakpoints were those defined by the CLSI [13].

2.5. Colistin administration

All patients in the current study received i.v. colistin (colistimethate sodium (CMS); Forest Laboratories UK Ltd., Dartford, UK) as a therapeutic intervention for infection due to MDR Gram-negative bacteria at a dose according to the protocol of the study. One milligram of the colistin base activity (CBA) formulation is approximately equal to 30 000 IU. The colistin preparation contains CMS, which is the active ingredient, as an amount of dry powder equivalent to one MIU (or equal to ca. 80 mg of CMS).

Adjustment of the i.v. dose is based on kidney function after consulting the ICU director or the infectious diseases specialists of the hospital. According to the protocol used [20,30], the loading dose of CBA was a constant single dose of 300 mg (5 mg/kg), whereas the maintenance dose was 100 mg (1.7 mg/kg) q8 h if the creatinine clearance (CL_{Cr}) was >50 mL/min. For a CL_{Cr} ranging between 20–50 mL/min the maintenance dose was 150 mg

(2.5 mg/kg) every 24 h (q24 h), and for a CL_{Cr} of <20 mL/min the maintenance dose was 150 mg (2.5 mg/kg) every 48 h. Patients who were on dialysis received the same single loading dose, whilst the maintenance dose was either 30 mg every 12 h (q12 h) for those who were on intermittent haemodialysis or 100 mg q12 h for those who were on continuous renal replacement therapy.

2.6. Meropenem administration

One-half of the enrolled patients received i.v. meropenem (Meronem™; AstraZeneca, Macclesfield, UK) as a combined therapeutic intervention for severe infection due to MDR Gram-negative bacterial infection at an i.v. dose of 1000 mg q8 h for patients with normal kidney function. Patients with a CL_{Cr} of 26–50 mL/min received 1000 mg q12 h, those with a CL_{Cr} between 10–25 mL/min received 500 mg q12 h and those with a CL_{Cr} of <10 mL/min were given 500 mg q24 h. Patients who were on dialysis treatment received meropenem 500 mg q24 h and another 500 mg post-dialysis [22].

2.7. Assessment of outcomes

The primary outcome measure was in-hospital mortality, whereas the secondary endpoints included clinical outcome of the infection and the occurrence of renal dysfunction. Improved outcomes were defined as follows: cured, if symptoms and signs of infection were resolved by the end of colistin treatment with a decrease in the SOFA severity of illness score for hospital mortality and the patient was discharged from the ICU [28]. Suspension of colistin or colistin–meropenem administration was determined based on improvement of the clinical condition with resolved symptoms and signs of infection combined with normalisation of total leukocyte count (TLC) and neutrophil percentage and improvement of C-reactive protein (CRP) serum values followed by confirmation by procalcitonin (PCT) value (<0.5 ng/mL). Improved patients were those who showed partial resolution of the presenting symptoms and signs of infection, whilst unresponsive patients were those with persistent or even worsening of the presenting symptoms and/or signs of infection during colistin administration. Renal function was stated as normal if the serum creatinine (SCr) level was ≤1.3 mg/dL, and the baseline SCr was defined as the SCr level on the first day of i.v. colistin administration. Deterioration of renal function during colistin treatment was accepted as an increase of >50% of the baseline SCr level to a value >1.3 mg/dL or as a decline in renal function requiring renal replacement therapy.

2.8. Data collection

Data were collected using a detailed case report form designed specifically for this study. These details cover the APACHE II and SOFA scores on the first day of colistin administration [27,28,31], site(s) of infection, duration of colistin treatment, concomitant antibiotic treatment, prior antibiotic or antifungal use, mechanical ventilatory support, renal support and duration of hospitalisation. In addition, microbiological data were reported, including the causative organism (*K. pneumoniae*) isolated from the site(s) of infection, date of isolation as well as the in vitro susceptibility to several antimicrobial agents, including colistin. Furthermore, data from laboratory tests, such as kidney/liver function tests [SCr, aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], CRP, haemoglobin, platelet count, TLC and percentage of neutrophils from the first to last day of treatment were recorded. In addition, the serum PCT value was evaluated once as a biomarker to rule out sepsis for patients with improved clinical condition with resolved symptoms and signs of infection combined with normal values of TLC and percentage of neutrophils and improvement of serum CRP value.

The information collected in the case report forms was processed through a computer database. Using random numbered cards for selection, 95% of the registered data were double-checked by an independent reviewer. In addition, the type of infection, causative pathogen(s) and clinical outcome were determined by two blinded reviewers.

2.9. Data analysis

Categorical variables were compared by Fisher's exact test. For continuous variables, Student's *t*-test or Mann–Whitney test and Wilcoxon matched-pairs signed-rank test were used for normally and non-normally distributed variables, respectively, whereas the Gehan–Breslow–Wilcoxon test was used for survival proportion. Variables associated with mortality in the univariable analysis ($P < 0.05$) were included in a backward stepwise multiple logistic regression model. One-way analysis of variance (ANOVA) followed by Dunnett's and Kruskal–Wallis followed by Dunn's test were used to analyse parametric and non-parametric data, respectively. The two-way ANOVA test was followed by Dunnett's or Šidák's multiple comparison tests. All statistical analyses and graphs were performed using GraphPad Prism v.6.01 (GraphPad Software, Inc., San Diego, CA).

3. Results

This study was conducted from the start of May 2016 to the end of October 2016 and included 60 patients infected with MDR *K. pneumoniae*. The patients received either colistin monotherapy or colistin–meropenem combination therapy. The current results were obtained from these 60 patients and the data were collected, documented and revised by the ICU medical staff.

3.1. Patient characteristics

Table 1 describes the demographic (age, sex) and clinical features, including patient co-morbidities, of the two groups who received either colistin alone ($n = 30$) or colistin–meropenem ($n = 30$). Statistical analysis revealed no significant differences between the two groups with regard to demographic data, type of infection and causative pathogen. In addition, 13 (21.7%) of the 60 patients had abnormal baseline SCr values at the onset of treatment (>1.3 mg/dL). The duration of administration was significantly ($P < 0.0001$) reduced in the combination treated group compared with the monotherapy group. None of the patients had undergone organ transplantation, radiotherapy or interferon treatment. All patients had received other antimicrobial agents prior to colistin treatment.

3.2. Incidence of mortality

As a primary outcome, colistin–meropenem combination therapy showed a significant ($P < 0.05$) survival proportion with lower mortality, where only 5 (16.7%) of 30 patients died compared with 13 (43.3%) in the colistin monotherapy group (Table 2A; Fig. 1). All of the patients who died had pneumonia and, in addition, six of them had bacteraemia, one had a surgical site infection and two had an abdominal infection (Table 1).

3.3. Renal function during treatment

Fig. 2A shows the incidence of SCr level elevation as proof of acute kidney injury (AKI); as shown in the figure, no significant difference was reported between the number of patients with and

Table 1
Comparison of demographics, illness severity and co-morbidities of patients treated with either colistin monotherapy or colistin–meropenem combination therapy.

Characteristic	*n* (%) or mean ± S.D.		*P*-value
	Colistin (*n* = 30)	Colistin–meropenem (*n* = 30)	
Demographic data			
Age (years)	56.20 ± 17.87	55.90 ± 15.61	0.8456
Sex (male)	16 (53.3)	12 (40.0)	0.4379
APACHE II score	18.13 ± 4.023	18.87 ± 5.463	0.5625
SOFA score	12.47 ± 2.649	11.70 ± 2.521	0.1944
Co-morbidities			
Malignancy	3 (10.0)	1 (3.3)	0.6120
Heart dysfunction	18 (60.0)	13 (43.3)	0.3015
Lung dysfunction	11 (36.7)	6 (20.0)	0.2516
Diabetes mellitus	7 (23.3)	9 (30.0)	0.7710
Urogenital disorder	4 (13.3)	7 (23.3)	0.5062
Chronic renal failure	2 (6.7)	4 (13.3)	0.6707
Baseline SCr ≥1.3 mg/dL	7 (23.3)	6 (20.0)	1.0000
Thrombocytopenia	3 (10.0)	3 (10.0)	1.0000
Elevated baseline AST and ALT	2 (6.7)	4 (13.3)	0.6707
Hepatic disease	2 (6.7)	3 (10.0)	1.0000
Haematological disorder	4 (13.3)	7 (23.3)	0.5062
Neurological disorder	15 (50.0)	9 (30.0)	0.1872
Previous hospitalisation in the preceding 90 days	18 (60.0)	23 (76.7)	0.2668
Duration of hospitalisation in the preceding 90 days (days)	15.00 ± 5.186	16.60 ± 6.157	0.3397
Previous antibiotic use	24 (80.0)	21 (70.0)	0.5520
Previous surgery	7 (23.3)	4 (13.3)	0.5062
Admission to the ICU			
Duration of ICU stay of survivors (days)	17.74 ± 2.401	13.92 ± 2.499	0.0001*
Mechanical ventilation	24 (80.0)	17 (56.7)	0.0946
Special treatments			
Anti-tumour treatment	4 (13.3)	1 (3.3)	0.3533
Steroid treatment	7 (23.3)	9 (30.0)	0.7710
Blood transfusion	9 (30.0)	6 (20.0)	0.5520
Haemodialysis	3 (10.0)	2 (6.7)	0.6120
Urinary catheter	30 (100)	29 (96.7)	1.0000
Gastrostomy/colostomy	2 (6.7)	1 (3.3)	1.0000
Type of infection			
Pneumonia	30 (100)	30 (100)	1.0000
Urinary tract infection	7 (23.3)	3 (10.0)	0.2990
Abdominal infection	2 (6.7)	5 (16.7)	0.4238
Surgical site infection	2 (6.7)	0 (0)	0.4915
Skin and soft-tissue infection	1 (3.3)	0 (0)	1.0000
Catheter-related infection	3 (10.0)	6 (20.0)	0.4716
Bacteraemia	11 (36.7)	17 (56.7)	0.1954
Pathogen isolated			
Klebsiella pneumoniae	30 (100)	30 (100)	1.0000
Antimicrobial susceptibility			
MDR (carbapenem-resistant *K. pneumoniae*)	26 (86.7)	23 (76.7)	0.5062
MDR (carbapenem-resistant *K. pneumoniae* susceptible only to colistin)	4 (13.3)	7 (23.3)	0.5062

S.D., standard deviation; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment SCR, serum creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICU, intensive care unit; MDR, multidrug-resistant.
Statistical analysis was carried out using Mann–Whitney test and Fisher's exact test.
* Compared with colistin monotherapy.

without kidney dysfunction among the two treatment regimens. Only five patients in the colistin-treated group had AKI, of which only one died during the study; likewise, in the colistin–meropenem group four patients had AKI and again only one died. Both patients who died had an abnormal SCr value.

It was observed that although there was a significant difference in the duration of treatment between the two groups, the incidence of AKI did not differ significantly, where AKI occurred after 4.2 ± 1.30 days (mean ± standard deviation) in the colistin-treated group compared with 2.75 ± 0.96 days in the colistin–meropenem-treated group. Similarly, no significant difference was detected between the two groups regarding SCr level during the study (Fig. 3A,D). However, studying the values in the surviving patients, a difference in the SCr level was noticed in the groups itself; there was always a significant difference between the values of SCr at the initiation/end of treatment and the maximum values for both regimens, as shown in Fig. 3B,C.

3.4. Changes in markers of sepsis

As shown in Table 2B, a gradual significant decrease ($P < 0.0001$) in TLC and percentage of neutrophils of the 42 cured patients was observed during the treatment with either regimen. However, improvement in the two assessed parameters was reported on Day 5 in the combination therapy group and on Day 6 in the colistin monotherapy group. Regarding CRP, its values also improved gradually in the surviving patients to reach 47.29 ± 39.00 mg/dL

Table 2A
Comparison of mortality and clinical morbidity outcomes in patients treated with colistin monotherapy versus colistin–meropenem combination therapy.

Clinical outcome	*n* (%)		*P*-value
	Colistin (*n* = 30)	Colistin–meropenem (*n* = 30)	
Mortality	13 (43.3)	5 (16.7)	0.0470*
Cured and survived	17 (56.7)	25 (83.3)	0.0470*
Reversible renal dysfunction	4 (13.3)	3 (10.0)	0.7210
Irreversible renal dysfunction	1 (3.3)	1 (3.3)	1.0000
Haemodialysis	3 (10.0)	2 (6.7)	0.6120

Statistical analysis was carried out using Fisher's exact test.
* Compared with colistin monotherapy.

(17/30 survivors) vs. 291.0 ± 113.5 mg/dL following colistin monotherapy and 54.68 ±39.13 mg/dL (25/30 survivors) vs. 299.4 ± 103.30 mg/dL following colistin–meropenem treatment. Of the 42 cured patients, 12 (28.6%; 5 treated with colistin and 7 treated with colistin–meropenem) had significantly elevated serum CRP levels with normal TLC values. Therefore, to rule out sepsis, PCT was evaluated in the 42 cured patients and its value among all these patients was 0.1614 ± 0.02179 ng/mL (Table 2C). This was in parallel with the improved clinical condition, negative sputum culture results and a significant ($P < 0.0001$) decrease in SOFA score of all patients treated with colistin monotherapy and colistin–meropenem combination therapy (Table 2B).

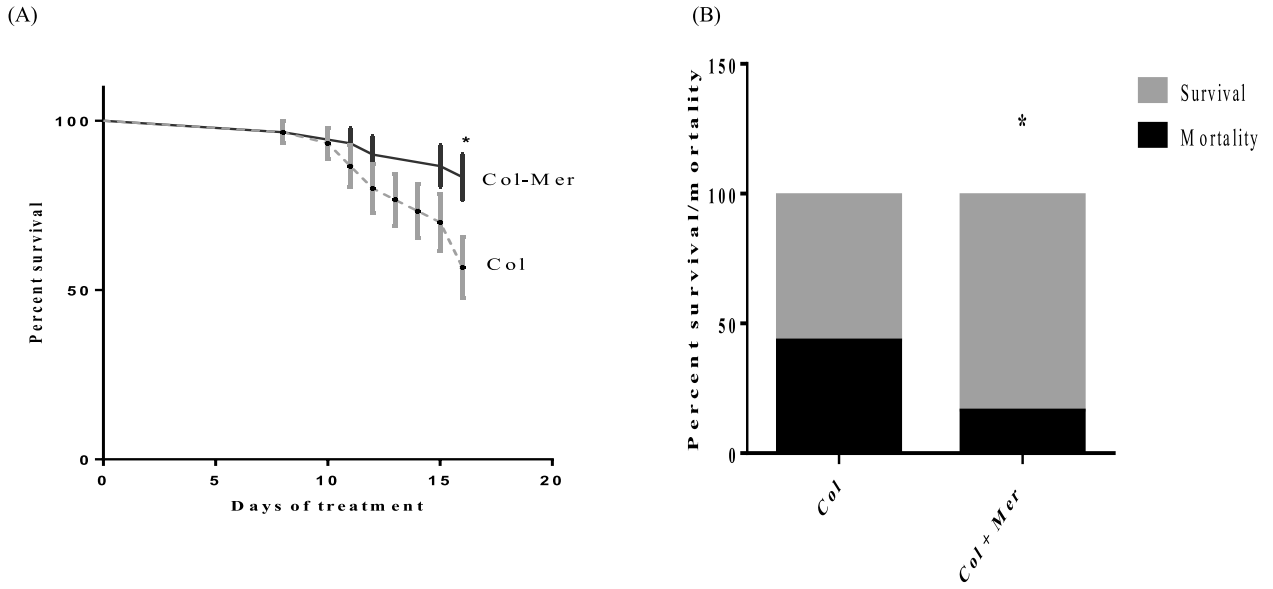

Fig. 1. Survival proportions: (A) percent survival in the colistin monotherapy (Col) and colistin–meropenem combination therapy (Col-Mer) groups; and (B) percent survival/mortality in the Col and Col–Mer groups. Statistical analysis was carried out using Gehan–Breslow–Wilcoxon test and Fisher's exact test. * $P < 0.05$ compared with colistin monotherapy.

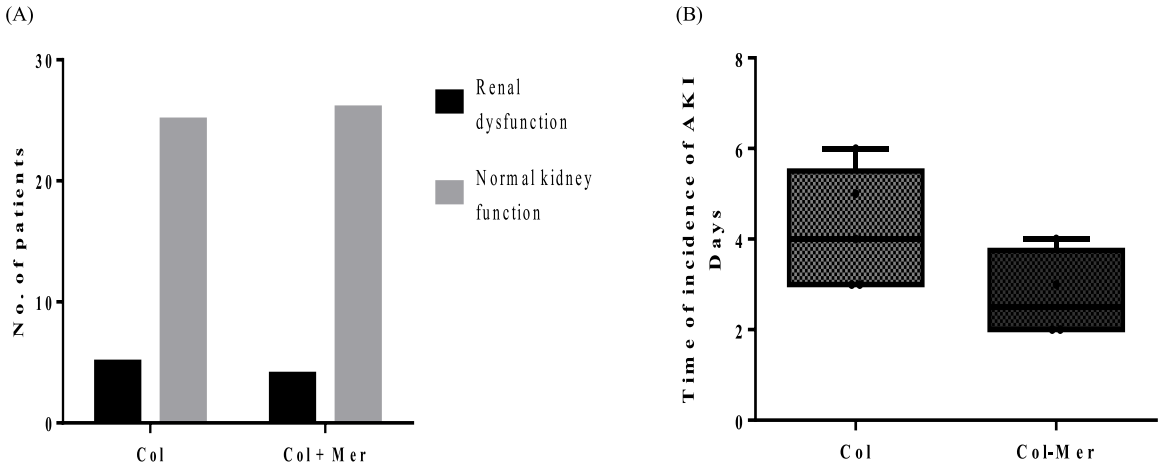

Fig. 2. (A) Distribution of patients with normal and abnormal kidney function in colistin (Col)- and colistin–meropenem (Col-Mer)-treated groups. (B) Time of acute kidney injury (AKI) incidence in Col- and Col-Mer-treated groups. Horizontal lines within the boxes represent the mean ± standard deviation number of days. Statistical analysis was carried out using Fisher's exact test and Mann–Whitney test.

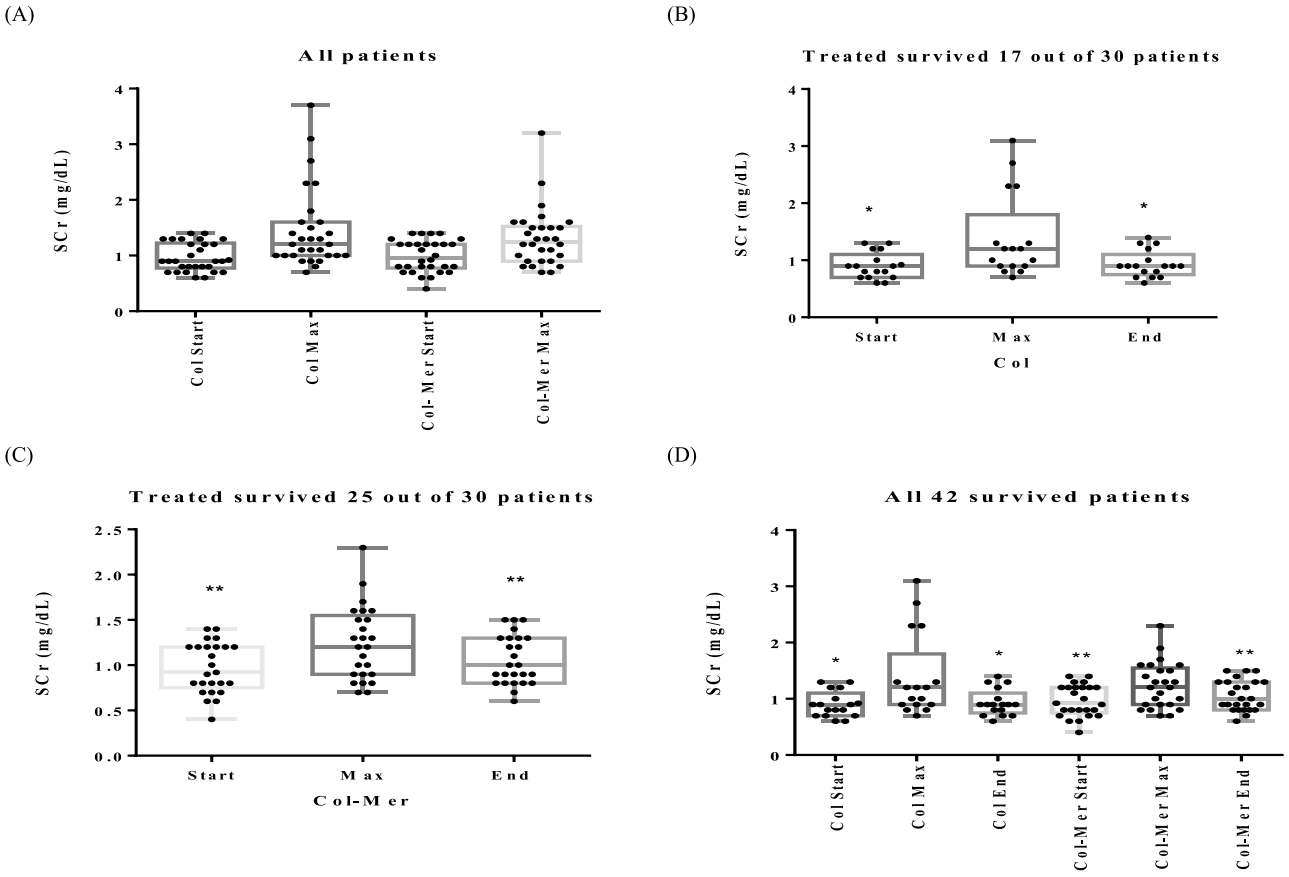

Fig. 3. Distribution of serum creatinine (SCr) levels on the first day of treatment (Start), the peak value (Max) and at the end of treatment (End) with colistin monotherapy (Col) or colistin–meropenem combination therapy (Col-Mer) in (A) all studied patients, (B) the group of survivors treated in the Col group, (C) the group of survivors in the Col–Mer group and (D) all 42 surviving patients in both treatment groups. Horizontal lines within the boxes represent the mean ± standard deviation. Statistical analysis was carried out using Wilcoxon matched-pairs signed-rank test. Significant difference ($P < 0.05$) from * Col Max and ** Col–Mer Max.

Table 2B
Comparison of data on the first day of treatment (Start) and at the end of treatment (End) of surviving patients treated with either colistin monotherapy or colistin–meropenem combination therapy.

Parameter	Start	End	*P*-value	End–Start
Colistin monotherapy ($n = 17$)				
SCr (mg/dL)	0.9012 ± 0.229	0.935 ± 0.234	0.0938	0.034 ± 0.079
TLC (WBC/μL)	20 906 ± 4492	8311 ± 682.10	<0.0001*	−12 595 ± 4596
Neutrophils %	86.82 ± 5.51	62.18 ± 5.69	<0.0001*	−24.65 ± 7.46
CRP (mg/dL)	291.0 ± 113.50	47.29 ± 39.00	<0.0001*	−243.70 ± 119.20
Procalcitonin (ng/mL)	–	0.1695 ± 0.02176	–	–
SOFA score	12.35 ± 3.121	1.882 ± 0.927	<0.0001*	−10.47 ± 3.125
Colistin–meropenem combination therapy ($n = 25$)				
SCr (mg/dL)	0.97 ± 0.28	1.056 ± 0.273	0.0817	0.087 ± 0.254
TLC (WBC/μL)	21 967 ± 5648	9446 ± 633.10	<0.0001*	−12 521 ± 5699
Neutrophils %	86.92 ± 4.609	67.32 ± 7.45	<0.0001*	−19.60 ± 8.37
CRP (mg/dL)	299.4 ± 103.30	54.68 ± 39.13	<0.0001*	−244.7 ± 114.10
Procalcitonin (ng/mL)	–	0.1558 ± 0.02224	–	–
SOFA score	11.64 ± 2.675	1.720 ± 0.843	<0.0001*	−9.920 ± 2.613

SCr, serum creatinine; TLC, total leukocyte count; WBC, white blood cells; CRP, C-reactive protein; SOFA, Sequential Organ Failure Assessment.
Data are presented as the mean ± standard deviation (S.D.).
Statistical analysis was carried out using Wilcoxon matched-pairs signed-rank test.
* $P < 0.05$ compared with colistin monotherapy.

3.5. Effect of different treatments on platelet count

As shown in Table 1, 10.0% (6/60) of the enrolled patients had thrombocytopenia (3 patients in each group). However, during treatment with either regimen, two (66.7%) of the three patients showed a further decrease in platelet count and died. In addition, 10 (18.5%) of the 54 patients with normal baseline platelet count experienced thrombocytopenia during treatment, 5 (50.0%) of whom died. These five patients included two in the colistin–meropenem combination group and three in the colistin monotherapy group (Table 3A).

Table 2C
Comparison of the number of surviving patients with abnormal C-reactive protein (CRP) and procalcitonin (PCT) values at the end of treatment with either colistin monotherapy or colistin–meropenem combination therapy

	n (%)		*P*-value
	CRP > 10 mg/dL	PCT > 0.5 ng/mL	
Survivors (*n* = 42)	12 (28.6)	0 (0)	0.0002*

Statistical analysis was carried out using Fisher's exact test.
* Compared with colistin monotherapy.

3.6. Effect of different treatments on liver function

Of the 60 enrolled patients, 6 (10.0%) had elevated serum AST and ALT levels (Table 1), of whom 2 continued to have elevated liver enzymes during treatment and died. In addition, as shown in Table 3A, seven (13.0%) other patients with normal baseline serum ALT and AST showed a significant elevation in their liver enzymes after treatment initiation (three patients by the third day, one patient by the fifth day, one patient by the seventh day, one patient by the ninth day and one patient by the eleventh day) but returned to normal values thereafter. Of the seven patients, three were receiving colistin monotherapy and one of them received micafungin sodium (antifungal); the other four patients received colistin–meropenem combination therapy and three of them received micafungin and voriconazole (antifungal). It is worth mentioning that there was no significant difference between the transaminase values between the end and the baseline values in either group (Table 3B).

3.7. Neurotoxicity

During treatment, all patients were closely monitored for possible neurological adverse episodes, including dizziness, weakness, paraesthesia and ataxia, as well as neuromuscular blockade and apnoea. Only three patients developed tonic–colonic seizures, and one of them had repeat symptoms while on her 5th, 8th and 12th day of treatment with colistin–meropenem combination therapy. From then on, although colistin–meropenem was continued, the symptoms gradually subsided. No confirmatory electromyography testing was performed.

4. Discussion

In an era of increasing antibiotic abuse/misuse, which leads to increased antimicrobial resistance, the use of neglected antibiotics for decades, such as colistin, is necessary to cure infections by resistant Gram-negative micro-organisms [32]. The current study was therefore conducted to evaluate the role of colistin with and without meropenem in treating MDR *K. pneumoniae* infections in 60 patients with HAP or VAP. In contrast to most recent retrospective clinical studies dealing with colistin as combination therapy, the present work is a prospective comparative study evaluating the safety and efficacy of a high dose of colistin as monotherapy versus combination therapy with meropenem.

The results revealed significant superiority of the colistin–meropenem combination therapy with regard to clinical response (cure and improvement) of the infection as well as decreasing duration of treatment and mortality rate as the primary endpoint; the combination regimen also showed no significant differences in the incidence of nephrotoxicity, hepatotoxicity and neurotoxicity as a secondary endpoint in comparison with high-dose colistin monotherapy.

In support of the present findings, previous in vitro [22] and in vivo [23] studies reported that the combination regimen is superior to colistin monotherapy. These authors used larger doses than the standard daily dose (6 MIU) against *K. pneumoniae* and other Gram-negative micro-organisms including *P. aeruginosa*, *A. baumannii* and *Escherichia coli*. However, using the standard daily dose of colistin (6 MIU) showed no significant difference between monotherapy and combination therapy [33]. Furthermore, a non-specific study of MDR *K. pneumoniae*-induced HAP or VAP conducted by Paul et al. revealed no significant difference between colistin monotherapy and combination therapy with meropenem [24]. In contrast to the current study, the inclusion criteria of the study conducted by Paul et al. included HAP, VAP, bloodstream infection and urosepsis induced by different types of MDR Gram-negative bacteria, including *Acinetobacter* spp., *P. aeruginosa* and Enterobacteriaceae (including but not restricted to *K. pneumoniae*, *E. coli* and *Enterobacter* spp.) with the same colistin loading dose (9 MIU) but different maintenance dose (4.5 MIU q12 h) [24]. Despite the similarity in the aim of the previously mentioned study and the current study, major differences in the inclusion criteria and the method of the study conducted by Paul et al. [24] lead to differences in the results and conclusions.

Colistin toxicity, especially nephrotoxicity, is one of the most important considerations when using colistin, as reported previously by Koch-Weser et al. [5]. Nevertheless, in the 1990s and thereafter this adverse effect became less severe, possibly because physicians became more focused on the potential nephrotoxicity and the use of improved supportive treatments and fluid supplementations. Hence, authors using colistin in doses of 3–6 MIU [20,34] who reported manageable nephrotoxicity led to the revival of colistin use. Subsequently, Dewan and Shoukat showed that colistin at a dose of 9 MIU had no significant increase in the incidence of nephrotoxicity to defend the present data [35]. Moreover, in the current results, combining colistin with meropenem did not cause significant irreversible kidney injury, in line with earlier retrospective studies [11,36].

On the other hand, neurotoxicity is the other less common toxicity associated with colistin administration, as reported by Koch-Weser et al. [5]. These authors reported few cases of paraesthesia, neuromuscular blockade or apnoea in patients receiving colistin. However, this was not the case in the present work, which coincides with the report of Falagas and Kasiakou [34]. Insignificant adverse effects involved the liver enzymes and thrombocytopenia with colistin monotherapy and with its combination with meropenem.

Regarding the termination of treatment, the present study showed that PCT sensitivity differs significantly from that of CRP in patients with sepsis. In line with the current results, Kaziani et al. highlighted the value of using PCT-based protocols in shortening the duration of antibiotic regimens in pneumonia in order to reduce antimicrobial resistance and costs and to improve clinical outcomes [37]. In addition, Hayashida et al. reported the superior

Table 3A
Comparison of clinical morbidity outcomes in patients treated with colistin monotherapy versus colistin–meropenem combination therapy.

Clinical outcome	*n* (%)		*P*-value
	Colistin (*n* = 30)	Colistin–meropenem (*n* = 30)	
Liver enzyme elevation with normal baseline by starting the treatment	3 (10.0)	4 (13.3)	1.000
Thrombocytopenia in patients with normal baseline by starting the treatment	4 (13.3)	6 (20.0)	0.731

Statistical analysis was carried out using Fisher's exact test compared with colistin monotherapy.

Table 3B
Comparison of laboratory data on the first day of treatment (Start) and at the end of treatment (End) of surviving patients treated with either colistin monotherapy or colistin–meropenem combination therapy.

Laboratory parameter	Start	End	*P*-value	End–Start
Colistin monotherapy ($n = 17$)				
Platelets	$317.8 \pm 111.6 \times 10^3$	$294.4 \pm 92.00 \times 10^3$	0.3116	$-23.47 \pm 80.88 \times 10^3$
AST	53.65 ± 46.42	54.94 ± 55.00	0.8119	1.294 ± 10.74
ALT	49.65 ± 36.80	48.41 ± 40.11	0.0977	-1.235 ± 15.52
Colistin–meropenem combination therapy ($n = 25$)				
Platelets	315.1 ± 112.1	263.7 ± 102.9	<0.0001*	-51.44 ± 96.18
AST	40.44 ± 30.58	39.04 ± 31.34	0.0567	-1.400 ± 5.025
ALT	34.12 ± 23.55	35.04 ± 25.93	0.3223	0.9200 ± 4.907

AST, aspartate aminotransferase; ALT, alanine aminotransferase.
Data are presented as the mean ± standard deviation (S.D.).
Statistical analyses was carried out using Wilcoxon matched-pairs signed-rank test.
* $P < 0.05$ compared with colistin monotherapy.

value of PCT as a biomarker for diagnosis and following the prognosis of sepsis instead of clinical parameters and traditional laboratory markers such as elevated TLC and CRP [38], which cannot differentiate infectious from non-infectious inflammation.

Moreover, PCT has superiority to differentiate between bacterial and viral infections in comparison with CRP [39]. In addition, PCT alone or combined with other scoring systems such as SOFA, as referred in the present study, has a significant value in determining the incidence of sepsis in patients with suspected sepsis [40].

5. Conclusions

In conclusion, colistin–meropenem combination therapy for patients with HAP or VAP due to MDR carbapenem-resistant *K. pneumoniae* infection showed significant superiority compared with colistin monotherapy in effectiveness as well as decreased morality and duration of treatment. In addition, consideration of PCT as a marker for eradication of sepsis and suspension of therapy showed significant superiority to CRP. These limited and preliminary data provide support for further studies of colistin combination therapy in this patient population.

Funding

None.

Competing interests

None declared.

Ethical approval

This study was approved by the 127 Ethics Committee of the Faculty of Pharmacy, Cairo University (Cairo, Egypt) [PT 1679].

References

[1] Carmeli Y., Troillet N, Karchmer AW, Samore MH. Health and economic outcomes of antibiotic resistance in *Pseudomonas aeruginosa*. Arch Intern Med 1999;159:1127–32.
[2] Hsueh PR, Teng LJ, Chen CY, Chen WH, Yu CJ, Ho SW, et al. Pandrug-resistant *Acinetobacter baumannii* causing nosocomial infections in a university hospital, Taiwan. Emerg Infect Dis 2002;8:827–32.
[3] Kasiakou SK, Michalopoulos A, Soteriades ES, Samonis G, Sermaides GJ, Falagas ME. Combination therapy with intravenous colistin for management of infections due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. Antimicrob Agents Chemother 2005;49:3136–46.
[4] Evans ME, Feola DJ, Rapp RP. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant Gram-negative bacteria. Ann Pharmacother 1999;33:960–7.
[5] Koch-Weser J, Sidel VW, Federman EB, Kanarek P, Finer DC, Eaton AE. Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy. Ann Intern Med 1970;72:857–68.
[6] Yow EM, Tan E, Shane L, Schonfeld S, Abu-Nassar H. Colistin (coly-mycin) in resistant bacterial infections. A clinical appraisal. Arch Intern Med 1961;108:664–70.
[7] Falagas ME, Kasiakou SK. Colistin the revival of polymyxins for the management of multidrug-resistant Gram-negative bacterial infections. Clin Infect Dis 2005;40:1333–41.
[8] Bergen PJ, Li J, Nation RL, Turnidge JD, Coulthard K, Milne RW. Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with *Pseudomonas aeruginosa* in an in vitro pharmacodynamic model. J Antimicrob Chemother 2008;61:636–42.
[9] Bergen PJ, Bulitta JB, Forrest A, Tsuji BT, Li J, Nation RL. Pharmacokinetic/pharmacodynamic investigation of colistin against *Pseudomonas aeruginosa* using an in vitro model. Antimicrob Agents Chemother 2010;54:3783–9.
[10] Dudhani RV, Turnidge JD, Coulthard K, Milne RW, Rayner CR, Li J, et al. Elucidation of the pharmacokinetic/pharmacodynamic determinant of colistin activity against *Pseudomonas aeruginosa* in murine thigh and lung infection models. Antimicrob Agents Chemother 2010;54:1117–24.
[11] Imberti R, Cusato M, Villani P, Carnevale L, Iotti GA, Langer M, et al. Steady-state pharmacokinetics and BAL concentration of colistin in critically ill patients after IV colistin methanesulfonate administration. Chest 2010;138:1333–9.
[12] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. EUCAST; 2017 http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf. [Accessed 2 August 2017].
[13] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. Document M100-S21. Wayne, PA: CLSI; 2011.
[14] Dudhani RV, Turnidge JD, Nation RL, Li J. *f*AUC/MIC is the most predictive pharmacokinetic/pharmacodynamic index of colistin against *Acinetobacter baumannii* in murine thigh and lung infection models. J Antimicrob Chemother 2010;65:1984–90.
[15] Markou N, Markantonis SL, Dimitrakis E, Panidis D, Boutzouka E, Karatzas S, et al. Colistin serum concentrations after intravenous administration in critically ill patients with serious multidrug-resistant, Gram-negative bacilli infections: a prospective, open-label, uncontrolled study. Clin Ther 2008;30:143–51.
[16] Plachouras D, Karvanen M, Friberg LE, Papadomichelakis E, Antoniadou A, Tsangaris I, et al. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by Gram-negative bacteria. Antimicrob Agents Chemother 2009;53:3430–6.
[17] University of California, Los Angeles. Health System Pharmaceutical Services Inc. UCLA Antimicrobial Stewardship Program. Colistimethate sodium. https://www.asp.mednet.ucla.edu/files/view/guidebook/Colistin_Dosing.pdf. [Accessed 17 May 2017].
[18] Tascini C, Ferranti S, Messina F, Menichetti F. In vitro and in vivo synergistic activity of colistin, rifampin, and amikacin against a multiresistant *Pseudomonas aeruginosa* isolate. Clin Microbiol Infect 2000;6:690–1.
[19] Gunderson BW, Ibrahim KH, Hovde LB, Fromm TL, Reed MD, Rotschafer JC. Synergistic activity of colistin and ceftazidime against multiantibiotic-resistant *Pseudomonas aeruginosa* in an in vitro pharmacodynamic model. Antimicrob Agents Chemother 2003;47:905–9.
[20] Conway SP, Pond MN, Watson A, Etherington C, Robey HL, Goldman MH. Intravenous colistin sulphomethate in acute respiratory exacerbations in adult patients with cystic fibrosis. Thorax 1997;52:987–93.
[21] Gheitani L, Fazeli H, Moghim S, Nasr IB. Frequency determination of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolated from hospitals in Isfahan of Iran and evaluation of synergistic effect of colistin and meropenem on them. Cell Mol Biol (Noisy-le-grand) 2018;64:70–4.

[22] Timurkaynak F, Can F, Azap OK, Demirbilek M, Arslan H, Karaman SO. 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. Int J Antimicrob Agents 2006;27:224–8.
[23] Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, et al. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother 2012;56:2108–13.
[24] Paul M, Daikos GL, Durante-Mangoni E, Yahav D, Carmeli Y, Benattar YD, et al. Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem-resistant Gram-negative bacteria: an open-label, randomised controlled trial. Lancet Infect Dis 2018;18:391–400.
[25] Mammina C, Bonura C, Di Bernado F, Aleo A, Fasciana T, Sodano C, et al. Ongoing spread of colistin-resistant *Klebsiella pneumoniae* in different wards of an acute general hospital, Italy, June to December 2011. Euro Surveill 2012;17: pii: 20248.
[26] 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.
[27] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985;13:818–29.
[28] Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working Group on 'Sepsis-Related Problems' of the European Society of Intensive Care Medicine. Crit Care Med 1998;26:1793–800.
[29] Chew KL, La MV, Lin RTP, Teo JWP. Colistin and polymyxin B susceptibility testing for carbapenem-resistant and *mcr*-positive Enterobacteriaceae: comparison of Sensititre, MicroScan, Vitek 2, and Etest with broth microdilution. J Clin Microbiol 2017;55:2609–16.
[30] Conway SP, Etherington C, Munday J, Goldman MH, Strong JJ, Wootton M. Safety and tolerability of bolus intravenous colistin in acute respiratory exacerbations in adults with cystic fibrosis. Ann Pharmacother 2000;34:1238–42.
[31] Damiano AM, Bergner M, Draper EA, Knaus WA, Wagner DP. Reliability of a measure of severity of illness: acute physiology of chronic health evaluation—II. J Clin Epidemiol 1992;45:93–101.
[32] Çetin ÇB, Özer Türk D, Şenol Ş, Dinç Horasan G, Tünger Ö. Colistin efficacy in the treatment of multidrug-resistant and extremely drug-resistant Gram-negative bacterial infections. Turk J Med Sci 2016;46:1379–84.
[33] 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.
[34] Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. Crit Care 2006;10:R27.
[35] Dewan A, Shoukat M. Evaluation of risk of nephrotoxicity with high dose, extended-interval colistin administration. Indian J Crit Care Med 2014;18:427–30.
[36] Chen Z, Chen Y, Fang Y, Wang X, Chen Y, Qi Q, et al. Meta-analysis of colistin for the treatment of *Acinetobacter baumannii* infection. Sci Rep 2015;5:17091.
[37] Kaziani K, Sotiriou A, Dimopoulos G. Duration of pneumonia therapy and the role of biomarkers. Curr Opin Infect Dis 2017;30:221–5.
[38] Hayashida K, Kondo Y, Hara Y, Aihara M, Yamakawa K. Head-to-head comparison of procalcitonin and presepsin for the diagnosis of sepsis in critically ill adult patients: a protocol for a systematic review and meta-analysis. BMJ Open 2017;7:e014305.
[39] Gendrel D, Raymond J, Coste J, Moulin F, Lorrot M, Guerin S, et al. Comparison of procalcitonin with C-reactive protein, interleukin 6 and interferon-α for differentiation of bacterial vs. viral infections. Pediatr Infect Dis J 1999;18:875–81.
[40] Kim H, Hur M, Moon HW, Yun YM, Di Somma S, GREAT Network. Multi-marker approach using procalcitonin, presepsin, galectin-3, and soluble suppression of tumorigenicity 2 for the prediction of mortality in sepsis. Ann Intensive Care 2017;7:27.