Original Article

TANAFFOS

Effect of Inhaled Colistin on the Treatment of Ventilator-Associated Pneumonia due to Multi-drug Resistant Acinetobacter

Omid Moradi Moghaddam [1], Mohammad Niakan Lahiji [1], Mahshid Talebi-Taher [2], Behnam Mahmoodiyeh [3]

[1] Trauma and Injury Research Center, Department of Critical Care, Iran University of Medical Sciences, Tehran, Iran, [2] Department of Infectious Disease, School of Medicine, Iran University of Medical Sciences, Tehran, Iran, [3] Department of Anesthesiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.

Correspondence to: Mahmoodiyeh B
Address: Department of Anesthesiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.
Email address:behnammahmoodiyeh@gmail.com

Background: Ventilator-Associated Pneumonia (VAP) is a hospital pneumonia that is considered in patients on mechanical ventilation for at least 48 hours with symptoms of new lower respiratory tract infections being reported in them. The present study reviews the effect of adding inhaled colistin in the treatment of ventilator-induced pulmonary infections in Intensive Care Unit (ICU) patients.

Materials and Methods: In this single blind clinical trial, patients admitted to the ICU with diagnosis of pulmonary infections caused by ventilator were investigated. In the treatment group, patients received 150 mg of colistin plus 1,000,000 units inhaled colistin every eight hours and in the control group only 300 mg of colistin every eight hours intravenously was given. Patients were followed up in terms of clinical findings for seven days after the initial diagnosis of infection.

Results: The results of this study showed that administration of inhaled colistin in patients admitted in ICU significantly improved culture indices, leukocyte, white blood cell count, chest X-ray, chest secretion, CPIS score and saccharification (SpO2) on the third and seventh days after treatment compared to the first day.

Conclusion: Considering the positive effect of adding inhaled colistin to the treatment of patients admitted to ICU with pulmonary infections caused by ventilator with multi-drug resistant Acinetobacter, the use of combination drug therapy is recommended.

Key words: Pulmonary infection, ICU, Inhaled colistin

INTRODUCTION

A large number of studies have shown that Ventilator-Associated Pneumonia (VAP) is the most common nosocomial infection and one of the most frequent complications among patients admitted to hospitals and particularly for Intensive Care Unit (ICU) patients (1-5). As a result of recent researches, intubation with mechanical ventilation raises the risk of pneumonia by 6 to 20 fold among patients and is associated with 20-40 percent crude mortality rates (6,7). Pneumonia caused by VAP is the most common fatal infection which demands for prescription of parenteral antibiotics (7,8). About 20% of hospital-acquired pneumonia is due to using mechanical ventilation (9). The rate of mortality for nosocomial pneumonia ranges from 38 to 70%, and even higher rates for Multidrug Resistant Gram-Negative (MDR-GN) organisms (10). Recent studies indicate that the incidence of lower respiratory tract infection due to MDR pathogens and gram-negative bacilli such as *Acinetobacter baumannii* has been increasing (6,7). Therefore, according to the 2005

American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) guidelines for management of VAP, improvement in patient outcome emphasizes the importance of appropriate antibiotic therapy based on patient risk factors for infection due to MDR pathogens (6).

Because of potent bactericidal action of colistin, low incidence of resistances, and excellent activity against gram-negative bacilli, even MDR strains, colistin was used as a polymycin antibiotic agent in the 1960s and 1980s (11). Colistin does not increase cross-resistance of gram-negative bacilli and its' mechanism of action makes it less susceptible to bacterial resistance mechanisms (12,13). Over 20 years, inhaled colistin has been used successfully to prevent and cure pulmonary infections in cystic fibrosis patients (14). There are only few studies available assessing the effectiveness of inhaled colistin for the treatment of nosocomial pneumonia and particularly VAP (15,16).

Inhaled colistin has been used for both early and chronic infection in Europe and in the United States. The evidence from large controlled trials would support its use (17-23). But there are limited and conflicting data on the efficacy and adverse effects regarding inhaled colistin in the treatment of VAP in patients (24-26). This study compared the efficacy and safety rate of administering inhalation and intravenous colistin versus only intravenous colistin in patients with MDR-GN VAP.

MATERIALS AND METHODS

Base on inclusion and exclusion criteria, the study population consisted of 114 VAP patients admitted in ICU of Hazrat-e Rasool General Hospital, Tehran, Iran (from March 2016 to April 2017). Ethical approval for the study was granted from the hospital's review board. The clinical trial of the present study was registered in the Iranian Registry of Clinical Trials (IRCT) with the code number of (IRCT2015071323184N1).

Inclusion criteria
- Age ranging: over 16 years
- Multi-drug resistant to MDR Acinetobacter

- Without sensitivity to polymyxins
- Positive culture (Colony count: Bronchoalveolar Lavage-BAL 10^4 or Mini BAL 10^5)

Exclusion criteria
- Patient death
- Incidence of sensitivity to polymyxins
- Reduced Glomerular Filtration Rate (GFR) <0.25
- Incidence of bronchospasm evidences in treatment group

Study design

In this blinded clinical trial, patients diagnosed with VAP entered the study. Patients who fulfilled all inclusion/exclusion criteria were randomly assigned into two groups. Randomization schedule was generated by using a random numbers table. In treatment group, patients received 150 mg colistin administrated intravenously in addition to 1,000,000 units inhaled colistin for one week every eight hours. The control group only received 300 mg of colistin every eight hours intravenously for one week (routine treatment).

On the first, third and seventh days after intervention, the patients were sampled and followed in terms of clinical and laboratory findings such as fever (>38⁰C), number of leukocytosis, plasma creatinine, plasma urea, SpO_2, FiO_2, blood pressure, heart rate, body temperature, and number of breaths per minute. Also on the seventh day, re-cultivation was carried out and in terms of changes in the nature of the patient's Clinical Pulmonary Infection Score (CPIS) score was checked by the doctor using the same procedure.

CPIS test Characteristic

The CPIS was calculated as an appropriate tool to facilitate the diagnosis of VAP (27). Luna et al. at baseline calculated and modified the CPIS by using the five variables (28). By adding the progression of the infiltrate and culture results of the BAL, CPIS at 72 hours was calculated based on seven variables (29). Papazian et al.

used the CPIS for 38 patients who died after more than 72 hours of mechanical ventilation, of which 18 patients had histological evidence of pneumonia (30). The CPIS test of tissue samples is used as a gold standard for diagnosis due to the strength of its analysis. The study findings showed that for the presence of VAP, at the threshold of 6 points, CPIS had a high sensitivity, specificity and overall accuracy (30). Another study conducted by Fabregas et al., validated the CPIS by the presence of both histological and positive microbiological evidence of pneumonia in patients receiving mechanical ventilation. In Fabregas study, 25 patients died while receiving mechanical ventilation for more than 72 hours and the sputum samples were obtained immediately after death and before discontinuation of mechanical ventilation. The rate of sensitivity, specificity and overall accuracy of the presence of VAP in CPIS were high and similar to the Papazian study (30,31).

Statistical analysis

Variables were evaluated for normality of distribution by the use of the Kolmogorov–Smirnov test. Quantitative variables are expressed as mean values ± SDs. Categorical variables were compared using Wilcoxon (to compare changes during treatment) or Mann-Whitney U test (to compare two groups), whichever was appropriate. Continuous variables were compared using paired sample t test (to compare changes during treatment) or student's *t* test (to compare two groups). Statistical significance was defined as P value<0.05.

RESULTS

One hundred and fourteen patients with MDR-GN pneumonia were studied: 57 patients received colistin intravenously in addition to inhaled colistin and 57 patients received only intravenous colistin. The average daily mortality rates for the first 7 days of the post-

treatment period were less in treatment group than in the control (14 vs.15.8%; P value=0.7). The side effect incidence rates (kidney diseases; urea level rise over 50%) were higher in control group on 3rd day (5.4 vs. 3.6%; P value=0.6) and 7th day (7.2 vs. 5.4%; P value=0.6). It should also be mentioned that one patient in the treatment group died after treatment.

Patient's demographic information is listed in table1. The two groups were similar in demographic characteristics. In terms of baseline characteristics, there were not significant differences between the two groups (P value > 0.05), except in case of Mean Arterial Pressure (MAP) and leukocyte count on 3rd day in which there was a significant difference between the two groups (P value<0.05) (Table 2). On other hand, significant changes were observed during the treatment days in clinical and laboratory characteristics (Paired sample t test; P value<0.05).

Table 1. Demographic data of participants

Parameter	Treatment group	Control group	P value
Age (y) ± SD	49.19 ± 21.6	47.3 ± 18.3	0.61
Male gender, n	40 (70.2%)	40 (70.2%)	1
Weight (kg) ± SD	72.38 ± 10.67	73.33 ± 8.9	0.6

P value: comparison of demographic parameters communication between two groups by t test or Mann-Whitney U

CPIS characteristics were assessed and the results demonstrated in table 3. The Mann-Whitney U test showed that there was a significant difference only in the position of CXR, secretion and sputum culture of patients between the two groups on 3rd and 7th days (P value < 0.05). Also the CPIS in treatment group had a better score as compared with the control group on 3rd and 7th days (t test; P value = 0.0001).

Table 2. Comparison of clinical and laboratory characteristics in treatment and control groups (T test) on 1st, 3rd and 7th day after treatment

Baseline characteristics	Days	Treatment group	Control group	P value
	1st day	97.2±21	92.6±17.7	0.2
MAP (Mean Arterial Pressure) ± SD	3rd day	92.9±16	100.4±15.4	0.01
	7th day	93±14.3	89.2±13	0.1
	1st day	99.7±26.2	107±26.7	0.1
Heart rate (beat/min) ± SD	3rd day	89.9±20.7	96.7±23.5	0.1
	7th day	86.8±20.4	91.3±20.8	0.2
	1st day	25.4±4	26±4.4	0.4
Respiratory Rate (beat/min) ± SD	3rd day	19.9±3.2	21.3±4.3	0.07
	7th day	18.1±3.9	18.3±4.5	0.7
	1st day	38.5±1.1	38.5±1	0.7
Temperature (°C) ± SD	3rd day	37.7±0.7	37.9±0.7	0.2
	7th day	37.3±0.6	37.3±0.6	0.8
	1st day	14145.6±4759	15687.7±3899	0.06
Leukocyte count (per mm^3) ± SD	3rd day	11303.5±4408	13635±4013	0.004
	7th day	10269.6±3446	11064.9±3588	0.2
	1st day	1.2±0.5	1.2±0.6	0.9
Plasma Creatinine ± SD	3rd day	1.2±0.6	1.1±0.8	0.8
	7th day	1.2±0.7	1.2±0.8	0.9
	1st day	30.2±10.8	26.5±11.2	0.07
Plasma urea± SD	3rd day	29.1±11.3	25.9±11.4	0.1
	7th day	29.4±13.3	25.8±12.7	0.1
	1st day	7.4±1	7.4±1	0.8
Arterial blood pH± SD	3rd day	7.3±0	7.4±0	0.6
	7th day	7.3±0	7.3±0	0.8
	1st day	84.7±9.9	83.1±7.8	0.3
Pressure of oxygen in arterial± SD	3rd day	87.9±9.9	86.5±7.3	0.3
	7th day	89.4±11.8	89.3±8.5	0.9
	1st day	92.1±6.3	89.7±6.4	0.052
Oxygen saturation (SpO$_2$) ± SD	3rd day	94.5±6	92.7±6.3	0.1
	7th day	95.1±7.4	94.7±6.9	0.8
	1st day	56±16.4	58.7±15.6	0.3
Fraction of inspired oxygen (*FiO$_2$*) ± SD	3rd day	51.7±17.7	54.9±16.5	0.3
	7th day	49.7±17.5	54.3±18	0.1

Table 3. Comparison of Clinical Pulmonary Infection Score (CPIS) in treatment and control groups (T test) on 1st, 3rd and 7th day after treatment

CPIS characteristics	Category	Treatment group			Control group		
		1st day	3rd day	7th day	1st day	3rd day	7th day
Temperature (°C)	Normal	6(10.5%)	43(75.4%)	50(87.7%)	8(14%)	32(56.1%)	49(86%)
	low-grade fever	27(47.4%)	8(14%)	2(3.5%)	23(40.4%)	20(35.1%)	4(7%)
	high fever	24(42.1%)	6(10.5%)	4(7%)	26(45.6%)	5(8.8%)	4(7%)
White blood cells	Normal	9(15.8%)	20(35.1%)	37(64.9%)	2(3.5%)	17(29.8%)	35(61.4%)
	<4	47(82.5%)	37(64.9%)	19(33.3%)	55(96.5%)	40(70.2%)	22(38.6%)
	>11						
	Band>500	1(1.8%)	-	-	-	-	-
PaO₂/FiO₂	Normal	49(86%)	51(89.5%)	51(89.5%)	48(84.2%)	49(86%)	49(86%)
	No ARDS	8(14%)	6(10.5%)	5(8.8%)	9(15.8%)	8(14%)	8(14%)
CXR	Normal	2(3.5%)	13(22.8%)	30(52.6%)	8(14%)	10(17.5%)	12(21.1%)
	Diffused/ patchy	22(38.6%)	19(33.3%)	11(19.3%)	8(14%)	8(14%)	7(12.3%)
	Localize	33(57.9%)	25(43.9%)	15(26.3%)	41(71.9%)	39(68.4%)	38(66.7%)
Secretion	Normal	-	4(7%)	27(47.4%)	-	-	2(3.5%)
	Non purulent	1(1.8%)	30(52.6%)	24(42.1%)	-	3(5.3%)	36(63.2%)
	purulent	56(98.2%)	23(40.4%)	5(8.8%)	57(100%)	54(94.7%)	19(33.3%)
Culture	Normal	-	10(17.5%)	44(77.2%)	-	-	30(52.6%)
	Positive	57(100%)	47(82.5%)	12(21.1%)	57(100%)	57(100%)	27(47.4%)
CPIS score		7.9±1.3	5.4±2.1	2.5±2.7	8.1±1.4	6.86±1.6	4.5±2.4

DISCUSSION

This study demonstrated that patients with MDR-GN pneumonia treated with both inhaled colistin and IV colistin compared to patients who received IV colistin had a more favorable outcome. The results of the study showed that during 7 days after treatment, inhaled colistin was well tolerated and the speed of recovery of patients in treatment group was higher than the control group which was in the same line with Korbila et al. (26). Also the mortality rate in both groups was approximately equal. It should be mentioned that the studied patients in both groups were matched in terms of age, gender and weight in the two groups.

Colistin or polymyxin E identified as a polymyxin antibiotic is produced by certain strains of Bacillus polymyxa var in 1949 (32). Because of the inherent resistant of Gram-positive bacteria, *Burkholderia cepacia*, *Serratia marcescens*, *Moraxella catarrhalis*, Proteus spp., Providencia spp., and *Morganella morganii* to the treatment with colistin, the limited range of colistin usage is only for treating the infections with *Pseudomonas aeruginosa* (*P. aeruginosa*) and *A. baumannii* (32). This polypeptide antibiotic is effective against most Gram-negative bacilli (33). Due to the absence of effective alternative cure, IV colistin has been used in patients with VAP caused by MDR-GN pathogens as salvage treatment (33,34).

Results of studies on the effect of direct delivery of antibiotics such as colistin were clinically beneficial to patients by increasing topical drug levels (35-37). Due to the increase of morbidity and mortality rate in patients with MDR-GN infections, it is important to determine if inhaled colistin had better healing results (25). Aside from the intravenous administration of colistin, it can be used as inhaled colistin whose effectiveness has been proven (18). Some studies reported that combination therapy (inhaled colistin as adjunctive therapy to IV colistin therapy) had

high clinical and microbiological responses to VAP (38-41), which was similar to the results of this study. By contrast, in some other studies no additional benefit was observed (25,42).

The result of the present study and Korbila et al. could not show any significant difference in mortality rate between the two groups (26). So it seems that this therapeutic approach does not have definite impact on the mortality rate. But adding inhaled colistin to IV could significantly improve negativization of respiratory cultures.

The most important side effects of colistin therapy are nephrotoxicity and neurotoxicity which is more prevalent in patients with abnormal creatinine levels. Even during 1970s and 1980s, colistin was forbidden because of these difficulties (43). Several published studies confirmed the safety and efficacy of colistin caused by MDR-GN bacteria (44,45); but renal dysfunction is the most frequent reported adverse effect in the critically ill patients in the ICU setting (46). In other studies, the incidences of nephrotoxicity were reported as the main limiting factor for colistin usage in adults (24,38,47). In the current study a total of 5 patients in treatment group and 7 patients in control group experienced nephrotoxicity after treatment. Although the incidence of nephrotoxicity was higher in control group but it was not statistically different.

The current study had some limitations that should be mentioned. It was not possible to control the particle size of real inhaled colistin which was administered via conventional nebulizers and as a result the exact amount of drug delivered to the lungs could not be exactly determined (48,49). We also should note that this was a retrospective study and only single-center site. This comparative evidence is only available to make a decision between IV antibiotics and inhaled colistin treatment to determine usefulness of inhaled colistin for VAP at that time.

CONCLUSION

The results showed that receiving 150 mg colistin in addition to 1,000,000 units of inhaled colistin (inhaled-

intravenous colistin therapy) had a better outcome compared to IV colistin alone in the treatment of VAP caused by MDR-GN Acinetobacter. On other hand, super-infection with inherently colistin-resistant pathogens and development of colistin option for multidrug resistant *P. aeruginosa* as an important issue should be assessed in future studies.

The patient's death, patient's withdrawal from the ICU and follow-up of patient's problems were the main reasons for not checking the 30-day outcome, which were the limitations of this study.

REFERENCES

1. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, et al. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. *JAMA* 1995;274(8):639-44.

2. Rello J, Quintana E, Ausina V, Castella J, Luquin M, Net A, et al. Incidence, etiology, and outcome of nosocomial pneumonia in mechanically ventilated patients. *Chest* 1991;100(2):439-44.

3. Celis R, Torres A, Gatell JM, Almela M, Rodríguez-Roisin R, Agustí-Vidal A. Nosocomial pneumonia. A multivariate analysis of risk and prognosis. *Chest* 1988;93(2):318-24.

4. Craven DE, Kunches LM, Lichtenberg DA, Kollisch NR, Barry MA, Heeren TC, et al. Nosocomial infection and fatality in medical and surgical intensive care unit patients. *Arch Intern Med* 1988;148(5):1161-8.

5. Jiménez P, Torres A, Rodríguez-Roisin R, de la Bellacasa JP, Aznar R, Gatell JM, et al. Incidence and etiology of pneumonia acquired during mechanical ventilation. *Crit Care Med* 1989;17(9):882-5.

6. 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(4):388-416.

7. Chastre J, Fagon JY. Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2002;165(7):867-903.

8. Bercault N, Boulain T. Mortality rate attributable to ventilator-associated nosocomial pneumonia in an adult intensive care unit: a prospective case-control study. *Crit Care Med* 2001;29(12):2303-9.

9. Niederman MS, Craven DE, Fein AM, Schultz DE. Pneumonia in the critically ill hospitalized patient. *Chest* 1990;97(1):170-81.

10. Laessig KA. End points in hospital-acquired pneumonia and/or ventilator-associated pneumonia clinical trials: food and drug administration perspective. *Clin Infect Dis* 2010;51 Suppl 1:S117-9.

11. Pérez-Pedrero MJ, Sánchez-Casado M, Rodríguez-Villar S. Nebulized colistin treatment of multi-resistant Acinetobacter baumannii pulmonary infection in critical ill patients. *Med Intensiva* 2011;35(4):226-31.

12. Rios FG, Luna CM, Maskin B, Saenz Valiente A, Lloria M, Gando S, et al. Ventilator-associated pneumonia due to colistin susceptible-only microorganisms. *Eur Respir J* 2007;30(2):307-13.

13. Evans ME, Feola DJ, Rapp RP. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. *Ann Pharmacother* 1999;33(9):960-7.

14. Ratjen F, Rietschel E, Kasel D, Schwiertz R, Starke K, Beier H, et al. Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. *J Antimicrob Chemother* 2006;57(2):306-11.

15. Kwa AL, Loh C, Low JG, Kurup A, Tam VH. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa. *Clin Infect Dis* 2005;41(5):754-7.

16. Hamer DH. Treatment of nosocomial pneumonia and tracheobronchitis caused by multidrug-resistant Pseudomonas aeruginosa with aerosolized colistin. *Am J Respir Crit Care Med* 2000;162(1):328-30.

17. Littlewood JM, Miller MG, Ghoneim AT, Ramsden CH. Nebulised colomycin for early pseudomonas colonisation in cystic fibrosis. *Lancet* 1985;1(8433):865.

18. Jensen T, Pedersen SS, Garne S, Heilmann C, Høiby N, Koch C. Colistin inhalation therapy in cystic fibrosis patients with chronic Pseudomonas aeruginosa lung infection. *J Antimicrob Chemother* 1987;19(6):831-8.

19. Valerius NH, Koch C, Høiby N. Prevention of chronic Pseudomonas aeruginosa colonisation in cystic fibrosis by early treatment. *Lancet* 1991;338(8769):725-6.

20. Frederiksen B, Koch C, Høiby N. Antibiotic treatment of initial colonization with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. *Pediatr Pulmonol* 1997;23(5):330-5.

21. Ratjen F, Döring G, Nikolaizik WH. Effect of inhaled tobramycin on early Pseudomonas aeruginosa colonisation in patients with cystic fibrosis. *Lancet* 2001;358(9286):983-4.

22. Ramsey BW, Pepe MS, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med* 1999;340(1):23-30.

23. Ryan G, Mukhopadhyay S, Singh M. Nebulised anti-pseudomonal antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2000;(2):CD001021.

24. Michalopoulos A, Fotakis D, Virtzili S, Vletsas C, Raftopoulou S, Mastora Z, et al. Aerosolized colistin as adjunctive treatment of ventilator-associated pneumonia due to multidrug-resistant Gram-negative bacteria: a prospective study. *Respir Med* 2008;102(3):407-12.

25. Kofteridis DP, Alexopoulou C, Valachis A, Maraki S, Dimopoulou D, Georgopoulos D, et al. Aerosolized plus intravenous colistin versus intravenous colistin alone for the treatment of ventilator-associated pneumonia: a matched case-control study. *Clin Infect Dis* 2010;51(11):1238-44.

26. Korbila IP, Michalopoulos A, Rafailidis PI, Nikita D, Samonis G, Falagas ME. Inhaled colistin as adjunctive therapy to intravenous colistin for the treatment of microbiologically documented ventilator-associated pneumonia: a comparative cohort study. *Clin Microbiol Infect* 2010;16(8):1230-6.

27. Zilberberg MD, Shorr AF. Ventilator-associated pneumonia: the clinical pulmonary infection score as a surrogate for diagnostics and outcome. *Clin Infect Dis* 2010;51 Suppl 1:S131-5.

28. Luna CM, Blanzaco D, Niederman MS, Matarucco W, Baredes NC, Desmery P, et al. Resolution of ventilator-associated

pneumonia: prospective evaluation of the clinical pulmonary infection score as an early clinical predictor of outcome. *Crit Care Med* 2003;31(3):676-82.

29. Fagon JY, Chastre J, Wolff M, Gervais C, Parer-Aubas S, Stéphan F, et al. Invasive and noninvasive strategies for management of suspected ventilator-associated pneumonia. A randomized trial. *Ann Intern Med* 2000;132(8):621-30.

30. Papazian L, Thomas P, Garbe L, Guignon I, Thirion X, Charrel J, et al. Bronchoscopic or blind sampling techniques for the diagnosis of ventilator-associated pneumonia. *Am J Respir Crit Care Med* 1995;152(6 Pt 1):1982-91.

31. Fàbregas N, Ewig S, Torres A, El-Ebiary M, Ramirez J, de La Bellacasa JP, et al. Clinical diagnosis of ventilator associated pneumonia revisited: comparative validation using immediate post-mortem lung biopsies. *Thorax* 1999;54(10):867-73.

32. Michalopoulos AS, Tsiodras S, Rellos K, Mentzelopoulos S, Falagas ME. Colistin treatment in patients with ICU-acquired infections caused by multiresistant Gram-negative bacteria: the renaissance of an old antibiotic. *Clin Microbiol Infect* 2005;11(2):115-21.

33. Falagas ME, Grammatikos AP, Michalopoulos A. Potential of old-generation antibiotics to address current need for new antibiotics. *Expert Rev Anti Infect Ther* 2008;6(5):593-600.

34. Levin AS. Treatment of Acinetobacter spp infections. *Expert Opin Pharmacother* 2003;4(8):1289-96.

35. Michalopoulos A, Papadakis E. Inhaled anti-infective agents: emphasis on colistin. *Infection* 2010;38(2):81-8.

36. Ioannidou E, Siempos II, Falagas ME. Administration of antimicrobials via the respiratory tract for the treatment of patients with nosocomial pneumonia: a meta-analysis. *J Antimicrob Chemother* 2007;60(6):1216-26.

37. Lesho E. Role of inhaled antibacterials in hospital-acquired and ventilator-associated pneumonia. *Expert Rev Anti Infect Ther* 2005;3(3):445-51.

38. Michalopoulos A, Kasiakou SK, Mastora Z, Rellos K, Kapaskelis AM, Falagas ME. Aerosolized colistin for the treatment of nosocomial pneumonia due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. *Crit Care* 2005;9(1):R53-9.

39. Falagas ME, Kasiakou SK, Kofteridis DP, Roditakis G, Samonis G. Effectiveness and nephrotoxicity of intravenous colistin for treatment of patients with infections due to

40. Mastoraki A, Douka E, Kriaras I, Stravopodis G, Manoli H, Geroulanos S. Pseudomonas aeruginosa susceptible only to colistin in intensive care unit patients. *Surg Infect (Larchmt)* 2008;9(2):153-60.

41. Lin CC, Liu TC, Kuo CF, Liu CP, Lee CM. Aerosolized colistin for the treatment of multidrug-resistant Acinetobacter baumannii pneumonia: experience in a tertiary care hospital in northern Taiwan. *J Microbiol Immunol Infect* 2010;43(4):323-31.

42. Kalin G, Alp E, Coskun R, Demiraslan H, Gündogan K, Doganay M. Use of high-dose IV and aerosolized colistin for the treatment of multidrug-resistant Acinetobacter baumannii ventilator-associated pneumonia: do we really need this treatment? *J Infect Chemother* 2012;18(6):872-7.

43. Wolinsky E, Hines JD. Neurotoxic and nephrotoxic effects of colistin in patients with renal disease. *N Engl J Med* 1962;266:759-62.

44. Kallel H, Bahloul M, Hergafi L, Akrout M, Ketata W, Chelly H, et al. Colistin as a salvage therapy for nosocomial infections caused by multidrug-resistant bacteria in the ICU. *Int J Antimicrob Agents* 2006;28(4):366-9.

45. Kallel 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(7):1162-1167.

46. Markou N, Apostolakos H, Koumoudiou C, Athanasiou M, Koutsoukou A, Alamanos I, et al. Intravenous colistin in the treatment of sepsis from multiresistant Gram-negative bacilli in critically ill patients. *Crit Care* 2003;7(5):R78-83.

47. Linden PK, Paterson DL. Parenteral and inhaled colistin for treatment of ventilator-associated pneumonia. *Clin Infect Dis* 2006;43 Suppl 2:S89-94.

48. Dhand R, Sohal H. Pulmonary Drug Delivery System for inhalation therapy in mechanically ventilated patients. *Expert Rev Med Devices* 2008;5(1):9-18.

49. Georgopoulos D, Mouloudi E, Kondili E, Klimathianaki M. Bronchodilator delivery with metered-dose inhaler during mechanical ventilation. *Crit Care* 2000;4(4):227-34.