

# EXPERT OPINION

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## Inhaled colistin for lower respiratory tract infections

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**Introduction:** Lower respiratory tract infections, due to *Pseudomonas aeruginosa* or *Acinetobacter baumannii*, are frequently encountered in patients with cystic fibrosis (CF) or in patients developing nosocomial pneumonias. Both of these conditions bear a high mortality risk and aggressive antibiotic therapy is necessary. Inhaled antibiotics might represent an effective therapeutic approach for these diseases as it has demonstrated good bactericidal efficacy and safety in both preclinical and clinical studies. This colistin formulation might be useful particularly in patients with respiratory tract infections due to multidrug-resistant Gram-negative bacteria. Its main advantages are a better safety profile with a minimal or absent risk of nephrotoxicity.

**Areas covered:** This paper discusses the available systemic formulations of colistin, with pharmacokinetic and safety profiles, followed by an overview of inhaled antibiotics in lower respiratory tract infections.

**Expert opinion:** Inhaled colistin should be used selectively as monotherapy in chronic infections with *P. aeruginosa* in CF patients, whereas in patients with hospital/ventilator-acquired pneumonia (HAP/VAP), it should be used in a combined regimen with systemic antibiotics.

**Keywords:** *Acinetobacter baumannii*, colistin, inhalatory, *Pseudomonas aeruginosa*, respiratory tract infections

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### 1. Introduction

Colistin is a cationic antibiotic, which was once more commonly used to treat various infections caused by Gram-negative bacteria. Due to the occurrence of side effects, such as nephrotoxicity or neurotoxicity, appearing at therapeutic doses when used for longer periods of time, colistin was abandoned in favor of other antibiotics with more favorable safety profiles. This less frequent use had, however, a good consequence: bacteria such as *Acinetobacter baumannii* or *Pseudomonas aeruginosa* kept their sensitivity to colistin and allowed its use for this selective therapeutic indication. New Delhi metallo- $\beta$ -lactamase multidrug-resistant *Enterobacteriaceae* were also reported to be sensitive to colistin [1].

*P. aeruginosa* and *A. baumannii* strains are involved in chronic colonization, frequent exacerbations and negative disease prognostic in cystic fibrosis (CF) (*P. aeruginosa*) and nosocomial pneumonia (hospital associated or ventilator associated), with high lethality risk (*P. aeruginosa* and *A. baumannii*) [2,3]. Nosocomial infections caused by multidrug-resistant (MDR) Gram-negative bacteria in particular pose a serious challenge in critical care settings worldwide. Carbapenem-resistant strains of *P. aeruginosa*, *A. baumannii* and *Klebsiella pneumoniae* that exhibit resistance to almost all antibiotics except polymyxins have emerged as important nosocomial pathogens with significant morbidity and mortality. The lack of new available antibiotics has prompted physicians to reevaluate the use of polymyxins (principally of polymyxin B and colistin). Recent studies have demonstrated that polymyxins remain active against most Gram-negative bacteria *in vitro*, and intravenous or inhaled polymyxins for the

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treatment of MDR Gram-negative bacterial infections have been found to be effective and considerably less toxic than reported in older studies [4].

Both CF exacerbations and hospital/ventilator-acquired pneumonia (HAP/VAP) are treated with systemic antibiotics as first intention, but the addition of inhaled antibiotics might increase the effectiveness of the therapeutic regimen. However, for these two conditions, the choice of inhaled antibiotics is limited because their topic formulations are poorly evaluated clinically, difficult to be effectively administered clinically despite good preclinical efficacy or because some of the initially promising formulations were subsequently discontinued.

In such lower respiratory tract infections, inhaled antibiotics can be given via nebulization or as dry powder inhaled formulations. This is the case of tobramycin and of colistin but for both nebulization still remains the most efficacious and the most feasible.

## 2. Systemic formulations of colistin: pharmacological properties and overview of use in lower respiratory tract infections

Colistin (or polymyxin E) is a cationic, lipopeptide antibiotic, which was isolated initially from *Bacillus polymyxa* subsp. *colistinus* with two major components: colistin A (polymyxin E<sub>1</sub>) and colistin B (polymyxin E<sub>2</sub>) [5,6]. The main difference between the molecules of polymyxin B and colistin is represented by the amino acid sequence: colistin consists of D-leucine, L-threonine and L- $\alpha$ , $\gamma$ -diaminobutyric acid, while polymyxin B contains D-phenylalanine instead of D-leucine [7].

There are two pharmaceutical forms of colistin: colistin sulfate (CS) and colistin methanesulfonate (CMS), colistin's prodrug, its molecule containing the colistin moiety with an added sulfomethyl group to the primary amines of colistin. CMS hydrolysis yields the active drug colistin along with other partially sulfomethylated derivatives. CMS is the CS prodrug, is less toxic and is given as an injectable formulation [4]. CS may be used to treat intestinal infections, to eradicate colonic flora or as various topic formulations (creams, powders or otic solutions) [4].

Colistin exerts a bactericidal effect on Gram-negative bacteria, and their mechanism of action consists of an interaction with lipopolysaccharides and phospholipids, which are structural components of the bacterial membrane, from which they displace competitively the calcium and magnesium cations [8]. This leads to a change in the membrane's tensioactive properties, damages the osmotic barrier and leads to extracellular leakage of intracellular contents [8].

Colistin has evolved as a potent therapy for respiratory tract infections caused by multidrug-resistant Gram-negative microbes, due to rapid bactericidal activity, low risk of emergence of resistant strains and a rather narrow spectrum of activity.

## 3. Inhaled antibiotics in lower respiratory tract infections: an overview

In lower respiratory tract infections, inhaled antibiotics have evolved as an alternative to the corresponding systemic formulations due to the fact that with such formulations it is possible to increase the local bactericidal efficacy and reduce the systemic exposure, the risk of side effects being considerably lower. In theory, an ideal inhaled antibiotic is given at lower doses as compared with parenteral or oral formulations, is able to reach the small caliber airways, is not inhibited by the sputum, is cheap and does not produce local side effects (Table 1) [9].

However, the development of an inhaled formulation of an antibiotic is very difficult as various issues should be addressed in this particular setting. For example, penetration to the small airways is an issue if the patient has bronchospasm or CF: in both cases, the antibiotic cannot reach the smaller caliber airways due to the airflow limitation or due to the viscid sputum. Furthermore, in a lung area with pneumonia, for example, the airflow can be reduced as compared with healthy zones and the antibiotic tends to go in the latter territories where it is not even needed [9].

Inhaled antibiotic might be in theory able to reach minimal inhibitory concentrations (MICs) at lower dosages than the corresponding systemic formulations. However, usually lung inflammation is associated with a sputum hyperproduction and sputum itself can contain molecules that inactivate the antibiotic and reduce the bactericidal effect locally, increasing the risk of development of resistant strains [9]. This is the case with aminoglycosides, which are inactivated by sputum mucin or DNA, and this limitation was overcome by increasing the aminoglycoside sputum concentrations up to 25 times the MIC [10].

Furthermore, the nebulization of antibiotics might be an expensive therapeutic intervention not because of the active substance but because of its requirements of administration: for example, the nebulized tobramycin can be delivered only with certain types of nebulizers.

Inhaled antibiotics might produce local inflammation, cough or bronchospasm often due to excipients/preservatives such as benzalkonium chloride or EDTA in the nebulization solution. If the antibiotic is diluted directly in normal saline and is not irritative for the airways in the inhaled formulation, such effects have at least in theory a low risk of development [9].

In lower respiratory tract infections associated or not with CF, inhaled antibiotics might be used as a prophylaxis to prevent infection, as a therapy for early infection (early eradication), as a therapy of chronic infection or as a therapy of manifest/symptomatic infection [10,11].

The most prototypal example of a disease in which all these indications are possible is the CF and a well-known antibiotic used with nebulized and dry powder inhaled formulation is tobramycin. This inhaled antibiotic was demonstrated to improve lung function and to reduce the rate of exacerbations with *P. aeruginosa* [10,11].

**Table 1. Qualities of the ideal inhaled antibiotic [1].**

Quality	Relevance
Penetrance	In cystic fibrosis or in patients with bronchospasm, this may be impaired, therefore, considering co- or prior administration of an inhaled bronchodilator might increase the penetrance
Not inhibited by sputum	This might be dependent on the physicochemical properties of the antibiotic and should be mandatorily tested during the drug development program
Thermic stability during aerosolization	Should not be influenced by the decrease in temperature during nebulization with jet nebulizers or by the increase in temperature during ultrasonic nebulizers
Cost-effective	Antibiotics nebulization procedure is currently quite costly due to its requirements: frequent daily administration, the nebulizer, etc. The dry powder inhaled antibiotics might be more cost-effective
Local toxic effects (cough, bronchospasm) Absent systemic exposure	These can be due to the antibiotic itself or to the excipients of the solution for nebulization In the case of aminoglycosides, the systemic exposure can lead to side effects such as nephro- or ototoxicity
Low environmental risk	Colistin and pentamidin are reported to be hazardous for health-care workers

Adapted from [9].

Inhaled colistin was also considered for the same indication, and in some countries including the USA, it is authorized as an inhaled antimicrobial to be used for CF patients. Apart from CF, inhaled colistin was also used for other types of lower respiratory tract infections [12].

#### 4. Inhaled colistin: preclinical evaluation

The efficacy of colistin in experimental models of lower respiratory tract infections was initially evaluated with systemic formulations. In an initial study performed in an animal model of pneumonia with three strains of *A. baumannii* (A, D, E) with carbapenem resistance, the bactericidal efficacy of imipenem, sulbactam, tobramycin, rifampin and CMS given intraperitoneally every 6 h after 4 h from inoculation and for a total period of 44 h after experimental infection was determined. The CMS dosage used was 500,000 U/kg. Measures of bactericidal efficacy were represented by the reduction of log(10) CFU/g in lung bacterial counts, by the reduction of bacteremia and by survival in treated animals compared with untreated animals with pneumonia (controls). Imipenem and sulbactam were found to be the most efficacious (reduction of lung bacterial counts: -5.38 and -4.64 log(10) CFU/ml) on susceptible and intermediate susceptible strains. They were followed by tobramycin and rifampin (-4.16 and -5.15 log(10) CFU/ml). Unfortunately, colistin demonstrated the lowest antibacterial efficacy (-2.39 log(10) CFU/ml;  $p < 0.05$ ) [13].

However, when combined with other antibiotics such as rifampin and in certain *A. baumannii* strains (strain E), CMS was able to exert a synergistic antimicrobial effect (reduction of the lung bacterial counts :  $5.62 \pm 0.26$  with rifampin,  $8.38 \pm 1.22$  with CMS, both significant when compared with controls,  $5.59 \pm 1.17$  with rifampin + CMS combination,  $p < 0.05$  when compared with controls) [14].

In a subsequent *in vivo* experiment performed in a rat model of acute pneumonia produced by an inoculum of  $2.5 \times 10^6$  colony-forming units of carbapenem-resistant *A. baumannii* (CRAB) strain Ab396, the efficacy of imipenem/cilastatin plus sulbactam 80/80 and 40 mg/kg every 8 h given intraperitoneally, that of CS 150,000 U/kg every 8 h given intraperitoneally and that of intratracheal CS 75,000 U/kg every 8 h given intraperitoneally at 2 h after intratracheal inoculation of CRAB were evaluated. The MICs of parenteral antibiotics on the Ab396 strains were 2 µg/ml for CS, 128 µg/ml for imipenem/cilastatin or 32 µg/ml. Animals receiving inhaled CS had the best survival rate at 72 h of therapy initiation (100% as compared with 5 in untreated animals, 10% for imipenem/sulbactam combination and 0% for parenteral CS). The same colistin formulation was reported to normalize the wet lung/body weight ratios [15]. Although pharmacokinetics of aerosolized colistin is not well known, a study performed in ventilated piglets with *P. aeruginosa*-induced pneumonia demonstrated that the topic administration of CMS is able to achieve a maximal lung concentration faster than the more traditionally used intravenous formulation. CMS, administered after 24 h from bronchial

inoculation of *P. aeruginosa* either by nebulization at a dose of 8 mg/kg every 12 h or by intravenous route at a dose of 3.2 mg/kg every 8 h, achieved a peak lung concentration of 2.8 µg/g with aerosolized formulation and undetectable levels with intravenous formulation. For the aerosolized CMS, the lung concentrations depended on the severity of local inflammation, the highest levels being reached in lung segments featuring mild pneumonia (10 µg/g) as compared with 1.2 µg/g in segments with severe. After 24 h of therapy, aerosolized CMS was associated with a significant rate of bacterial clearance, 67% of the pulmonary segments having a bacterial count of  $< 10^2$  CFU/g as compared with 28% with intravenous administration ( $p < 0.001$ ) [16].

Combination therapy with other antipseudomonal antibiotics was demonstrated to exert a synergistic bactericidal effect. In a preclinical study in which *P. aeruginosa* biofilms were generated *in vitro* and *in vivo* in rat lungs, CS-tobramycin combination reduced the *P. aeruginosa* counts in the biofilm models *in vitro*. *In vivo*, in the rat model of lung *P. aeruginosa*-related biofilms, antibiotic combination therapy was associated to lower mortality rates, lower rate of lung damage and fewer bacterial colony-forming units after 7 days of therapy when compared with single antibiotics [17].

## 5. Inhaled colistin: pharmacokinetic profile

Pharmacokinetics of inhaled colistin was evaluated after disease was stable again in CF patients, both children and adults ( $n = 30$ ), hospitalized for a CF exacerbation and it was possible to discontinue the antibiotic therapy 5 days prior to the initiation of the study. At baseline, blood, urine and sputum samples were collected before inhalation of antibiotic. The dose to be assessed was 2 million international units of CMS (158 mg of colistimethate-Na or 66 mg of colistin) dissolved in 6 ml of isotonic saline, which was nebulized and other blood samples were taken at 0.25, 0.5, 1, 2, 3, 6, 8 and 12 h after inhalation. Urine was collected up to 12 h after administration of inhaled colistin at 4-h interval. Sputum specimens were collected after 1, 4 and 12 h from nebulization. Polymyxin E1, which is the main component of colistin, was used to evaluate its pharmacokinetics. Serum polymyxin E1 concentration reached the  $t_{max}$  at 1.5 h after inhalation and subsequently decreased. The  $AUC_{0-12}$  was  $0.865 \pm 0.187$ , the half-life ( $t_{1/2}$ ) was  $4.51 \pm 0.57$  and the mean residence time (MRT) was about 7 h. When compared with those of the systemic formulations, the serum concentrations of inhaled colistin were lower. CMS cumulative concentrations in urine ranged from 0.18 to 16.13 mg, and the mean urine concentration was 4.3%. Sputum concentrations reached a maximum 1 h post-inhalation and this was about 10 times higher than the MIC breakpoint for *P. aeruginosa* and decreased thereafter, their levels being above 4 mg/l after 12 h. In the same study, in a subset of eight patients, it was possible to evaluate the physicochemical properties of the same inhaled CMS dose, which was given via an electronic nebulizer. This solution for inhalation (2 millions of units of

inhaled colistin dissolved in 2.5 ml normal saline) was found to have a surface tension of 44.2 mN/m, dynamic viscosity 1.3 mP/s and osmolality 334 mosmol/kg, pH 6.7. The percentage of small diameter droplets was found to be 79.2, the mass median diameter (MMD) was 3.4 µm and the total output rate (mg/min) 150 [18].

## 6. Inhaled colistin: clinical use in lower respiratory tract infections

Intravenous formulation of colistin methanesulfonate has been traditionally used as a therapy for the lower respiratory tract infections such as pneumonia, infected bronchiectasis associated or not with CF caused by Gram-negative bacteria. However, in inflamed tissue, colistin achieves effective bactericidal concentrations when given at higher dosages, and this might be associated with an increased risk of side effects.

In order to reduce the systemic toxicity, inhaled formulations of CS and CMS were developed and evaluated mostly in *P. aeruginosa* infections in CF and are used in Europe rather than in the US for this indication. Furthermore, inhaled colistin was also used in HAP/VAP as a monotherapy or as an add on to other, systemic, less toxic antibiotics.

### 6.1 Cystic fibrosis

One of the initial clinical reports on inhaled colistin was from a study that involved 40 CF patients with *P. aeruginosa* chronic infections who were evaluated in a prospective double-blind placebo-controlled study. They were randomized to receive either inhaled CMS 1 million of units twice daily for 3 months or placebo (isotonic saline). Inhaled colistin improved the respiratory symptoms, lung function and reduced the inflammation significantly [19].

In CF, colistin given via inhalatory route as nebulized aerosols or as dry powder inhaled formulation has been so far evaluated as a therapy directed against chronic colonization with various Gram-negative bacilli, most commonly represented by *P. aeruginosa*. Some studies used CMS, whereas others used CS, which is the most active antibacterial between the two compounds.

In a double-blind, randomized crossover study, two preparations of nebulized colistin, colistin sulfate and colistin sulfomethate were evaluated in nine CF patients with chronic *P. aeruginosa* infection in the airways and FEV1 of 57.8%. Patients were given at the first visit either nebulized colistin sulfate or colistin sulfomethate and vice versa at the second visit, which was scheduled after at least 5-day interval. Lung function was measured before and after nebulization, and tolerance to the nebulized solution was evaluated with short, specific questionnaire. Colistin sulfomethate at a dose of 160 mg (Colistin parenteral®, Grünenthal GmbH, Aachen, Germany) and CS at a dose of 100 mg dissolved in 6 ml normal saline to obtain a solution for inhalation with a pH of 7.4 and an osmolality of 366 mOsm/kg for the CMS and,



respectively, a pH of 5 and an osmolality of 306 for CS. Nebulization of CS caused a significant larger mean decrease in lung function compared with nebulized CMS. In seven patients, completion of nebulization with CS was not possible because of throat irritation and severe cough [20].

Inhaled colistin administration was demonstrated to be associated with improvement of various disease outcome measures in adult CF patients. In a study, 14 stable CF patients (6 males and 8 females) having a mean FEV1 approximately 45% predicted with concomitant rDNAse treatment received an antibiotic regimen consisting of inhaled tobramycin (cycles lasting 28 days every other month) and of inhaled CMS (2,000,000 IU  $\times$  b.i.d. during the months without tobramycin) given on alternate basis for 6 months. Exercise capacity was tested with cardiopulmonary exercise testing procedure before and after inhaled CMS. Inhaled colistin was found to improve clinical status, peak  $\text{VO}_2$  (18.3 vs 19.2,  $p < 0.02$ ) and  $\text{VO}_2/\text{t}$  slope (0.41 vs 0.48,  $p < 0.07$ ) [21].

When evaluated in 10 CF children, inhaled colistin (1,000,000 bid) associated with ciprofloxacin (30 mg/kg/24 h) given for 21 days in newly diagnosed *P. aeruginosa* infections ( $n = 8$ ) or given alone at the same dosage in children with chronic *P. aeruginosa* infection ( $n = 2$ ), both subsets of CF patients being followed up for 1 year for thriving, exacerbations rate, sputum bacterial load, weight gain, no exacerbations and improvement in respiratory symptoms were reported. Sputum *P. aeruginosa* eradication was reported in six out of eight children with new bacterial infection and in all children with chronic infection [22].

Inhaled colistin might also be effective as a prophylaxis of chronic *P. aeruginosa* infection in CF patients with initial *P. aeruginosa* (PA) airways colonization. In a study comparing such CF patients treated with inhaled colistin and oral ciprofloxacin with 43 historic controls, the rate of chronic *P. aeruginosa* development was 16% in the treated patients after 42 months compared with 72% in the historical control sample ( $p < 0.005$ ). This regimen also increased pulmonary function throughout the first initial year post-enrollment as compared with the control group who experienced lung function decline ( $p < 0.01$ ). In patients receiving the active treatment and who developed chronic *P. aeruginosa* infection, a better lung function was found at the onset of this infection when compared with historical controls ( $p < 0.001$ ). A 3-month regimen consisting of inhaled colistin and oral ciprofloxacin produced a significant delay in the development of chronic *P. aeruginosa* infection ( $p < 0.05$ ) [23].

In other studies, it was demonstrated that colistin is less efficacious than other nebulized antibiotics in improving the lung function: when nebulized colistin 80 mg/3 ml or nebulized tobramycin 300 mg/5 ml given twice daily on random basis in 115 CF patients for 4 weeks, and FEV1 change from baseline was compared between groups, it was demonstrated that only nebulized tobramycin improved the lung function significantly. Both nebulized antibiotics, however,

reduced the sputum PA counts and were associated with no emergence of a resistant strain [24].

Another formulation of inhaled colistin, CMS dry powder, was subsequently investigated. In an initial deposition study performed in healthy volunteers ( $n = 8$ ) inhaled, a single dose of 25 mg CMS was given via an inhaled device called Twincer inhaler and the median diameter of the dry powder particles measured by laser diffraction technique was 1.6  $\mu\text{m}$ . Pulmonary function tests were performed before, and 5 and, respectively, 30 min after colistin inhalation. Serum samples were drawn at 15, 45 min, 1.5, 2.5, 3.5, 5.5, 7.5 and 24 h after inhalation. Dry powder CMS was found to exert no significant effect on lung function and was well tolerated [25].

These results prompted the performance of a pilot single dose study in 10 CF patients with chronic *P. aeruginosa* infection in which the efficacy of this formulation was compared with that of the nebulized colistin. This was a randomized crossover study. During each of the two visits, patients inhaled either inhaled CMS as 25 mg dry powder (Twincer) or 158 mg nebulized colistin sulfomethate solution. Pulmonary function tests were performed and serum samples were collected as in the initial study. No significant impairment in lung function and side effects were reported with both formulations. Relative bioavailability of DPI to nebulization was approx. 140% based on actual dose [26].

Chronic infection with *P. aeruginosa* is associated with progressive deterioration in lung function in CF patients. The purpose of this trial was to assess the efficacy and safety of tobramycin nebulizer solution (TNS) and nebulized colistin in CF patients chronically infected with *P. aeruginosa*. One hundred and fifteen patients, aged  $> \text{or} = 6$  years, were randomized to receive either TNS or colistin, twice daily for 4 weeks. The primary end point was an evaluation of the relative change in lung function from baseline, as measured by forced expiratory volume in one second % predicted. Secondary endpoints included changes in sputum *P. aeruginosa* density, tobramycin/colistin minimum inhibitory concentrations and safety assessments. TNS produced a mean 6.7% improvement in lung function ( $p = 0.006$ ), while there was no significant improvement in the colistin-treated patients (mean change 0.37%). Both nebulized antibiotic regimens produced a significant decrease in the sputum *P. aeruginosa* density, and there was no development of highly resistant strains over the course of the study. The safety profile for both nebulized antibiotics was good. Tobramycin nebulizer solution significantly improved lung function of patients with CF chronically infected with *P. aeruginosa*, but colistin did not, in this study of 1-month duration. Both treatments reduced the bacterial load [17].

## 6.2 Non-CF bronchiectasis

Nebulized colistin was also evaluated for its bactericidal effects in patients with non-CF bronchiectasis alone or in combination with inhaled tobramycin. For example, in an observational study performed in 81 adults with non-CF-related lower

respiratory tract infections, who received between January 2004 and December 2008 a total number of 97 courses of inhaled colistin alone, inhaled colistin plus tobramycin or inhaled tobramycin alone, the frequency and duration of hospitalizations for respiratory exacerbations, as well as development of antibiotic resistance, antibiotic use during hospitalization, sustained *P. aeruginosa* eradication in the airways and mortality were evaluated among the three treatment groups. Both inhaled antibiotics were found to exert comparable effects on the outcome measures analyzed. Inhaled colistin therapy was associated with a lower risk of development of antibiotic strain when compared with tobramycin (hazard ratio 0.09, 95% confidence interval (CI) 0.03 – 0.32). Compared with inhaled tobramycin, inhaled antibiotics combination was demonstrated to be associated with a more prominent therapeutic effect, leading to a reduced duration of hospitalization (relative risk (RR) 0.33, 95% CI 0.10 – 1.12) and of antibiotic use (RR 0.27, 95% CI 0.08 – 0.93, respectively) [27].

In a prospective study involving 18 patients with bronchiectasis treated with nebulized colistin 30 mg/day, it was found that inhaled colistin reduced significantly the lung function decline (44 ml/year compared with 104 ml/year prior to colistin therapy,  $p = 0.035$ ) and improved significantly the quality of life (3.6 vs 6.2,  $p = 0.001$ ) [28].

### 6.3 Hospital-/ventilator-associated pneumonia

Inhaled colistin was also claimed to be efficacious in patients with severe forms of pneumonia such as HAP/VAP targeting multiresistant germs such as *P. aeruginosa* or *A. baumannii*, both adults and children (Table 2). Some other isolated reports suggest that it might also be effective in pneumonias in immunosuppressed patients [29].

In an initial report on the efficacy and safety of inhaled colistin added to parenteral antibiotic therapy in patients ( $n = 8$ ) with hospital-associated pneumonia and APACHE scores on the day of the admission to intensive care unit 14.6, most of the patients (7; 87.5%) had VAP and *A. baumannii* infection and the other case had *P. aeruginosa*. Fifty percent of the bacterial strains identified were sensitive to colistin. Inhaled colistin was given as a daily dose of 1.5 – 6 million IU three or four times a day, the mean duration of therapy being 10.5 days. Seven (87.5%) patients also received concomitant intravenous treatment with colistin. Therapeutic response rate was 87.5% (50% were cured, 27.5% improved) and one death due to sepsis and multiple organ failure was also reported [30]. In another series of five patients, three with VAP and two with nosocomial pneumonia due to *A. baumannii* ( $n = 3$ ), *P. aeruginosa* ( $n = 1$ ) and *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* combined infection ( $n = 1$ ) who were previously treated with systemic antibiotic therapy (piperacillin/tazobactam, meropenem, ceftriaxone and ciprofloxacin) and who received subsequently nebulized colistin, a cure rate of (80%) was reported [31].

In another retrospective study, VAP outcome was compared in patients with concomitant intravenous and nebulized

colistin ( $n = 78$ ) and in patients with intravenous colistin alone ( $n = 43$ ). Mean daily dose of i.v. colistin was 7.0 respectively 6.4, whereas the mean daily dose of inhaled colistin was 2.1 millions IU. The cure rates were 79.5% in 62 patients who received i.v. plus inhaled colistin compared with 60.5% in 26 patients who received intravenous colistin only ( $p = 0.025$ ). Mortality rates were comparable between groups. Addition of inhaled colistin was identified to be independently associated with VAP cure (OR 2.53). Predictors of mortality were a higher APACHE II score at ICU admission (OR 1.12), presence of malignancy (OR 4.11) and lower daily dosage of i.v. colistin (OR 0.81) [32].

In a prospective study with a larger sample ( $n = 60$ ), the efficacy and safety of inhaled colistin were evaluated in patients with VAP due to multidrug-resistant pathogens such as *A. baumannii* ( $n = 37$ ), *P. aeruginosa* ( $n = 12$ ) and *K. pneumoniae* strains ( $n = 11$ ). Inhaled colistin mean daily dose was 2.2 million IU, and the mean duration of therapy was 16.4 days. Most of the patients ( $n = 57$ ) received concomitant intravenous colistin. Bacteriological and clinical response of VAP was reported in 50 (83.3%) patients and no adverse effects related to inhaled colistin were recorded. Mortality rate was 16.7% [33]. In more severe and older VAP patients ( $n = 45$ ), mean age 71, mean APACHE II scores on the day of admission 22.5; a mean ICU hospitalization duration of 34 days was reported. A higher mean daily dose of inhaled colistin was also reported (4.29 millions IU), and the mean duration of inhaled colistin therapy was 10.29 days. Microbiological response was reported in only 37.8% whereas clinical response was reported in 57.8%. Mortality rate was 42.2% and no adverse effects were reported [33].

Inhaled colistin was also found to be effective in patients with cancer and VAP due to Gram-negative bacteria (mostly *P. aeruginosa*): in a retrospective analysis in which pneumonia outcome was compared in patients receiving colistin or aminoglycoside systemically ( $n = 16$ ) and in patients treated also with corresponding inhaled formulations ( $n = 16$ ). Addition of inhaled antibiotics reduced the need of corticosteroids significantly (13% compared with 50% in patients with systemic antibiotic therapy only;  $p < 0.02$ ) and reduced the risk of renal impairment (31% in patients with systemic antibiotic therapy versus 0% in patients receiving inhaled antibiotic therapy  $p \leq 0.04$ ). Patients receiving inhaled antibiotics had a higher rate of complete clinical (81 vs 31%  $p < 0.01$ ) or microbiologic response (77 vs 8%  $p < 0.0006$ ). Inhaled antibiotic therapy predicted complete clinical resolution (OR 6.3,  $p < 0.04$ ) and microbial eradication (OR 36.7,  $p < 0.003$ ) [34].

More recently, however, it was demonstrated that addition of inhaled CMS did not influence significantly the disease outcome in patients with VAP due to multidrug-resistant Gram-negative bacteria [35].

Based on these results, it might be concluded that topic therapy with inhaled colistin might be effective in patients with non-CF-related severe forms of lower respiratory tract

**Table 2. Nebulized colistin and disease outcomes in adult hospital/ventilator-acquired pneumonia (HAP/VAP) patients.**

Authors	Number of patients	Daily dose	Bacterial strains	Monotherapy/ combined regimen	APACHE score	Duration of therapy	Therapeutic response rate
Michalopoulos et al. 2005 [1]	8	1.5 – 6 mil IU	<i>Acinetobacter baumannii</i> <i>Pseudomonas aeruginosa</i>	Nebulized colistin–intravenous colistin (n = 7)	14.6	10.5	87.5%
Falagas et al. 2009 [2]	5	0.5 – 4 times daily 1 mil IU three times daily	<i>A. baumannii</i> <i>P. aeruginosa</i> <i>Klebsiella pneumoniae</i>	Nebulized colistin piperacillin/tazobactam, meropenem, ceftriaxone, ciprofloxacin	11-27	6-11	80
Michalopoulos et al. 2008 [3]	n = 78 with nebulized colistin n = 43 without nebulized colistin	2.1 mil IU	<i>A. baumannii</i> <i>P. aeruginosa</i> <i>K. pneumoniae</i>	Nebulized colistin/ piperacillin/tazobactam, meropenem, gentamicin	11-24	6-11	80%
Lin et al. 2010 [4]	n = 45	4.29 million IU	<i>A. baumannii</i> <i>P. aeruginosa</i>		22.5	34	37.8% microbiological response 57.8% clinical response

Adapted from [30,31,33].

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* 43(4):323-331.

infections. However, the presence of an underlying pulmonary disease or smoking might be associated with a negative therapeutic outcome [36].

Nebulized colistin might also be used in pediatric population with HAP/VAP or other severe forms of lower respiratory tract infections in which inhaled colistin was associated with higher cure rates and earlier discharge even when given as monotherapy [37,38].

## 7. Colistin: safety profile

Intravenous colistin is known for its significant toxic potential, which is particularly high when the parenteral therapy is prolonged more than 4 weeks. Its main manifestations are nephrotoxicity and neurotoxicity. In various studies involving different types of infections, nephrotoxicity was found to be up to 36% when the mean duration of therapy was below 30 days [39-42].

Neurotoxicity is thought to occur less frequently than nephrotoxicity and can manifest with signs of neuromuscular junction blockade (paresthesias, limb weakness) or with severe polyneuropathy [40].

In CF patients in particular (n = 21), in whom the duration of colistin therapy ranged from 6 to 35 days, there were 26 reports of reversible neurologic manifestations that included paresthesias, headache or lower limb weakness. One event of colistin-induced nephrotoxicity was also reported. These events were not related to colistin plasma levels of pharmacokinetics [43].

In terms of allergic reactions, in a retrospective analysis of these events in CF patients (n = 180) who received intravenous colistin, 51 individuals (28%) developed drug-related adverse events that led to early therapy discontinuation. Lymphocyte transformation test (LTT) was performed in 11 patients with rash, headache or paresthesia to colistin and was found to be positive in 9 patients [44].

The bronchoconstriction induced by nebulized colistin in CF patients has been reported in several studies performed in children or in adults: in a study that enrolled 58 CF children with a mean age of 12 and a mean FEV1% predicted 59.3, most of them (n = 55) being regular users of colistin, bronchoconstriction measured with FEV1 fall was significant at 0 respectively 15 min after colistin nebulization (5.9, p < 0.001; 3.3, p < 0.004), and 34% of CF children had an FEV1 fall of more than 10% bronchoconstriction following nebulized colistin in CF [45].

In adults (n = 27), with CF and a mean FEV1% predicted of 54% in whom nebulizations with hypertonic, isotonic or hypotonic inhaled colistin solution was given over 3 consecutive days randomly, the bronchoconstrictive effect was measured before and at 0, 15, 30, 60 and 90 min after nebulization. Nebulized colistin was associated with bronchoconstriction irrespective of the tonicity of the nebulized solution, the mean time to the maximum fall in FEV1% predicted being found to increase with the decrease in tonicity (7.8 min with hypertonic solution, 19.2 min with isotonic solution and 34.2 min with hypotonic solution) [46].

However, this bronchoconstrictive effect might also be related and correlated to the severity of preexisting lung function impairment and to the existence of bronchial hypersensitivity [47]. In fact, in a prospective placebo-controlled crossover clinical trial, 75 mg colistin diluted in 4 ml normal saline solution or placebo solution of the same osmolarity was given via inhalatory route in CF patients who were classified into two groups according to their likelihood to develop bronchospasm: a high-risk (HR) and a low-risk (LR) group. The fall in FEV1 in HR group was 12% after placebo and 17% after colistin, whereas in LR group it was 9% after placebo and 13% after colistin. The proportion of patients experiencing a mean FEV1 fall of  $\geq 15\%$  after inhaling colistin was significantly higher in the HR group ( $p < 0.01$ ) as compared with LR group [48].

Inhaled colistin was suspected to exert a pro-inflammatory effect at the airways level [49]. In an *in vitro* study performed with sputum collected from CF patients with demonstrated *P. aeruginosa* colonization, colistin but not ceftazidime, gentamicin or tobramycin significantly stimulated the lytic activities of purified human neutrophil elastase and of *P. aeruginosa*-derived elastase, and this was due to the inhibition of counteracting smaller molecules. However, it might be possible that this stimulatory effect is rather a consequence of the presence of increased quantities of NE substrates such as albumin in these specimens, rather than the true enhancing action of colistin, because in a subsequent study, in which these findings were challenged, it was demonstrated that in fact colistin inhibited the activity of neutrophil elastase *in vitro* and this inhibitory effect was related to the absence of albumin [50].

Another issue is related to the potential hazards arising from the exposure of health-care workers to such formulations. This was demonstrated not only with the use of systemic platinum containing cytotoxic agents used in oncology but also with inhaled pentamidine or inhaled CS.

### 8. Expert opinion

The existing preclinical and clinical data support the use of inhaled colistin in lower respiratory tract infections, prophylaxis or therapy. Both CS or CMS can be used as inhaled formulations, but CMS is better tolerated than CS at the airways level and easier to be dosed. However, given CS, which is the real antibactericidal, its efficacy depends on the amplitude of CMS hydrolysis at the lung level and this is something that so far was not evaluated. On the other hand, the direct

CS administration in the airways is associated with airways irritation and with increased risk of other side effects.

The data discussed above suggest that inhaled colistin monotherapy can be used in certain settings to prevent chronic infection and in others only to treat the manifest infections. The main bacterial 'targets' are represented by *A. baumannii* and *P. aeruginosa* usually resistant to other antibiotics. In HAP/VAP, however, inhaled colistin monotherapy is not usually indicated and its association with a systemic antibiotic increases the chance of eradication and reduces the duration of antibiotic therapy. However, it is not known whether in patients with HAP/VAP with sensitive strains, inhaled colistin should be given after the initiation of systemic antibiotic therapy when signs of its limited efficacy are documented and resistance to systemic antibiotics is suspected, or concomitant systemic inhaled antibiotic therapy should be started. It is not known also whether inhaled colistin is able to penetrate in smaller caliber airways, which is expected for pneumonia, and which are the delivery devices meeting this requirement.

The discussed clinical studies used a dosage of 1 – 2 millions of IU of inhaled colistin given three or four times daily. However, it is not known whether, in this case too, colistin is inhibited or not by sputum, and achieving an acceptable bactericidal effect would require an increase in sputum levels above the MICs for systemic formulations.

The inhaled formulation of CMS, the most commonly used, is administered via nebulization, but about one decade ago, a dry powder formulation was also assessed. The fact ever since no new data have been reported might mean that the development of that formulation was abandoned for various reasons. However, the idea of giving colistin via a device different from a nebulizer still remains appealing and should be further ascertained.

To conclude, nebulized colistin might be an effective antibiotic to prevent chronic *P. aeruginosa* infection in CF patients and to treat, as a part of a combined regimen, the severe pneumonias with multidrug-resistant strains such as *A. baumannii* or *P. aeruginosa*.

### Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.



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