AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews

Inhaled Antibiotics for Gram-Negative Respiratory Infections

Eric Wenzler,[a] Dustin R. Fraidenburg,[b] Tonya Scardina,[c] Larry H. Danziger[a,d]

University of Illinois at Chicago, College of Pharmacy, Chicago, Illinois, USA[a]; Department of Medicine, Division of Pulmonary, Critical Care, Sleep and Allergy Medicine, University of Illinois at Chicago, Chicago, Illinois, USA[b]; Loyola University Medical Center, Chicago, Illinois, USA[c]; University of Illinois at Chicago, College of Medicine, Chicago, Illinois, USA[d]

(continued)

Published 25 May 2016

Citation Wenzler E, Fraidenburg DR, Scardina T, Danziger LH. 2016. Inhaled antibiotics for Gram-negative respiratory infections. Clin Microbiol Rev 29:581–632. doi:10.1128/CMR.00101-15.

Address correspondence to Larry H. Danziger, danziger@uic.edu.

SUMMARY

Gram-negative organisms comprise a large portion of the pathogens responsible for lower respiratory tract infections, especially those that are nosocomially acquired, and the rate of antibiotic resistance among these organisms continues to rise. Systemically administered antibiotics used to treat these infections often have poor penetration into the lung parenchyma and narrow therapeutic windows between efficacy and toxicity. The use of inhaled antibiotics allows for maximization of target site concentrations and optimization of pharmacokinetic/pharmacodynamic indices while minimizing systemic exposure and toxicity. This review is a comprehensive discussion of formulation and drug delivery aspects, *in vitro* and microbiological considerations, pharmacokinetics, and clinical outcomes with inhaled antibiotics as they apply to disease states other than cystic fibrosis. In reviewing the literature surrounding the use of inhaled antibiotics, we also highlight the complexities related to this route of administration and the shortcomings in the available evidence. The lack of novel anti-Gram-negative antibiotics in the developmental pipeline will encourage the innovative use of our existing agents, and the inhaled route is one that deserves to be further studied and adopted in the clinical arena.

INTRODUCTION

> **If I had asked people what they wanted, they would have said faster horses.**
> —Henry Ford

The concept of delivering therapeutic compounds directly to the respiratory tract has been around for thousands of years. In early mythology, Pythia, the Oracle of Delphi, inhaled emanations from the temple of Apollo. Claudius Galen, physician to the gladiators in Pergamon, instructed his patients to breathe fumes carrying sulfuric vapors from Mount Vesuvius. In history and medicine, Pedanius Dioscorides, the father of the science of pharmacy, initially prescribed inhaled sulfur vapors in the first century (1). It was not until 1932 that the word aerosol was coined by Whitlaw and Patterson based on "aer," meaning air, and "sol," meaning solution (2). Long before the adoption of this word, the process of delivering medicinal agents to the lungs via inhalation had been in use for decades in one form or another. From Native American shamans harnessing the psychotropic effects of *Datura* to physicians attempting to treat tuberculosis with inhaled iodine and sulfuric acid, aerosolized therapy has played a role in the treatment of pulmonary ailments long before the advent of the antimicrobial. Nebulized therapeutics have fallen in and out of favor throughout the decades, gaining a rebirth at the time of the First World War with the commercialization of ephedrine, which was nebulized through primitive devices akin to perfume vaporizers. World War II brought about major advancements in aerosol expertise owing to technological improvements related to chemical warfare. These evolutions improved the understanding of the qualities necessary for an inhaled agent to reach the lower airway, the site of therapeutic efficacy, and in 1947, Tiffeneau and Brun first reported the diameter of droplets necessary to avoid pharyngeal impaction (3). This progression paved the way for the use of inhaled antibiotics to treat acute and chronic bacterial infections of the airway. The 1940s and the late 1950s gave rise to attempts to aerosolize neomycin, polymyxin, and even penicillin G for patients with pneumococcal pneumonia (4, 5). The use of aerosolized antimicrobials eventually stagnated due to the lack of reliable delivery devices (6) and the conclusion from the 1975 article by Feeley et al. that "continuous use of polymyxin B aerosol appears to be a dangerous form of therapy" (7), a conclusion based on results observed with suboptimal methodologies and techniques for the administration of inhaled antibiotics. More recent technological advances occurring in the late 1990s and 2000s led to the introduction of tobramycin specifically manufactured for inhalation in patients with cystic fibrosis (CF) and chronic *Pseudomonas aeruginosa* colonization. Despite the revival of interest in aerosolized drug delivery, there remains a paucity of data on the appropriate formulation, optimal delivery device, pharmacokinetics (PK), pharmacodynamics (PD), safety, and clinical efficacy of the inhalation route for the treatment of non-CF patients.

Infections of the respiratory tract are some of the most common causes of human illness and the leading cause of death from infectious diseases worldwide (8). The lung is constantly exposed to the mixture of gases, particulate matter, and microbes that con-

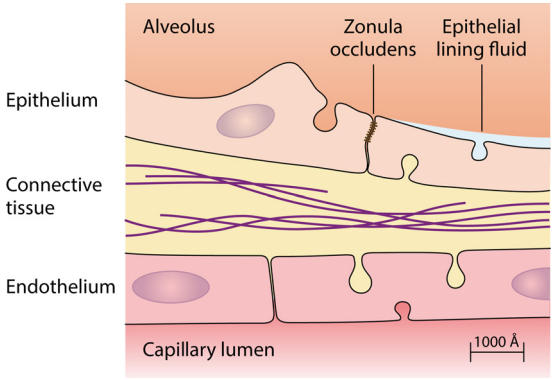


FIG 1 Representation of the alveolar capillary barrier. The barrier consists of three layers, of which the epithelium constitutes the least permeable layer because of the presence of numerous zonula occludens. Epithelial lining fluid lies in pools on the inside surface of the alveolus. 1,000 Å equals 100 nm. (Republished from reference 15 with permission.)

stitute inspired air. Pneumonia is most commonly defined as an infection of the lung parenchyma, or the portion of the lung involved in gas transfer (the respiratory bronchioles, alveolar ducts, and alveoli) (9). Pneumonia results from the proliferation of microbial pathogens at the alveolar level and the concomitant host response to these pathogens. In general, lower respiratory tract infections (LRTIs) are difficult to treat due to the sequestration of microorganisms deep within the airways, where only limited portions of drug gain access after traditional systemic therapy. Systemically delivered antimicrobials, in particular those used to treat LRTIs due to Gram-negative organisms (Gram-negative LRTIs), such as aminoglycosides and β-lactams, often have poor pulmonary penetration into the lung parenchyma (10). When hypoxia occurs in response to pneumonia, the pulmonary vasculature vasoconstricts in order to shunt blood away from areas of low oxygen toward areas maintaining adequate ventilation gas exchange (11). This shunting, as well as chemokine-induced inflammation, can further reduce the amount of drug that is systemically delivered to the lung parenchyma. In addition to the poor penetration of systemic antimicrobials, commonly encountered respiratory pathogens can escape the innate pulmonary defenses and avoid phagocytosis by alveolar macrophages (AMs) while surviving and proliferating in the epithelial lining fluid (ELF) (12–14). In the case of systemically administered antimicrobials, they must distribute to the alveoli and ELF from the blood, requiring them to pass through the alveolar barrier of the capillary lumen, connective tissue, and alveolar epithelial cells. Alveolar epithelial cells are a particular challenge given their connection via zonula occludens, which provide a barrier between plasma and ELF (Fig. 1). This barrier is also fortified by efflux pumps, including multidrug resistance protein 1 and breast cancer-resistant protein (15–18). Delivering antibiotics directly to the site of infection, the lung parenchyma, via inhalation could overcome the obstacles to pulmonary drug deposition faced by systemically administered antimicrobials.

Over 1 million people are admitted to hospitals in the United States each year for pneumonia (19). Gram-negative organisms account for about 11% of community-acquired bacterial pneumonia isolates and 33% of isolates in cases of nosocomial pneumonia (20, 21). Five of the top six bacteria that cause nosocomial infections are Gram-negative bacteria (22, 23), while *P. aeruginosa* represented nearly 25% of ventilator-associated pneumonia (VAP) isolates in one study, more than any other single bacterium (24). In the intensive-care setting, Gram-negative pathogens account for ~65% of pneumonia cases (25). Nosocomially acquired pneumonias due to Gram-negative pathogens, particularly *P. aeruginosa*, are difficult to treat and exceptionally problematic to eradicate, leading to high rates of recurrent infection despite adequate systemic antimicrobial therapy (26, 27). The consistent and alarming rise in the rate of antimicrobial resistance, particularly among these Gram-negative pathogens, represents a formidable threat to public health, and the demand for new antimicrobials is ever intensifying (28, 29). Although we have made tremendous strides in our knowledge of infectious diseases and our understanding of optimal antimicrobial therapy, the number of deaths due to drug-resistant bacteria continues to rise (8). Despite this, there has been a scarcity of novel antimicrobials available to fight this growing epidemic (30). The advent of life-saving technologies such as the mechanical ventilator has extended our ability to treat critically ill patients while also effectively inventing VAP, an infection associated with a 2- to 10-fold increase in mortality rates, affecting up to 20% of critically ill patients (24, 31). The reduction in the efficacy of our existing antimicrobials due to increasing bacterial resistance and the lack of new therapeutic agents have encouraged the innovative use of existing antibiotics.

It is well understood that effective antimicrobial therapy requires adequate drug concentrations at the target site of infection (32). To reach the deep airways in sufficient concentrations, high, often toxic doses of drugs would need to be given systemically. The inhalation of antibacterial agents allows higher concentrations to be deposited directly in the lungs so that pathogens are exposed to supralethal concentrations while minimizing potential systemic toxicity by limiting absorption and avoiding unfavorable PK/PD consequences (33–35). The large alveolar surface area (100 m^2) and thin epithelial layer (0.2 to 0.7 μm) of the lungs provide an advantageous environment for pulmonary deposition of inhaled compounds for which lung penetration after systemic administration is problematic (36, 37). Inhalation therapy has the capability of directly targeting the airways, creating increased and more sustained local concentrations and thereby increasing the therapeutic index, improving efficacy, minimizing toxicities, and decreasing the time of onset for the administered drug. Despite these theoretical advantages, practical issues concerning the use of nebulized drugs, in particular the ideal method of delivery, and an overall lack of robust clinical data have become hurdles to their widespread adoption (38). In addition, many clinicians rarely appreciate the complexities associated with inhaled therapy due in part to misconceptions based on inadequate techniques performed in the past, including instilling antibiotic solutions through the endotracheal (ET) tube (39, 40) and using existing parenteral formulations for inhalation (41–43). Finally, developing new inhaled products remains tremendously challenging due to the intricacies of particle engineering and the necessity for an effective drug-device combination.

This review provides an in-depth discussion of inhaled antibacterials in the treatment of non-CF patients with Gram-negative LRTIs. The use of inhaled antibiotics in CF patients is excluded from this work, as this topic was reviewed in depth recently (44). Formulation considerations, including particle characteristics and drug delivery systems, along with microbiological and *in*

vitro concerns, PK/PD parameters, and a review of safety and clinical outcomes are discussed. Throughout this review, the word "inhaled" is used to describe the pulmonary delivery of antibiotics, as it most accurately represents the physiological action required to deposit drugs into the lungs. Other words, such as "nebulized" and "aerosolized," are often used interchangeably, although they more accurately describe the characteristics and delivery mechanisms for inhaled antimicrobials. In addition, wherever possible, we avoid discussing studies utilizing intratracheal (i.t.) instillation of antibacterial solutions as a means of intrapulmonary delivery, as this method has shown nonuniform deposition in both animals and humans and is no longer commonly employed. Intratracheal delivery is mentioned as it applies to animal and human studies in which antibiotics are administered to the lungs without a delivery device but are sprayed or aerosolized in the lungs.

FORMULATION AND DRUG DELIVERY

Barriers to the delivery of inhaled antibiotics include the natural pulmonary physiology, administration techniques, tolerability, physical characteristics of the aerosolized particles, and specifications of the delivery device, among many others. Considerations for optimal pulmonary drug delivery include overcoming the inhibitory effect of sputum, rapid delivery to reduce treatment burden and increase patient convenience, and effective particle distribution to critical areas of the lungs.

Inhaled drug formulation considerations are extremely important when considering administering inhaled antimicrobial therapy to a patient with a Gram-negative LRTI. Even differences in chemical entities of the same compound can have important implications when utilized as inhaled agents, as has been shown with colistin (45), highlighting the importance of understanding the nuances of inhaled antimicrobials. In addition to the detailed considerations discussed below, drugs prepared and manufactured specifically for inhalation should be pyrogen free, isotonic, sterile, pH balanced to the airway epithelium (pH 6), and dispensed in unit-dose, single-use containers. Importantly, preservatives should be avoided if possible, as they have been specifically associated with adverse effects when inhaled.

This section focuses on the local delivery of inhaled antibiotics to the surface of the lungs and does not discuss issues related to achieving adequate systemic absorption via the inhalation route. (For a detailed review of this concept, see reference 46.)

Pulmonary Physiology

The natural physiology of the human pulmonary system makes efficient delivery of inhaled antibacterials to the target site of action extremely challenging. As air is inhaled through the mouth and nose, it passes through the larynx and the trachea and eventually passes through 16 bifurcations of rapidly dividing bronchi and bronchioles (Fig. 2). The alveoli begin at the 17th generation of bronchioles and end at the 23rd generation, transitioning from respiratory bronchioles to alveolar ducts and finally to alveolar sacs. Inhaled particles that are able to traverse the labyrinth of the upper airway are then deposited and efficiently transported out of the lungs by active mucociliary clearance prior to reaching the respiratory bronchiole and subsequent alveoli (46). The mucociliary elevator carries mucus covering the airways toward the mouth, where >500 ml of airway fluid can be swallowed daily. In addition, the air-facing sides of the lungs' ~500 million alveoli are each

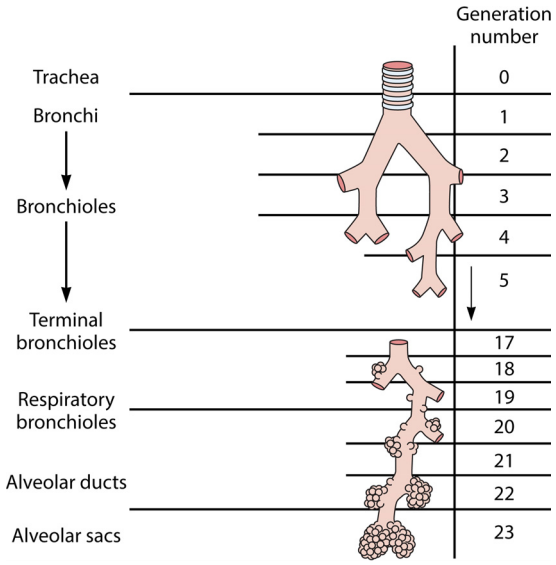


FIG 2 The airways branch roughly 16 to 17 times before alveolar sacs are encountered. The surface area of the human airways averages ~2 to 3 m^2, compared with roughly 100 m^2 for the alveolar surface. In the upper airways, the inertia of the larger particles causes them to break free of the streamlines of the flow and collide with a wall to be deposited. As impaction clears these larger particles in the upper airways, slightly smaller particles are filtered out of the airstream in the middle airways by gravitational sedimentation. Finally, for very small particles, particle motion is determined by Brownian diffusion, which accounts for the dominant mechanism of deposition in the alveolar region. (Reprinted from reference 46 by permission from Macmillan Publishers Ltd.)

policed by macrophages designed to phagocytize and digest any insoluble particles deposited there (47). The pattern of deposition of drugs in the lungs also changes as the internal landscape changes from thick-walled ciliated central airways to bronchioles and alveolar sacs. This armamentarium of innate pulmonary defenses designed to clear exogenous debris and bacterial microbes can also effectively eliminate antibiotic particles delivered via inhalation, particularly if these particles are not optimally formulated for this environment.

While the natural, healthy physiology of the lungs presents difficulties for developers of inhaled antibacterials, the pathophysiological changes in diseased lungs can be dramatic. These changes also make it challenging to extrapolate data from healthy volunteers or from CF patients to other disease states, as the distribution of inhaled antibiotics is uneven due to numerous factors, including areas of airway contraction secondary to edema, increased secretions, or smooth muscle constriction. This could in turn impact clinical outcomes by reducing the amount of drug available for distribution to the distal airway and lead to differences in outcomes observed in clinical evaluations (48). Future studies evaluating both the deposition and the safety and efficacy of inhaled antimicrobials are needed for patients suffering from Gram-negative LRTIs in order to properly formulate inhaled agents for the pulmonary physiological changes occurring in these disease states.

Drug Deposition Considerations

The efficacy of inhaled antibiotics correlates with the amount of drug deposited into the patient's lungs, which in turn depends on three main parameters: airway anatomy, patient ventilation, and

aerosol characteristics. The aerosol characteristics of the inhaled particle are the most modifiable factor and have the largest impact on satisfactory drug deposition. An inhaled medicine designed to penetrate deeply into peripheral lung regions rich in alveoli with ≥90% efficiency should be manufactured to deliver aerodynamic particles between 1 and 5 μm in size, known as the mean mass aerodynamic diameter (MMAD). The MMAD is the mean particle size produced by the combination of the medication and nebulizer (34). Particles that are ≤1 μm may be removed during exhalation, and particles that are ≥5 μm are deposited into the oropharynx (49). Particles of ≥5 μm may also rain out in the nebulizer circuit before reaching the upper airway. Therefore, deposition of particles into the lung parenchyma and alveolar space in humans occurs ideally when particles are between 1 μm and 5 μm (50–52). These optimally sized particles should be combined with low tidal breathing rates of at least 6 liters/min in order to achieve adequate delivery to the deep lower airway.

In order to utilize the potential of inhaled agents for their quick onset and high pulmonary concentrations, they must reach the appropriate target site and remain there in therapeutically significant concentrations for a sufficient time period. This concept is beginning to be appreciated and studied as it pertains to systemically administered antibiotics, but there are very limited data about how this process occurs with regard to inhaled antimicrobials.

Optimal properties of a drug that is designed to be systemically absorbed and active when administered via the inhaled route are low molecular mass, hydrophilicity, and a net negative charge (46). For antibacterial agents, the goal is to maintain drug concentrations within the lungs and on the appropriate pulmonary surface in order to combat bacterial pathogens that reside there during infection. This means that these drugs need to have the opposite properties of those of compounds developed specifically for systemic activity after inhalation (i.e., inhaled insulin). The ideal inhaled antimicrobial would therefore be manufactured to be lipophilic and positively charged and to have a high molecular mass. In addition, inhaled compounds intended for local treatment should also have high first-pass hepatic elimination to circumvent systemic effects after oral uptake of the inhaled dose deposited in the mucosal cavity and swallowed. These properties, balanced against an appropriate particle size, would allow optimum antibacterial efficacy of an inhaled antibiotic within the lung parenchyma and maximization of the agent-specific PK/PD index by both providing high local concentrations and avoiding immediate diffusion into the bloodstream and removal by lung defenses.

Particle Engineering

There are several methods available to manufacture inhalable particles, including micronization, precipitation, freeze-drying, and spray-drying. To obtain fine drug powders in the appropriate size range for optimal lung deposition, jet milling has been traditionally used by the pharmaceutical industry. However, jet milling often produces cohesive particles due to the high surface energies of these milled particles. A carrier powder can be added to improve flow, although this increases the overall powder volume, the number of inhalations, and the time needed to receive a dose. Spray-drying has become the state-of-the-art method for engineering aerosolized particles, as it is a one-step, high-throughput process able to engineer particles in a much more tightly controlled manner. The process of spray-drying allows optimization of particle size, size distribution, and surface morphology (53). This advanced technique has allowed consistent aerosol performances across temperatures, humidities, and airflow rates (54).

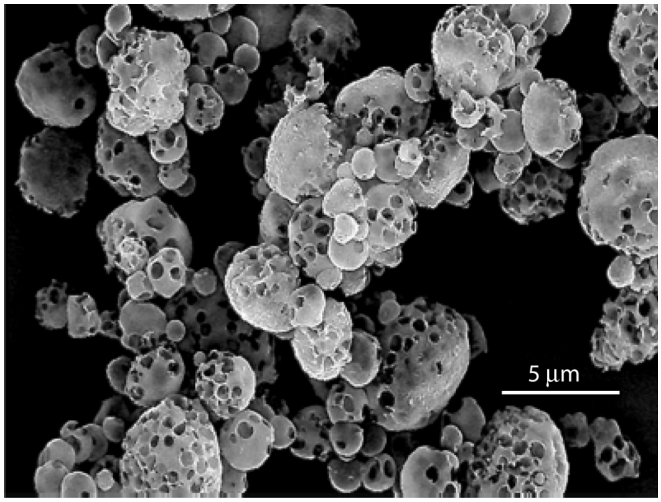


FIG 3 Scanning electron microscope image of tobramycin inhalation powder. (Reprinted from reference 56 with permission of the publisher. Copyright 2015 American Chemical Society.)

A recent example of the advancements made in particle engineering comes in the form of the Pulmosphere technology developed for tobramycin inhalation powder (55). Briefly, Pulmosphere particles are manufactured by rapidly evaporating an atomized liquid stock with a heated gas to form a spray-dried powder. The key to this process is the formation of the emulsion-based feedstock, which is an oil-in-water emulsion stabilized by a surfactant. High-pressure homogenization is then used to form submicrometer emission droplets, which are then mixed with a drug annex solution comprised of an aqueous solution of free-base tobramycin sulfate. This same process has also been completed by using organic alcohols such as methanol in order to decrease surface tension, minimize residual water content, and further decrease particle size (53). The culmination of mixing this feedstock with hot air and atomizing leaves small porous particles with tobramycin concentrated at the core of the particle and the excipients within the outer walls. The result of this highly advanced particle engineering process also allows improved control over the particle surface properties. By intentionally manufacturing the morphology of these particles, the outer hydrophobic surfaces can be used to decrease cohesive forces between the individual particles. This improves aerosol performance and eliminates the need for lactose carriers. A high drug load can be achieved with these specifically designed particles, enabling higher doses of drug per inhalation and better lung deposition. Additionally, this formation of tobramycin inhalation powder into an amorphous solid improves the physiochemical stability of the powder over those of other crystalline powder formulations (Fig. 3). (For a more in-depth discussion, including the methods used to characterize the tobramycin inhalation powder on the particle level, see reference 56.)

Although small aerosol particles (<1 μm) are usually exhaled during breathing, nanoparticles (~100 nm) are able to be deposited in the alveolar space by sedimentation due to an accumula-

tion process in the lung. Nanoparticles are colloidal particles ranging from 50 to 500 nm in diameter that are often encapsulated in liposomes made from phospholipid bilayers than can entrap both hydrophilic and lipophilic drugs in the core and bilayer, respectively. Polymeric nanoparticles have also been explored, although they typically involve the use of organic solvents in order to dissolve the polymers. These nanoparticles are able to persist in tracheobronchial secretions for longer periods of time, improving the local exposure of the delivered antimicrobial agent. The key benefits of nanoparticle-based antibiotic formulations include prolonged retention at the target site and decreased systemic exposure (57, 58). (For a detailed discussion on nanoparticle formulations, see reference 59.)

Surface coating of fine particles with force control agents such as magnesium or sodium stearate can also improve flow and aerosolization. (For a detailed discussion of this and other finer aspects of particle considerations, see reference 60.)

Many inhaled antibiotics are hydrophilic and have a low molecular mass, making them easily systemically absorbed and eliminated after inhalation (61). Low solubility (high hydrophobicity) is required to decrease the dissolution rate and prolong lung surface contact before systemic absorption. Therefore, in order to maintain high drug concentrations within the lungs, liposomal formulations have been explored. These liposomal formulations are equivalent to systemic sustained-release products, allowing prolongation of drug concentrations at the target site, which leads to enhanced killing of bacteria and a reduced dosing frequency for the patient. Using liposomal or encapsulated formulations to develop controlled-release particles can also increase residence time and improve the ability to target intracellular organisms as liposomes are engulfed by macrophages (62).

Although diffusion is minimized by formulation within a liposome, the particle is then increasingly exposed to removal by the lungs via mucociliary clearance and phagocytosis by alveolar macrophages. In healthy lungs, mucociliary clearance can transport mucus containing the drug upward at a speed of 20 cm/h, clearing 80% of undissolved particles within 24 h (51). In patients undergoing intubation via a cuffed endotracheal or tracheostomy tube, this mucociliary clearance may be markedly impaired (63), resulting in impaired clearance of bacteria and particles, including drugs. Despite this natural lung defense, currently available liposomal formulations, such as liposomal amphotericin B, have been shown to persist in the lungs for several days after inhalation (64). The potential long-term deleterious effects of the prolonged persistence of these molecules on lung tissues are not known. In addition to liposomal particles, polymeric formulations have also been explored, although their extended drug release time (>24 h) makes them particularly susceptible to mucociliary clearance and phagocytosis.

In addition to altering hydrophobicity, charge can play a key role in the diffusion of inhaled antimicrobials from the lung parenchyma into the bloodstream. Ideally, particles should be nonnegatively charged, since positively charged molecules, such as the polycation tobramycin, bind to lung tissue and are slowly absorbed (65).

Finally, increasing the molecular mass of the inhaled particle to help slow diffusion has also been explored. This strategy is achieved by conjugating the antibiotic with a water-soluble inert ligand such as polyethylene glycol. This approach has proven to be inefficient due to the absorptive capabilities of the lungs. Compared to the gastrointestinal tract, which is limited to absorption of molecules of <600 Da, the lung's epithelial surface is capable of utilizing transport mechanisms to absorb molecules as large as 160,000 Da (46). The very high molecular masses required to slow diffusion through the lungs dramatically increase the powder volume needed to be inhaled, in turn decreasing the ease of administration and patient compliance.

Delivery Devices

It was not until the 1990s that investigators began to determine the actual amount of drug able to be delivered by compressed-air nebulization. With most conventional commercial nebulizers not specifically being designed for inhaled antimicrobials, the fraction of the dose emitted can be quite small. It is now understood that there is great variability in the amounts of drug delivered through different drug delivery devices, yet most hospitals today still use disposable nebulizers that were designed in the 1950s. While drug manufacturers and particle engineers are able to design compounds and particles for optimal lung deposition, as clinicians, our ability to optimize inhaled antibiotic therapy lies primarily in the choice of delivery device.

Inhaled medications consist of a triad of a drug, a formulation, and a delivery device specifically engineered to guarantee accurate delivery of a dose to the lungs. There are several types of delivery devices: soft-mist nebulizers (jet, vibrating mesh, or ultrasonic), pressurized metered-dose inhalers (MDIs), and dry-powder inhalers (DPIs) (66, 67). Like any pharmaceutical product, an inhaled agent must conform to two primary categories: pharmaceutical performance and quality (dose reproducibility, manufacturability, and stability) and clinical performance (safety and efficacy). An ideal inhalation product should be uncomplicated and portable, need minimal cleaning, deliver the same dose and flow rate consistently, and propel a significant fraction of the delivered dose to the lung. Achieving adequate lung deposition after inhalation from any drug delivery device is challenging. As opposed to systemic agents, the dose delivered via inhalation is usually very small, down to micrograms, and highly dependent upon patient factors such as appropriate inhalation techniques. Multiple dose measures must be considered when dealing with inhaled antimicrobials, that is, the lung-deposited dose, the delivered dose, and the metered dose. The metered dose is the dose designed to be expelled from the device, while the delivered dose is the actual dose that escapes the device. The lung-deposited dose is the dose that reaches the lung past the mouth and throat and is typically estimated through inhalation of a radiolabeled tracer followed by gamma scintigraphy or positron emission tomography (68).

When developing delivery devices for inhaled compounds, manufacturers must consider particle size, drug distribution at variable flow rates, the volume of powder needed to be inhaled, and the number and complexity of manual manipulations required by the patient to use the device correctly. The intricacy of aerosol delivery can be summed up by the need to quickly convert formulations into fixed-dose aerosol clouds with optimal delivery properties efficiently and in phase with inhalation and then deliver these clouds in a minimum number of inspirations. Adding commercial stability requirements, cost restrictions, and device portability makes the task of developing these drugs an immense undertaking.

Nebulizers. Three types of nebulizers to administer inhaled an-

tibiotics are currently available. Jet nebulizers produce an aerosol when compressed gas is forced through a small hole into an adjacent reservoir containing medication in solution. Advantages to jet nebulizers are their low cost, efficiency, and disposability. In addition, large particles within the reservoir condense on the top of the chamber and drip back into the reservoir, minimizing medication waste. Disadvantages include wide variability in performance between manufacturers and lengthy administration time.

Fill volume, airflow and pressure, the choice of continuous or intermittent use, placement within a ventilation circuit, solution properties, and the use of a spacer can all affect output from a jet nebulizer (69, 70). The Pari LC Plus jet nebulizer is currently the only device recommended in the European CF Society's consensus document for the administration of inhaled tobramycin, as it is the nebulizer that was used in pivotal trials. Pari LC Plus is a reusable jet nebulizer, which means that it requires cleaning, necessitating disassembly and reassembly. Single-use, disposable jet nebulizers have been developed to eliminate the need for cleaning and to reduce the risk of bacterial contamination. When disposable and reusable nebulizers were compared in both *in vitro* and *in vivo* models, the Pari LC Plus jet nebulizer demonstrated the highest mass of tobramycin deposited in the lungs, performing better than both of the disposable nebulizers (71).

The need for a power source and long treatment times, the required setup and cleaning, significant variations in performance between manufacturers, and the loss of expensive drug in considerably high residual volumes are significant drawbacks to jet nebulizers (72–75). *In vitro* data have demonstrated that drug delivery from a jet nebulizer can be optimized via operation with a nitrogen-oxygen mixture with particles entrained in a helium-oxygen operating circuit (76).

Ultrasonic nebulizers use a piezoelectric crystal that vibrates at high frequency to break the medication into a microscopic fog, resulting in aerosolization. Medication output is therefore directly proportional to the crystal's vibration amplitude, while droplet size is inversely proportional to vibration frequency. The output is additionally affected by the source of flow gas used to carry the aerosol. Advantages of ultrasonic nebulizers include consistency, efficiency, and short administration time. Disadvantages include cost, routine cleaning, and potential deleterious effects on the stability of medications due to the heat generated by vibration. In comparison to jet nebulizers, ultrasonic nebulizers utilize a slightly larger particle size but have a high rate of nebulization and a short operating time.

Finally, vibrating-mesh or -plate nebulizers use a vibrating mesh or plate to pump liquid droplets through multiple tapered apertures to produce aerosol particles. In this machine, the size of the particles is determined by the diameter of the holes in the mesh or plate. Modern vibrating-mesh nebulizers have a higher rate of nebulization than, and 2 to 3 times the drug output of, jet nebulizers (77, 78). Advantages of this device include less heat and consistency in particle size. Disadvantages include the potential for obstruction and damage to the nebulizer through the use of a highly concentrated or viscous solution. Vibrating-mesh nebulizers also do not alter the temperature of the solution like jet nebulizers, which can adversely affect the stability of the inhaled drug (79).

All nebulizers suffer from the contribution of humidity and its ability to increase the hygroscopic growth of aerosolized particles, potentially causing them to impact or rain out of the circuit (80). Renewed attention to the scientific foundation of aerosol therapy and the increased understanding of the relationship between the amount of drug deposited in the lower airways and the corresponding therapeutic response have led to improvements in drug delivery devices. Modern delivery devices are able to achieve lung deposition fractions of up to 50%, compared to the 10 to 15% attained previously (81). Nebulizers typically generate aerosol during the entire respiratory cycle of the patient, leading to significant drug loss during exhalation. Newer breath-actuated jet nebulizers counteract this issue. Even more novel nebulizers utilize electronic control systems to personalize aerosolizations to the individual patient's breathing pattern. These nebulizers are known as adaptive aerosol delivery (AAD) systems, such as the Activareo Akita Jet system and the Philips Respironics iNeb system. Although these systems have not yet been used for antimicrobials, they have been shown to shorten treatment time and improve lung deposition of therapeutic pharmaceutical agents.

Furthermore, nebulization devices are available for the sinonasal inhalation of antibiotics in the treatment of upper airway colonization. The Pari Sinus vibrating-mesh nebulizer has successfully been used in this fashion to effectively reduce the quantity of *P. aeruginosa* bacteria and increase quality of life while maintaining undetectable serum concentrations in patients with CF (82).

Inhalers. The use of nebulizers requires disassembly and cleaning of after each dose and extended administration times, causing a significant treatment burden to those patients using this method long term. MDIs are small, portable devices typically used to deliver aerosolized formulation of β-agonists, anticholinergics, and corticosteroids (83). Limitations of MDIs include difficult administration techniques and high particle exit velocity (83). Other factors to consider are the small quantity of medication delivered (<1 mg per puff), the need for the medication to be stable in a multidose canister, and compatibility with the propellant (83). Because of these issues, MDIs are not typically manufactured to administer antibiotics (84, 85). Similar to MDIs, DPIs are small and portable, do not require extensive cleaning after use, and are disposable (83, 84). In DPIs, the dry formulation of the medication is enclosed in a capsule, which is punctured and then inhaled into the lungs (84). DPIs are smaller and portable, take less time to use, and do not require special cleaning. The decreased administration time and lack of a need for a power supply improve the freedom and portability for patients using this delivery device. However, this DPI method requires good inspiratory effort, which may not always be possible for patients with advanced lung diseases (51). In addition, patients need to be adequately educated on the appropriate use of these devices, as lung deposition is highly dependent on the inhalation technique (84). Finally, for antibiotics, the loading dose within the capsule ranges from 20 to 150 mg, and multiple capsules are often required for inhalation of a sufficient amount of antibiotics into the lungs (84).

The introduction of the Tobi Podhaler provides an example of an appropriately designed and manufactured DPI. The Tobi Podhaler combines three key elements of an efficient DPI: a powder specifically engineered for use in a DPI, a hard-capsule package containing the drug, and a drug-specific inhalation device (56). In this system, the tobramycin inhalation powder is packed into hard-capsule shells that are individually packaged into blister packs. A single dose is delivered via puncturing and inhaling four capsules of tobramycin inhalation powder, and

TABLE 1 Brief overview of the properties of inhalation devices

Device	Advantages	Disadvantages
Jet nebulizer	Delivery independent of inspiratory effort, able to deliver high doses without reloading	Large device, disassembly and cleaning required after each dose, complicated to use, long delivery times, low-efficiency delivery
Ultrasonic nebulizer	Consistent dose delivery, faster delivery time than jet nebulizers, more efficient dose delivery than jet nebulizers	Expensive, disassembly and cleaning required after each dose, heat generated may damage medication
Vibrating nebulizer	Smaller than jet nebulizers, faster delivery time than jet nebulizers, more efficient dose delivery than jet nebulizers	Large size, expensive, disassembly and cleaning required after each dose, some suspensions may clog mesh holes
MDI	Handheld, multidose, activity independent of inspiratory effort	Difficulty coordinating actuation and inhalation, typically requires shaking and priming actuations, ill suited for high-dose delivery
DPI	Handheld, most are multidose, no disassembly required for cleaning, dose delivered upon inspiration	Dose delivery dependent on good inspiratory effort, requires manual refill prior to each dose

efficiency is maintained through proprietary technology used to engineer the powder.

In addition to the ease of use compared to nebulizers, inhaled antibiotic powders have demonstrated better outcomes than inhalation solutions. For example, inhaled tobramycin powder has been shown to improve adherence, decrease the need for adjunct intravenous (i.v.) antibiotic courses, and improve patient preference compared to a tobramycin inhalation solution in adult patients with CF (86). In contrast, an older study showed that tobramycin inhalation powder achieved similar reductions in *P. aeruginosa* density and increases in lung function (percent forced expiratory volume in 1 s [FEV_1]) compared to the solution but caused more coughing and had a higher discontinuation rate. Despite this, subjective treatment satisfaction was higher, likely owing to the almost 4-fold-shorter administration time for tobramycin inhalation powder (87). Although the administration time may be reduced, more adverse events have been reported with the use of DPIs than with nebulizers due to the need for rapid inhalation of highly concentrated solutions or powders that may cause coughing due to the sheer volume of inhalation or changes in the osmotic environment of the airway. Safety challenges with smaller doses should be considered first before administering the full dose, and pretreatment with a bronchodilator has been shown to reduce the incidence of bronchospasm (88).

DPIs can represent an option for transition of care for patients being treated using a nebulizer while undergoing mechanical ventilation in a hospital. Much like transitioning from i.v. to oral systemic antibiotics, patients can be switched to a DPI for administration in the hospital or as an outpatient. Unfortunately, not all inhaled antibiotics are available as a DPI. Tobramycin and colistin are available as both inhaled solutions and powders, while aztreonam is available only as a solution. As mentioned above, the formulation used for inhalation administration is extremely important, and different formulations of the same drug are not interchangeable. For example, aztreonam lysine is designed for inhalation, whereas the i.v. form of aztreonam contains arginine, which has been associated with pulmonary inflammation after long-term inhalation (89). Table 1 shows a summary of the major advantages and disadvantages of commonly used inhaled delivery devices. (For a comprehensive review, see reference 66.)

Administration Technique

An appropriate administration technique for inhaled antibiotics is essential for achieving therapeutic concentrations within the respiratory tract. Optimally, an inhaled antibiotic should be administered during a slow and deep inhalation (84). This increases the probability that larger particles containing more drug will bypass the upper airways and be distributed into the smaller airways (84). To decrease the risk of cough or bronchoconstriction, the composition of inhaled antibiotics should include an osmolarity of between 150 and 1,200 mosmol/liter (90–93), and normal saline should be used as the diluent (90–92). The drug should be diluted in a volume that fills the nebulizer (94–96). Patients and their family/caregivers should be adequately trained on how to properly administer antibiotics via the specified delivery device and how to clean it properly. As only ~10% of the nominal dose is actually delivered to the lungs by any of the delivery devices discussed, the room for technique error is uncomfortably low.

IN VITRO, PK/PD, AND MICROBIOLOGICAL CONSIDERATIONS

Inhaled Antibiotic Admixtures

Multidrug-resistant (MDR) Gram-negative pathogens often develop resistance via a variety of different resistance mechanisms. As such, it is rare for a single antimicrobial agent to have the ability to neutralize or avoid all of these resistance mechanisms. Combination antimicrobial therapy offers a powerful means of combating these MDR pathogens by decreasing the probability that a given pathogen may develop resistance to all the antibiotics in a given combination (97). In addition, antimicrobial combinations may provide synergistic activity to further enhance the bactericidal activity and improve the rate of killing over those attainable with individual agents. Utilizing antimicrobial combinations to combat MDR Gram-negative pathogens has become commonplace with systemic agents (98) and is beginning to be explored as it applies to inhaled antibiotics.

Several combinations have been explored, including combining multiple antimicrobial agents and combining antibiotics with agents that reduce mucus viscosity, such as dornase alfa. Mixing of drugs for simultaneous nebulization is also commonly done by patients with CF to limit the frequency and time required for treatment (99). The compatibility of these admixtures is mostly unknown with respect to antimicrobials. In some studies, combinations of antibiotics such as tobramycin have been shown to be incompatible with dornase alfa due to bisulfite excipients used in specific products. This incompatibility often results in the loss of activity of one of the two compounds, which could obviously have

deleterious effects on treatment outcomes (100). More recently, the stability of neither dornase alfa nor tobramycin (Bramitob or Tobi) was affected by admixing them for up to 24 h, potentially allowing simultaneous nebulization. Although dornase alfa has been used primarily in the treatment of CF, it has been explored for other disease states with impaired mucus clearance in which antimicrobials could also be used (101).

The majority of work examining drug admixtures has focused on nebulizer solutions. Several studies have attempted to combine antimicrobial agents in DPI formulations but have failed to demonstrate synergy *in vitro* (102, 103). A recent study examined the combination of ciprofloxacin and gatifloxacin and the combination of ciprofloxacin, gatifloxacin, and the naturally occurring mucolytic lysozyme against Gram-negative respiratory tract pathogens *in vitro* (104). The combination of ciprofloxacin and gatifloxacin demonstrated synergy against *P. aeruginosa* but only indifferent activity against *Klebsiella pneumoniae* and *Acinetobacter baumannii*. The combination of colistin and rifampin has been shown to be synergistic *in vitro* and has been explored systemically *in vivo* in patients with serious infections due to *A. baumannii* (105). When combined in a dry-powder formulation via spray-drying of rifapentine particles suspended in an aqueous colistin solution, the combination produced enhanced antimicrobial activity against both planktonic and biofilm cultures of *P. aeruginosa in vitro*. The combination showed high aerosol performance, and the addition of rifampin to the surface coating contributed to the moisture protection of colistin by minimizing contact between hygroscopic colistin particles (106). This same co-spray-dried combination of colistin and rifampin has also shown *in vitro* synergy against *A. baumannii* (107). The combination product of fosfomycin and tobramycin has been explored clinically in a phase II trial in CF patients with chronic *P. aeruginosa* airway infections (108). A phase II study evaluating the efficacy and safety of the combination of inhaled amikacin and fosfomycin in mechanically ventilated patients with Gram-negative pneumonia is currently recruiting patients (ClinicalTrials registration number NCT01969799). Several other combinations targeted at patients with CF are being explored *in vitro* (109).

Nebulized combinations such as the combination of tobramycin and clarithromycin have been considered for use in CF patients (110). Tobramycin has long been the inhaled antibiotic of choice for treating lung infections due to *P. aeruginosa* in patients with CF, while macrolides such as clarithromycin have been shown to be effective immunomodulators, bactericidal enhancers, and suppressors of virulence factors of *P. aeruginosa* (111–113). Given these factors, deposition of both of these agents simultaneously in the lungs of patients with infections due to *P. aeruginosa* could be an optimal therapeutic strategy. In a study examining the deposition of spray-dried tobramycin inhalation powder with amorphous clarithromycin present in the particle coating, identical depositions of the two drugs were achieved, lending credence to this combination idea (110). In addition, the coformulation of azithromycin and colistin into liposomes allows colistin to permeate the liposome and accelerate the rate of azithromycin release from the liposome (114). This research may eventually lead to the ability to utilize colistin to tailor the rate of release of other agents from liposomes after inhalation, potentially allowing less frequent inhalation dosing.

Other unique combinations have also been explored. A novel steroid-antibiotic dry-powder formulation consisting of ciprofloxacin and beclomethasone was developed via spray-drying (115). The combination of ciprofloxacin and beclomethasone showed increased drug release and a high fine-particle fraction compared to those of the individual agents. The combination also had good activity against *P. aeruginosa* and *K. pneumoniae in vitro*. Combining inhaled anti-infective and anti-inflammatory agents could hold promise for future formulations, as corticosteroids have been shown to benefit patients with pneumonia and exacerbations of obstructive airway diseases when used in combination with systemic antibiotics (116, 117). Iron has been shown to play an essential role in the formation of biofilms by Gram-negative pathogens (118). A recent *in vitro* investigation examined the effect of combining an iron-binding glycoprotein and the bactericidal agent hypothiocyanite (ALX-109) with tobramycin or aztreonam on biofilm production by *P. aeruginosa*. The combination of ALX-109 and tobramycin or aztreonam reduced biofilm formation and disrupted established biofilms on CF airway epithelial cells. Importantly, ALX-109 reduced the concentration of tobramycin required to eradicate *P. aeruginosa* biofilms by 5-fold (119).

A novel combination of an antibiotic and compounds exhibiting mucolytic properties and the ability to suppress quorum sensing has been investigated. Lee et al. combined ciprofloxacin and gatifloxacin with ambroxol hydrochloride to investigate antimicrobial synergy along with quorum quenching, mucoactive properties, and pulmonary protective effects of ambroxol (120). This combination showed adequate lung deposition that was higher in the triple combination than with any agent alone while also significantly increasing microbiological activity against *P. aeruginosa* in artificial sputum medium when ambroxol was added to the antibiotic combination.

Despite the lure of inhaled combination therapy, it is important to note that not all antibiotics are compatible and stable in the presence of an admixture (121). Antimicrobial agents without reported data confirming their stability and compatibility should not be mixed in a nebulizer solution for use as combination therapy.

Pharmacokinetics and Pharmacodynamics of Inhaled Antibiotic Compounds

The efficacy of an inhaled antibiotic compound is difficult to assess in an *in vitro* or PD system. Unlike systemic antimicrobials, *in vitro* and/or PK/PD data are less easily attainable and translatable as they pertain to inhaled antibiotics. Serum concentrations cannot be linked directly to target concentrations on the surface of the lung or in the ELF, and it is nearly impossible to accurately estimate the concentration-time profile in the lungs, as the concentrations cannot be directly or practically measured. Therefore, establishing the link between different pharmaceutical properties and clinical performance is extremely complex compared to systemically delivered agents. Although multicompartment models have been developed to model the variability and mucociliary clearance of inhaled corticosteroids (122), similar models as they relate to inhaled antimicrobials are scarce. These difficulties culminate in a lack of reliable and accurate preclinical data with which to move forward to animal and/or human studies. From a drug approval process standpoint, this creates tremendous problems for pharmaceutical companies attempting to navigate the regulatory system and move to phase II and III trials without the necessary *in vitro*, PK/PD, and animal data that agencies such as

the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) are accustomed to seeing with systemic antimicrobials.

Correlation of the *in vitro* and *in vivo* activities of a drug is crucial if *in vitro* methods are to be used to predict performance. In addition, realistic testing conditions that mimic different patient scenarios are also necessary if these models are to be used to forecast clinical outcomes. Unlike models evaluating systemically administered agents, this testing environment involves simulating the oropharyngeal geometry, matching inspiratory flow rates in different patient populations and disease states, and considering different delivery devices and individual patient inhalation techniques, among many other factors. In addition, the concentration-time profile that has become so familiar within PK modeling of systemic antimicrobials is complicated as it applies to inhaled antibiotics by aerosol deposition, particle dissolution, permeation into lung tissue, binding, and transfer to the systemic circulation. Each component within this equation is further complicated by specific variables. For example, aerosol deposition of the inhaled product depends on the patient-specific respiratory tract physiology, product-dependent variables such as the emitted dose, particle size distribution, and device resistance.

In simple cases, solutes administered to the lungs follow what would be similar to a one-compartment plasma model. Absorption occurs in a dose-dependent, first-order fashion, with no evidence of binding or metabolism. In the case of nonmetabolized drugs that are absorbed following first-order kinetics, sequestration to lung tissue occurs and can be modeled similarly to a two-compartment model where the second compartment is of "bound drug" and association and dissociation constants are calculated along with rates of transfer to the systemic circulation. Variables that must also be considered in the inhalation PK model include the difference between the dose administered and the absorbable amount, i.e., subtracting the dose lost in the inhalation process, and the amount of drug that is systemically absorbed versus bound to tissue. Advanced modeling techniques such as the one-dimensional algebraic approach, International Commission on Radiological Protection (ICRP)-96, and computational flow dynamic methods have been developed to improve predictions of aerosol deposition on lung surfaces (123, 124). Several studies have also attempted to create *in vitro* models, not specifically with antibiotics, to simulate the clinical use of inhaled antimicrobials along with a mass-balance-type technique in patients receiving mechanical ventilation (95, 125–128).

In addition to the lack of adequate *in vitro* approaches, there is no clear consensus on the site of action within the lungs that is most predictive of an optimal PK/PD response. Reported studies have evaluated PK/PD parameters in respiratory sputum, tracheobronchial secretions, and ELF and have often arrived at different conclusions. Moreover, the residence time in and rate of clearance of these compounds from the lungs are difficult to assess in humans but are essential to our understanding of how these agents should be dosed. For time-dependent drugs such as β-lactams, it is imperative to know the rate of clearance of the agent from the lungs in order to maximize the known PK/PD index of antibacterial efficacy for these agents. This rate is often not known, and dosing regimens are based arbitrarily on systemic doses or those used in animal studies without proper allometric scaling. Furthermore, different PK/PD targets may exist for the same agent when used for inhalation compared to systemic administration. For example, maximal tobramycin concentrations need to exceed 25 times the pathogen MIC to overcome the inhibitory effect of sputum in CF patients (129), compared to 10 times the MIC in human plasma.

Microbiological Considerations

As discussed above, antibiotics developed for inhalation need to be designed and formulated specifically for this purpose. This manufacturing process may lead to differences in the preparations of the drug, and these alterations may have an impact on the intrinsic antibacterial efficacy of the compound and therefore should be explicitly tested *in vitro* for their microbiological efficacy. In the only head-to-head comparison of different inhalation formulations, the *in vitro* efficacies of Bramitob versus Tobi inhalation solutions were tested against *P. aeruginosa* isolates collected from patients with CF. The mean MIC values of the two formulations were similar, at 0.42 and 0.45 mg/liter (130). The above-discussed combination of amikacin and fosfomycin designed for inhalation in patients with Gram-negative pneumonia has been tested extensively *in vitro* in order to solidify the bactericidal activity of this fixed-dose combination prior to entry into clinical trials. The amikacin-fosfomycin inhalation system (AFIS) is a fixed-dose combination of 300 mg of amikacin and 120 mg of fosfomycin administered via the Pari eFlow Inline nebulization system. This combination of amikacin and fosfomycin in a 5:2 ratio has been shown to significantly increase the potency of amikacin against amikacin-nonsusceptible Gram-negative isolates (131). *In vitro* checkerboard synergy assays demonstrated no antagonism between the amikacin-fosfomycin combination and other Gram-negative antibacterials commonly used to treat pneumonia, including aztreonam, cefepime, and meropenem (132). A 4:1 fixed-dose combination of fosfomycin and tobramycin has also been explored in *in vitro* synergy assays and an *in vivo* murine pneumonia model (133).

These combination agents allow increases in the antibacterial spectrum, a reduction in the required dose of aminoglycoside and therefore an improved safety profile, synergistic and mutant prevention activities, and finally an additive effect of combining agents with different PK/PD indices.

Finally, the currently approved and accepted MIC breakpoints for antibiotics used to treat Gram-negative LRTIs are developed in part from achievable plasma concentrations after systemic administration. These concentrations are not representative of the concentrations achieved at the target site of infection, the lung parenchyma, after either systemic or inhaled administration. Inhaled administration of antimicrobials achieves exponentially higher lung concentrations than administration of the same agent via the systemic route. This means that antibiotics administered via the inhalation route may still achieve the required PK/PD index of antibacterial efficacy even if the target pathogen is reported to be resistant by using conventional interpretations. For example, lung concentrations of gentamicin after systemic and inhaled administrations were $<$1.0 mg/liter and 400 mg/liter, respectively. A maximal effect of aminoglycosides is demonstrated when the achieved maximum concentration of drug in serum (C_{max}) is at least 10 times the MIC of the infecting pathogen. Therefore, in this example, an isolate of *P. aeruginosa* with an MIC of up to 40 mg/liter could be treated via inhalation, but only an isolate with an MIC of up to 0.1 mg/liter could be treated via systemic therapy. The vast majority of qualitative interpretations used by clinical microbiol-

ogy laboratories for susceptibility reporting are developed in relation to systemically achievable concentrations after parenteral and/or oral administration of an antibiotic and do not consider the high local concentrations obtained when inhaled therapy is used. The Spanish Antibiogram Committee has recommended specific breakpoints for inhaled tobramycin against *P. aeruginosa* that differ from breakpoints for systemic therapy (134). This group recommends that the resistant breakpoint be set at ≥128 mg/liter, compared to the Clinical and Laboratory Standards Institute (CLSI) resistance breakpoint of ≥16 mg/liter. In one study comparing the susceptibilities of *P. aeruginosa* isolates obtained from CF patients to tobramycin based on these different breakpoints, an additional 14% of isolates would have been deemed susceptible by using the Spanish Antibiogram Committee breakpoints (135). Reporting of these isolates as being resistant to inhaled tobramycin based on plasma-derived breakpoints may force clinicians to avoid inhaled administration unnecessarily when it is likely to still be effective and when few other options exist. In the future, organizations like the CLSI should adjust breakpoint interpretations to be specific for the site and route of administration in order to assist clinicians in making decisions about the utility and effectiveness of alternative routes of administration.

A recent study examined the pharmacodynamic profile of amikacin as the inhalation product BAY41-6551 in concentrations representative of those achieved in two phase II trials (INHALE 1 and 2) against organisms commonly encountered in mechanically ventilated patients with pneumonia (136). This study examined the simulated human ELF concentrations of amikacin after delivery via the pulmonary drug delivery system (PDDS) against susceptible and resistant *K. pneumoniae* and *P. aeruginosa* isolates. Mean steady-state concentrations of amikacin in ELF after administration of 400 mg every 12 h delivered via the PDDS were simulated by using a previously unreported pharmacokinetic model in a virtual population of 1,000 patients built from the INHALE trials. Resulting peak and trough ELF concentrations used were 5,252 and 507 mg/liter, respectively, with an area under the concentration-time curve from 0 to 12 h (AUC_{0-12}) of 17,940 mg · h/liter. Amikacin achieved maximal killing effect within 6 to 12 h and sustained bactericidal activity over 24 h for all the isolates tested with an amikacin of MIC ≤256 mg/liter. Importantly, when MICs were retested over the 72-h experiment, amikacin MICs did not change across all models. The isolates for which amikacin activity was limited in this study had MICs of up to 32,768 mg/liter and possessed dozens of resistance mechanisms. These isolates are extremely rare in clinical practice outside areas of endemicity, and surveillance studies examining amikacin MICs have consistently reported a maximal MIC_{90} of 32 mg/liter against *Enterobacteriaceae*, even against carbapenem-resistant strains, and an MIC_{90} of 8 mg/liter against *P. aeruginosa*. Given the current CLSI resistance breakpoint for amikacin against *Enterobacteriaceae* and *P. aeruginosa* of ≥64 mg/liter, the rare occurrence of isolates with MICs of ≥256 mg/liter, and the fact that commonly utilized automated broth microdilution systems can often report MICs of only up to 256 mg/liter, *in vitro* susceptibility testing by clinical microbiology laboratories for inhaled amikacin currently provides very little clinically relevant information. This same situation is likely applicable to all inhaled antibiotics given the extremely high intrapulmonary concentrations.

PULMONARY PHARMACOKINETICS

Aminoglycosides have several characteristics that make them attractive options for aerosol delivery. First, their antibacterial efficacy is concentration dependent, meaning that the high concentrations of drug achieved in the lungs after inhalation could lead to increased killing of bacteria and therefore improved clinical efficacy. Second, they have dose-dependent systemic toxicities such that the highest doses that can be tolerated when given intravenously lead to low pulmonary penetration ratios. Finally, aminoglycosides often retain activity against MDR Gram-negative pathogens and therefore are viable therapeutic options from a microbiological susceptibility standpoint. For these reasons, the majority of the literature concerning antimicrobial delivery via the inhalation route has focused on the aminoglycosides.

The erratic and often low-level penetration of systemically administered aminoglycosides, such as gentamicin, into pulmonary secretions and subsequent treatment failures have been recognized since the late 1960s and early 1970s (137, 138). These findings prompted clinicians and investigators to explore the direct administration of gentamicin into the lungs to help improve clinical responses. Indeed, more recent investigations have supported the findings of poor penetration of aminoglycosides into the lungs (139, 140). In contrast to intravenously administered aminoglycosides, which often achieve maximal plasma concentration values of 20 to 40 mg/liter, delivery of inhaled aminoglycosides to the lungs has consistently demonstrated lung concentrations of >200 mg/liter, although these studies were performed primarily with patients with CF. More advanced bioanalytical assays and PK procedures have allowed researchers to better characterize the degrees of exposure, distribution, and elimination of inhaled antimicrobials in both animal and human models.

Animals

Tobramycin. *Ex vivo* animal models have been used to examine the physiochemical properties of antimicrobials that lead to sustained effects between doses. In one such model utilizing tobramycin administered to an *ex vivo* rat lung, the magnitude of absorption of tobramycin was clearly dose dependent, while the rate constant for absorption was dose independent (141).

As mentioned above, liposomes can be used to encapsulate hydrophilic drugs in the core and to form bilayers with lipophilic drugs in order to produce sustained release within the lungs and reduce system absorption, thereby reducing the adverse-effect profile. In rats infected with *P. aeruginosa*, liposomal concentrations of tobramycin in the lungs were consistently ~5 times those of free tobramycin after intratracheal (i.t.) instillation. Systemic concentrations remained undetectable for both the free and liposomal formulations. Surprisingly, despite the increased concentrations of liposomal tobramycin over the sampling period, no differences in bacterial colony count reductions between liposomal and free tobramycin were seen. This may have been due to a lack of liberation of tobramycin from liposome encapsulation.

In a similar study examining single and multiple doses of conventional and liposomal tobramycin in rats with pulmonary infections due to *P. aeruginosa*, the liposomal formulation showed a significantly longer residence time and a significantly higher degree of exposure. Total pulmonary exposure as measured by the AUC was significantly higher for liposomal tobramycin than for conventional tobramycin (3,890 mg · h/liter versus 663 mg · h/liter). Despite this difference, the overall numbers of CFU of *P.*

aeruginosa were similar after single or multiple doses of either formulation, although a higher percentage of observations fell below 10^3 CFU for the liposomal formulation after multiple doses (142).

In contrast to these results, i.t. administration of both liquid and dry-powder formulations of liposomal tobramycin demonstrated improved antibacterial efficacy in rats with *P. aeruginosa* lung infections compared to the administration of free tobramycin (143, 144). In addition, liposome-encapsulated tobramycin has been shown to have improved *in vitro* activity over free tobramycin, even at sub-MICs, which is not entirely explained by an increased residence time (145). The lipid composition of the carrier vesicle is an important consideration and may have many variable downstream effects, such as lung tissue binding and engulfment by alveolar macrophages.

Similar to the nebulized admixtures discussed above, combinations of drugs within a single liposome have been explored. Bismuth is a compound that has been used for years to treat gastrointestinal ulcers due to *Helicobacter pylori*, although it has also demonstrated activity against Gram-negative organisms, reduced *P. aeruginosa* biofilms, and acted synergistically with tobramycin against *P. aeruginosa* (146–150). Despite these advantageous properties, low concentrations of bismuth have been shown to be toxic to human lung cells (151). One study combined bismuth ethanedithiol (BiEDT) and tobramycin coencapsulated in a liposome in order to decrease the toxic effects of BiEDT while preserving the antimicrobial activity (152). This liposomal combination of BiEDT and tobramycin was able to reduce the virulence factors and quorum-sensing molecules of *P. aeruginosa* and enhance the antimicrobial activity and anti-inflammatory properties when given to rats with simulated chronic *P. aeruginosa* lung infections. The combination successfully reduced the number of CFU per gram of *P. aeruginosa* in the lungs of rats, without evidence of significant systemic absorption or toxicity. In contrast, concentrations of free tobramycin were found only in the kidneys and not in the lung, indicating a higher potential for systemic toxicity, with minimal to no sustained lung deposition.

Gentamicin. Liquid perfluorocarbons (PFCs) have demonstrated therapeutic promise in their ability to support gas exchange and have been used experimentally as a protective strategy for patients with acute respiratory distress syndrome (153, 154). PFCs have also demonstrated effectiveness as a vehicle for distributing drugs throughout the lungs as a mixture of aqueous drug solutions. PFC vehicles serve as an advantageous medium for pulmonary drug delivery due to their biochemical inertness, low surface tension, and high respiratory gas solubility. In addition, their established benefit in improving ventilation-perfusion mismatch in human lungs provides an additional impetus to evaluate them for pulmonary drug delivery. In healthy rabbits receiving gentamicin in a PFC vehicle, the C_{max} and AUC_{0-8} in the lung after i.t. administration were 1,928 mg/liter and 680,540.2 mg · liter/h, respectively (155). i.t. administration resulted in a plasma C_{max} of 5 mg/liter, with an absolute bioavailability over 4 h of 57%. The bioavailability of gentamicin in the lung following intramuscular (i.m.) administration compared to i.t. administration was only 0.73%. At 1 week, there were still detectable lung concentrations after i.t. administration, whereas concentrations were undetectable 4 h after i.m. administration. This study again reveals the low level of lung penetration after systemic administration and demonstrates the high concentrations of gentamicin in lung tissue achieved by instillation of gentamicin in a PFC vehicle, which may allow increased local pulmonary delivery of antibiotics while also simultaneously improving ventilation and minimizing systemic toxicity.

As discussed above, lung injury in response to infection can create hypoperfusion and shunting away from the infected area, in turn affecting antibiotic supply to the infected area when given systemically. A model was created in order to simulate this by utilizing newborn lambs with induced acid lung injury undergoing partial liquid ventilation. The induced lung injury created pulmonary hypoperfusion secondary to hypoxia and impaired the distribution of oxygen resulting from consolidation and pulmonary edema, causing a ventilation-perfusion mismatch, as is commonly seen in pneumonia. Injury was induced until the animals' lung function was ~50% of the baseline value. Gentamicin was given both i.v. and i.t. at 5 mg/kg of body weight, and lambs were studied for 4 h postadministration, after which lungs were harvested and homogenized. In this study, serum concentrations of gentamicin were almost identical between i.t. and i.v. administrations, while mean tissue lung concentrations for the entire lung were significantly higher after i.t. administration. In contrast to the above-described study utilizing i.m. administration, lung concentrations of i.v. gentamicin were adequate throughout the lung, ranging from 14.6 to 21.8 μg/g (156). Interestingly, the concentrations were consistent and ubiquitous throughout the lung tissues sampled despite differences in ventilation-perfusion mismatches. These differences are likely due to the use of an injured-lung model, allowing increased drug permeation from plasma to the lung parenchyma and vice versa. Although this model attempts to more accurately reflect the ventilation-perfusion mismatch occurring in human lungs during infection, the use of homogenized lung tissue and collection of only a single concentration in pulmonary tissue inhibit the ability to thoroughly extrapolate these results to patients.

A similar study performed on newborn lambs undergoing liquid ventilation without acute lung injury given i.t. or i.v. gentamicin in the same dose showed that lung tissue concentrations were again significantly higher after i.t. administration than after i.v. administration (157). These findings confirm the differences in diffusion occurring between diseased and healthy mammalian lungs.

Like tobramycin, liposomal encapsulation of gentamicin has been shown to prolong the residence time, increase concentrations within the lungs, and minimize systemic absorption compared to conventional formulations (158).

Amikacin. The most modern animal model for studying the deposition, PK, and clinical outcomes of inhaled antimicrobial therapy is the ventilated-piglet model. In mechanically ventilated piglets with induced *Escherichia coli* pneumonia, lung deposition and efficacy of inhaled amikacin were studied. An ultrasonic nebulizer was positioned in the inspiratory line and filled with 45 mg/kg of amikacin in 12 ml of saline, and the drug was nebulized for ~20 min in 10 piglets. Eight piglets received a 15-mg/kg i.v. dose of amikacin. Amikacin concentrations were measured in lung tissue, plasma, and urine. Of the initial amikacin nebulizer charge, only 38% of the initial dose reached the respiratory tract, equivalent to a mean dose of 17 mg/kg. The remainder of the dose was either retained in the chamber and reservoir or fixed in the ventilator circuit, endotracheal tube, or expiratory filter. Lung tissue concentrations determined 1 h after the second amikacin dose

were significantly higher for inhaled amikacin than for i.v. amikacin in all sections of lung tested. Aerosol concentrations were highest in the lower lobe, with a maximal concentration of ~100 μg/g. Lung samples from the aerosol treatment group also had lower bacterial burdens than did those from the i.v. treatment group, and 71% of the tissues cultured in the aerosol treatment group were sterile, compared to only 16% in the i.v. treatment group. Surprisingly, the mean peak plasma concentrations were similar between the aerosol and i.v. treatment groups (22.5 mg/liter versus 36.4 mg/liter), with trough concentrations falling below 5 mg/liter after 6 h in both groups. These concentrations equated to a plasma AUC of 87.9 ± 42 mg · h/liter for inhaled amikacin, versus 72 ± 19 mg · h/liter for i.v. amikacin, and half-lives of 3.37 ± 0.93 and 4.7 ± 0.3 h, respectively. Based on the amount eliminated in the urine, the systemic bioavailability of inhaled amikacin was 70% ± 11%. This study clearly demonstrates improved microbiological eradication and lung concentrations after inhaled administration of amikacin compared to i.v. delivery. This is true even though <50% of the actuated dose reached the respiratory tract and despite the severe pulmonary consolidation and lack of aeration induced by inoculation (159). Interestingly, inhaled amikacin achieved a higher plasma AUC than did i.v. amikacin, and concentrations remained above the 4-mg/liter MIC for *E. coli* through the 6-h sampling window, indicating the potential lack of a need for both modes of administration when treating a systemic infection. This efficient systemic absorption can likely be attributed to the fact that these animals had diseased lungs. Conversely, the same extent of systemic absorption has not been seen in patients with pneumonia given inhaled amikacin.

The same group of investigators attempted to specifically examine the influence of lung aeration on the pulmonary concentrations of inhaled and i.v. amikacin, at 40 and 15 mg/kg, respectively, by histologically studying areas of induced bronchopneumonia. In this study, amikacin concentrations in lung tissue were 197 ± 165 μg/g for the aerosol treatment group and 6 ± 5 μg/g for the i.v. treatment group in lung areas with focal bronchopneumonia, compared to 40 ± 62 μg/g and 5 ± 3 μg/g, respectively, in areas of severe bronchopneumonia. Importantly, pulmonary concentrations of inhaled amikacin increased significantly in areas of adequate lung aeration. In contrast, pulmonary concentrations of i.v. amikacin decreased in areas of improved aeration. This demonstrates that the severity of lung disease is inversely proportional to the concentration of inhaled amikacin achieved in these lung tissues. This is likely due to the ability of particles to penetrate the alveoli via open distal bronchioles and the lack of penetration through purulent bronchial plugs induced by bacterial inoculation. Additionally, in nondiseased lungs, drug penetration is prevented by a healthy alveolar epithelium and vascular endothelium, explaining why more aerated areas had decreased i.v. amikacin concentrations. Despite this, lung concentrations achieved via the inhalation route were >20-fold higher than those achieved by the i.v. route (160).

Imipenem-cilastatin. The concentrations of imipenem-cilastatin in the bronchoalveolar lavage (BAL) fluid of rats after administration via continuous i.v. infusion and inhalation were compared. In this study, imipenem-cilastatin was also examined for its ability to affect bacterium-induced lung injury due to *P. aeruginosa* when given preinoculation. Imipenem-cilastatin was administered via aerosol at 200 mg over 1 h or by continuous i.v. infusion at 25 mg/kg/h over 5 h before pneumonia was induced via instillation of *P. aeruginosa*. The i.v. dose was designed to mimic human plasma concentrations, while the aerosol dose was based on data from studies utilizing tobramycin. Of note, rodents possess dehydropeptidase, the enzyme responsible for the degradation of imipenem, in their lungs, whereas primates primarily produce it almost exclusively in the kidneys. After an hour of administration, the mean concentration of imipenem-cilastatin in BAL fluid was >4-fold higher with inhaled imipenem than with i.v. imipenem (107.9 μg versus 25.8 μg), while the plasma concentrations were 200-fold lower (0.3 mg/liter versus 56 mg/liter). Inhaled imipenem-cilastatin demonstrated significantly higher concentrations in rat BAL fluid than did continuous systemic infusion in healthy rats. Four hours after inoculation of bacteria, both the i.v. and aerosol pretreatments reduced the inoculum to $<0.1 \times 10^8$ CFU, compared to the control arm of 6.9×10^8 CFU (161). These data suggest that inhalation of imipenem-cilastatin might prove useful for prophylactic treatment of patients at risk for colonization and subsequent infection by Gram-negative pathogens such as *P. aeruginosa*. This study again demonstrates that intrapulmonary concentrations are exceedingly high after inhaled administration, while systemic concentrations remain almost undetectable, thereby optimizing efficacy and minimizing toxicity.

Ceftazidime. In both humans and animals undergoing mechanical ventilation, vibrating-plate and ultrasonic nebulizer delivery demonstrated better lung deposition than did jet nebulizers. In animals, the efficiency of a vibrating-plate nebulizer was compared to that of an ultrasonic nebulizer for the ability to aerosolize and deposit ceftazidime into healthy mechanically ventilated piglets (162). One gram of ceftazidime was nebulized continuously into ventilator circuits by the respective nebulizers without humidification. Of note, a 65% helium–35% oxygen mixture (heliox) was used to enhance lung deposition, with ventilator settings optimized for nebulization. The respiratory fractions of ceftazidime available after administration via two different nebulizer types were similar, at 62% and 66% for the ultrasonic and vibrating-plate nebulizers, respectively. Mean lung tissue concentrations were also similar, at 452 μg/g and 553 μg/g, respectively, which equates to ~100 mg (10% of the nebulized dose) of ceftazidime reaching the lungs given the weight of the piglets included. Given their above-mentioned simplicity of use, vibrating-plate nebulizers may be the optimal selection for patients who are undergoing mechanical ventilation. Considerations for inhaled-drug delivery in patients undergoing mechanical ventilation are discussed in detail below.

In a similar study by the same group, i.v. and inhaled ceftazidime were compared in piglets with and those without *P. aeruginosa* pneumonia undergoing mechanical ventilation (163). This study utilized an ultrasonic nebulizer and heliox to improve lung deposition. In noninfected animals, mean lung concentrations of ceftazidime after i.v. and inhaled administrations were 17 μg/g and 576 μg/g, respectively. In piglets with induced pneumonia, concentrations were significantly lower after i.v. and inhaled administrations, at 10 μg/g and 111 μg/g, respectively. The heliox mixture also increased lung deposition of inhaled ceftazidime by 33% in noninfected animals but had no effect in those with pneumonia, likely due to the loss of alveolar aeration. The etiology and severity of lung disease are important considerations when determining the appropriate dose of inhaled antibiotics, as evidenced

by the dramatic difference in lung tissue concentrations of ceftazidime between healthy an infected animals in this study.

Fluoroquinolones. In one of the only studies evaluating both the PK and PD of an inhaled antimicrobial in an *in vivo* model, ciprofloxacin was administered to healthy rats as a dose of 200 μg/kg via the nasal cavity by using a liquid microsprayer, similar to an atomizer, and as an oral dose of 10 mg/kg. Plasma and BAL fluid concentrations were determined at specified time points after drug administration. Concentrations in the ELF and alveolar macrophages (AMs) were markedly higher after inhalation than after oral administration, while plasma concentrations were lower. The AUCs of ciprofloxacin in the ELF and AM following inhalation were 103 mg · h/liter and 244 mg · h/liter, respectively, compared to 0.74 mg · h/liter and 14.1 mg · h/liter after oral administration, respectively. Given the PK/PD index associated with efficacy in the treatment of *Streptococcus pneumoniae* and *P. aeruginosa* infections, intrapulmonary administration would achieve optimal activity, with MICs of up to and equal to 0.5 and 0.25 mg/liter, respectively, if the currently approved breakpoints are applied to the inhalation route.

In rats given ciprofloxacin, moxifloxacin, and grepafloxacin, the mean ELF concentration of ciprofloxacin 30 min after nebulization was 10-fold higher than that after i.v. administration (10.5 mg/liter versus 1.03 mg/liter), although there were no significant differences in ELF concentrations between inhaled and i.v. administrations at 2, 4, or 6 h postdose. Compared to ciprofloxacin, moxifloxacin and grepafloxacin showed no significant differences at any time point. This is true even when considering the potential overestimation of ELF concentrations due to the possibilities of lysis of macrophages and leakage of intracellular drug, given that the fluoroquinolones have been shown to accumulate intracellularly within macrophages (10). In this study, no obvious benefit of administering these fluoroquinolone agents via nebulization was seen. Although extremely high intrapulmonary concentrations of ciprofloxacin were initially achieved, which may be advantageous due to its primarily concentration-dependent activity, these concentrations eventually showed a time course and magnitude similar to those of i.v. administration. Of note, this study was conducted in healthy animals, which could have impacted the diffusion of drug both into and out of the lung (164).

In contrast to ciprofloxacin, aerosol administration of levofloxacin achieved an AUC that was ~9-fold higher than those achieved by intraperitoneal administration in healthy mice given a single dose of levofloxacin at 60 mg/kg via a microspray device or an intraperitoneal dose of 20 mg/kg. Neutropenia was then induced, and mice were infected with *P. aeruginosa* 24 to 48 h prior to the administration of an inhaled antibiotic twice daily for 24 or 48 h. The reduction in numbers of bacteria with inhaled levofloxacin was greater than that with intraperitoneally administered levofloxacin on a per-dose basis. Finally, compared to aztreonam and tobramycin, in a model of acute lethal lung infection, levofloxacin produced the greatest decrease in the amount of CFU per lung. Surprisingly, levofloxacin also provided the highest survival rate (100%) compared to tobramycin (60%) and aztreonam (20%) (165). The reason for this dramatic difference in survival is unclear, and few animal studies have evaluated both PK and survival as they relate to the treatment of induced infections with inhaled antibiotics, especially in neutropenic animals.

Colistin. Throughout this review, the doses of colistin and polymyxin B are reported as they were in the studies being discussed. The confusion surrounding the decision to dose these agents in units of milligrams or international units was detailed previously elsewhere (166–168). The currently recommended conversion factors between units and milligrams are 12,500 U colistin methanosulfate (CMS)/mg or 30,000 U colistin/mg for colistin and 10,000 U/mg for polymyxin B.

Although CMS (supplied commercially as either Coly-Mycin M or Colomycin) has long been administered to CF patients for airway colonization due to *P. aeruginosa*, it was only recently introduced as an adjunct for patients with Gram-negative LRTIs. After initial studies, a more comprehensive population PK study was designed in order to develop a model to better characterize CMS-to-colistin conversion and to understand its disposition in plasma and ELF in rats. This model would ultimately assist in identifying the targeted advantage achieved by delivering CMS directly to the lungs. In this study, CMS and colistin sulfate were administered both intravenously and via i.t. instillation. Rats were administered either 14 or 28 mg/kg of CMS or 0.41 mg/kg, 0.62 mg/kg, 0.99 mg/kg, or 1.49 mg/kg of colistin intratracheally (3 rats per dosing level), and blood samples were obtained serially. BAL fluid was collected only for the 14-mg/kg and 0.62-mg/kg doses of CMS and colistin, respectively. A C_{max} of 21,391 mg/liter was observed 5 min after pulmonary administration of CMS, with a maximum concentration of formed colistin occurring 4 h after CMS dosing of ~1,000 mg/liter. Concentrations of colistin were maintained above 200 mg/liter throughout the 12-h sampling period. Based on the results of the established three-compartment PK model for plasma data, the respiratory fractions of CMS and colistin were estimated to be 40.9% and 48.5%, respectively. Importantly, the fraction of colistin converted from CMS in ELF was 0.226%, which was ~9-fold higher than the conversion rate of 0.0255% in plasma after i.v. administration. Maximum concentrations of CMS and colistin achieved in plasma after i.t. administration were roughly 2 mg/liter (AUC, 3.01 ± 0.476 mg · h/liter). The AUC of formed colistin in ELF was 11,245 mg · h/liter, of which only 0.03% was absorbed into plasma. This study demonstrates that ELF concentrations of active formed colistin were extremely high and maintained above the MIC_{90} of 1 mg/liter for *P. aeruginosa* and *A. baumannii* (169) for the entire 12-h dosing interval, while concentrations in plasma remained low and below the toxic limit. Importantly, more of the CMS was converted to colistin in the lungs than in plasma, likely due to the longer residence time. Of note, at the highest i.t. dose of CMS (14 mg/kg), systemic exposure of formed colistin was 2-fold higher than that after the same dose was given i.v., likely due to the extensive conversion in the lung prior to passive diffusion into the systemic circulation. These data suggest that a lower dose could be given intratracheally to achieve adequate lung exposure while reducing systemic exposure; however, this needs to be confirmed with inhaled delivery of CMS (170). Data from this study must also be interpreted in light of the method of pulmonary delivery. Previous studies in rats and hamsters utilizing technetium-labeled particles demonstrated nonuniform distribution patterns after i.t. administration (171). As mentioned above, this delivery strategy can no longer be recommended in humans, and future animal studies should be performed only with true antibiotic inhalation in order to provide data that are the most accurate and translatable to humans.

A similar study evaluated i.v. and inhaled colistin sulfate at a dose of 0.35 mg/kg with serial blood samples and BAL fluid

collections at 0.5, 2, and 4 h postdose. The plasma concentration-time profiles were similar between i.v. and inhaled administrations, with estimated unbound concentrations at 2 h of 0.05 mg/liter and 0.06 mg/liter, respectively. Conversely, ELF concentrations of colistin after i.v. administration were undetectable, while mean concentrations after nebulization were 153 mg/liter, 112 mg/liter, and 20 mg/liter at 0.5, 2, and 4 h postdose, respectively. In this analysis, inhalation of formed colistin equated to lower plasma concentrations than with CMS after inhalation, which may limit toxicity. Then again, the i.v. dose given in this study may have been too low at 0.35 mg/kg, as doses of at least 1 mg/kg were used previously (172). Given the higher rate of adverse effects with nebulization of formed colistin, as discussed below in this review, and the high concentrations of formed colistin in the lungs after inhalation of CMS, CMS should almost certainly be used in clinical practice.

In a model of lung deposition and the bactericidal effect of inhaled versus i.v. colistin, VAP was induced in 12 piglets by instillation of *P. aeruginosa* into both lungs. Twenty-four hours after inoculation, half of the piglets received i.v. colistin at 40,000 U every 8 h, while the other half received 100,000 U via inhalation every 12 h by using a vibrating-plate nebulizer over 30 min. The respiratory fraction of colistin in this study was 60% of the initial nebulized dose, equaling ~9.6 mg/kg. The median peak lung tissue concentration in the inhalation treatment group was 2.8 μg/g, while significantly lower concentrations were observed in lung areas with severe pneumonia than in those with mild pneumonia. Lung concentrations of colistin after i.v. administration were undetectable even at steady state and using homogenized lung tissue. When bacterial counts in homogenized lungs were compared, inhaled colistin effectively sterilized 67% of piglet lungs at 24 h postdose, versus only 28% in the i.v. treatment group. Serum concentrations were also significantly lower after aerosol administration, with a mean C_{max} of 1.6 mg/liter, compared to 6.0 mg/liter after i.v. administration. Trough concentrations were virtually undetectable after inhalation, and the serum AUC was approximately half that with i.v. administration (7.5 mg · h/liter versus 13.5 mg · h/liter) (173). High concentrations of colistin were observed in the lungs after aerosol administration, along with low serum concentrations, and the antibacterial effect was significantly improved. Given that colistin is negatively charged at human physiological pH, the negative charge of the alveolar basement membrane likely contributes to the slow systemic passage of colistin into the systemic circulation (174). The decreased lung concentrations in areas of severe pneumonia may require increased dosages of inhaled colistin in these situations to achieve the required AUC/MIC ratio, which may be possible while avoiding toxicity given the low systemic concentrations. Given that lung concentrations were minimal after i.v. administration and serum concentrations after aerosol administration were too low to treat a systemic infection, it may be prudent to combine these two routes when treating the most severely ill patients with Gram-negative LRTIs. This combination may also allow for lower i.v. doses, thereby decreasing the rate of toxicities. Further data are needed regarding the treatment of Gram-negative LRTIs with inhaled antibiotics alone as monotherapy, especially in critically ill patients, before systemic therapy should be limited or abandoned completely.

Finally, a study examining the performance of jet and vibrating-mesh nebulizers for the pulmonary deposition and PK of CMS and colistin sulfate was performed in baboons, whose airway structures most closely resemble that of a human child. In this study, CMS and colistin sulfate were radiolabeled and given via nebulization at a dose of 26.6 mg. Plasma samples were obtained, and the lungs were scanned by using a gamma camera to determine lung deposition of the radiolabeled nebulization. Greater aerosol deposition was achieved via the Pari LC jet nebulizer, with a mean aerosol fraction deposited in the lung of 3.5%, versus 1.3% with the vibrating-mesh nebulizer. Plasma concentrations were detectable after nebulization but were never above 0.3 mg/liter and were roughly equivalent between CMS and formed colistin. The low lung deposition fractions in this study were attributed to the use of a conical mask over the mouth of the baboons, allowing escape of the delivered dose prior to lung deposition (175).

The observed efficacy of several different inhaled antibiotics in diverse animal models demonstrates the ability of locally administered agents to achieve extremely high concentrations within the lungs while maintaining low systemic exposure. Differences in diffusion to and from plasma and alveoli have been demonstrated in animals with healthy and diseased lungs, while the inhalation route has consistently achieved improved microbiological eradication in infection models. These studies demonstrate the ability of this route to bypass problematic physiological barriers and access deep-seated sites of infection in the lung parenchyma. These animal models help provide valuable insight into the potential clinical utility of inhaled antibiotics in humans with Gram-negative LRTIs.

Humans

As discussed above, the difficulty in constructing a PK model of inhaled antibiotics makes allometric scaling and extrapolation from animal to human studies challenging. Fortunately, many studies that observed the PK of inhaled antibiotics in both healthy and infected humans have been completed.

Tobramycin. In an open-label crossover study of 12 healthy volunteers, subjects inhaled a radiolabeled tobramycin inhalation solution via a jet nebulizer, while whole-lung deposition via gamma scintigraphy and serum concentrations were measured. After inhalation of 25 mg of radiolabeled tobramycin via a dry-powder inhaler, the average lung deposition among all subjects was 34.3%. Approximately 22% of the dose was retained within the inhaler, while 44% was deposited in the oropharynx. In contrast, after inhalation of 300 mg of a commercial tobramycin inhalation solution via a jet nebulizer, only 5% was deposited in the lung, and 8% was deposited in the oropharynx; >50% of the dose was retained within the nebulizer cup. For both formulations, ~60% of the total dose that reached the lungs was deposited in the alveoli. Finally, after inhalation of six 25-mg capsules via a DPI every 3 min over 15 min and one nebulization of unlabeled tobramycin over 15 min, serial blood samples were obtained for determination of tobramycin concentrations. The C_{max} of tobramycin was almost 2-fold higher with the DPI than with nebulized tobramycin, although the concentrations were negligible (0.60 mg/liter versus 0.28 mg/liter). Serum concentrations were undetectable in nearly all subjects (85%) by 18 h with either device. These healthy subjects were trained in the appropriate use of both the DPI and the nebulizer, although the nebulizer was not run to dryness (30 min), which likely contributed to the low lung deposition. There were no adverse events reported in this study. The improved efficiency and ease of use of the DPI make it an advantageous selection for patients requiring inhaled tobramycin, while the advent of

tobramycin inhalation powder allows larger doses to be delivered in a lower powder volume, helping to decrease adverse effects from use (176).

The lung, plasma, and urine PK of tobramycin have been investigated for both spontaneously breathing healthy volunteers and mechanically ventilated patients without pneumonia. One group studied included 10 patients who inhaled tobramycin 4 to 12 h before a planned pneumonectomy for lung cancer, after which a sample of lung was removed and homogenized for determination of drug concentrations. A commercially purchased pneumatic nebulizer was utilized to nebulize 300 mg of a technetium-labeled tobramycin inhalation solution. The amount of tobramycin exiting the nebulizer device in this study was 49% of the original dose, and maximum plasma concentrations were undetectable in 6/10 subjects in this study (3 in each group). Average lung concentrations 4 and 12 h after nebulization were 5.57 μg/g and 3.61 μg/g, respectively. Based on urine concentrations, the bioavailabilities were estimated to be ~4.3% and 6.6% for health volunteers and patients, respectively (177). Older studies of patients with lung disorders demonstrated mean pulmonary concentrations of tobramycin after inhalation of 2 ± 2.26 mg/liter, as assessed by using BAL fluid (178), although these studies were performed early in the history of ELF studies, and tobramycin concentrations were obtained from the first BAL fluid aliquot, a technique that is no longer used.

The negligible systemic absorption following inhalation of inhaled tobramycin has also been proven in a prospective study of hospitalized patients with an endotracheal tube or tracheotomy who received 300 mg of inhaled tobramycin twice daily. Of the 9 patients included, 92% of trough concentrations were below 0.5 mg/liter, and only one patient experienced a decline in renal function. This study suggests a lack of need for serum therapeutic drug monitoring when administering inhaled tobramycin and demonstrates a lack of systemic toxicity when monotherapy is used, as was observed in animal and preclinical studies (179).

Finally, a prospective, randomized trial was designed to assess the difference in lower respiratory tract concentrations of tobramycin and imipenem-cilastatin after nebulization or instillation in patients with suspected or proven respiratory infections who were undergoing mechanical ventilation. Imipenem-cilastatin was delivered at 1,500 mg every 8 h for two doses, and tobramycin was given at 200 mg every 12 h for two doses. Patients were randomized to receive either instilled or inhaled drug via injection into the tracheal tube or inhalation with an ultrasonic nebulizer, respectively. BAL fluid concentrations of imipenem-cilastatin after 1 h were remarkably higher after instillation than after nebulization (4,695 mg/liter versus 72 mg/liter), although inhaled concentrations still exceeded 20 times the MIC for most respiratory pathogens. There were no marked differences in tobramycin concentrations by either route after 2 h (102 mg/liter after nebulization versus 142 mg/liter after instillation). The majority of patients had undetectable plasma concentrations of both drugs at all time points, although 3 patients given instilled tobramycin had detectable concentrations at 12 h, compared to none who received inhaled tobramycin. These three patients all had renal failure at baseline in this study. There were no adverse events recorded in this study. Both serum and pulmonary concentrations of imipenem-cilastatin were significantly higher after instillation than after nebulization, although inhaled concentrations were more than adequate to treat a Gram-negative LRTI. Despite the higher concentrations of imipenem-cilastatin after instillation, nebulization likely achieves a more homogenous and uniform distribution throughout the lungs than instillation (180).

Gentamicin. Five patients with healthy lungs and a recent tracheostomy received 2 mg/kg of gentamicin either i.m. or via instillation through a polyethylene catheter introduced into the deep trachea. Four hospitalized patients without tracheostomy tubes were also given 40 mg of gentamicin via nebulization, and concentrations were evaluated in the same fashion. Bronchial secretion concentrations in four of the five patients after i.m. injection of gentamicin were <1.0 mg/liter, while the mean peak plasma concentration after endotracheal administration was 1.04 mg/liter. Despite low serum concentrations, the mean urine concentration was 19.3 mg/liter after endotracheal administration, indicating some systemic absorption. In contrast, peak concentrations in bronchial secretions exceeded 400 mg/liter and remained above 10 mg/liter 6 h after endotracheal administration. In the four patients administered gentamicin via nebulization, mean tracheal aspirate and induced sputum concentrations were 22.2 mg/liter and 17.8 mg/liter, respectively. Peak concentrations in both matrices were also significantly lower than those after endotracheal administration, while plasma concentrations were virtually undetectable (181). As demonstrated previously, local administration achieved supratherapeutic concentrations of antibiotic at the target site while maintaining negligible plasma concentrations. Given the substantial recovery of gentamicin in the urine of patients after endotracheal instillation, inhaled delivery is likely a safer and equally effective method. Compared to the typical 96% urinary excretion observed after i.v. dosing, only 11% of the dose was recovered in the urine after nebulization in healthy subjects (182).

Amikacin. In order to reduce the treatment burden of traditionally marketed inhaled products such as tobramycin inhalation powder, which require up to 4 administrations per day, amikacin was developed into a liposomal carrier form in order to create a more-sustained-release product. Not only does this liposome-encapsulated formulation have the ability to improve treatment adherence, but the extended lung residence time may also improve the antibacterial efficacy. As discussed previously, liposomal formulations have shown improved activity over free inhaled drug in animal models (142, 183). These liposomal formulations may play a particularly advantageous role in critically ill patients with accelerated antimicrobial elimination and impairment in passive diffusion borders (183). Remarkably, the virulence factors present in biofilms of *P. aeruginosa* have been shown to promote the release of amikacin from the liposome *in vitro*, allowing improved site-specific delivery of the antimicrobial (184). In order to evaluate the lung disposition and elimination of liposome-encapsulated inhaled amikacin, 3 healthy male volunteers inhaled a single radiolabeled dose of 120 mg of amikacin via a Pari LC Plus nebulizer over a 20-min period. In this study, ~30% of the emitted dose was deposited in the lungs, while >50% was trapped in the exhalation filter as a result of continuous output from the jet nebulizer. Gamma scintigraphy images at 24 h postdose showed significant pulmonary radioactivity in the lung periphery, with 75% and 38% of the initially deposited dose remaining after 3 and 48 h, respectively. There were no adverse events reported in this trial. Extrapulmonary deposition was high, as expected with the Pari LC Plus device, as outflow is continuous, even during exhalation by subjects. The majority of the liposomes that were inhaled were

deposited in the lungs, the bulk of which made it to the lung periphery. The use of liposome-encapsulated amikacin in this study allowed a sustained residence time of up to 48 h (184).

In an important proof-of-concept study, six healthy volunteers underwent noninvasive pressure-support ventilation and administration of i.v. and inhaled amikacin. A vibrating-mesh nebulizer was placed on the inspiratory limb with a standardized ventilation pattern. Four dosage sequences were used, with a 7-day washout period in between. The first sequence consisted of a 1-h infusion of 15 mg/kg of i.v. amikacin, while the following three sequences were nebulization of 40 mg/kg, 50 mg/kg, and 60 mg/kg of amikacin. Serial blood samples were measured over 24 h postdose. There were no adverse events reported, and no changes in renal, audiological, or respiratory function were observed. At all inhaled doses and time points assessed, serum concentrations were less than or equal to those observed after i.v. administration. All plasma concentrations at 24 h were below 2.5 mg/liter after inhalation, while C_{max} and AUC values were higher after i.v. infusion. In this study, median doses as high as 3,510 mg were inhaled, with a median maximal serum concentration of 9.2 mg/liter. The plasma AUC after inhalation was roughly half of that achieved after systemic administration (66 mg · h/liter versus 138 mg · h/liter). The maximum median bioavailability reported was 13% for inhaled amikacin. Given that toxicity associated with aminoglycosides is most closely associated with the plasma AUC value, inhaled amikacin may provide a less toxic option for treatment of Gram-negative LRTIs. The mean absorption time in this study (time needed for an inhaled molecule to reach the systemic circulation) was >2 h for all subjects. Given the concentration-dependent activity of amikacin, this residence time within the lung parenchyma may allow exponentially better antibacterial efficacy after inhaled administration than after parenteral administration. These results may also be due to the extremely high inhaled dosages given in this study, which may be prudent to maximize the PK/PD index of efficacy. The subjects in this study were not mechanically ventilated, making it impossible to control specific factors known to influence lung deposition, such as tidal volume and inspiratory flow (185).

BAY41-6551 is a drug-device combination of amikacin specifically formulated for inhalation and a gasless piezoelectric vibrating-mesh nebulizer. This nebulizer is also designed to be integrated with standard mechanical ventilation equipment so that drug release occurs only during the first 75% of inspiration and has been shown to deliver up to 70% of the nominal dose in laboratory settings. A multicenter study evaluated the penetration of amikacin into the ELF of patients with Gram-negative nosocomial pneumonia who were mechanically ventilated using the BAY41-6551 system. Patients received 400 mg of inhaled amikacin over 45 to 60 min twice daily for 7 to 14 days. Patients had to receive positive-pressure ventilation during nebulization, and a heated humidifier was permitted. If patients were extubated during the study, they completed therapy with a handheld version of the BAY41-6551 system. All patients underwent BAL after the end of the first inhaled dose on day 3, along with serial plasma and urine sampling. The median plasma PK parameters for the 28 patients included in the study were a C_{max} of 0.85 mg/liter, a time to maximum concentration of drug in serum (T_{max}) of 1 h, and an AUC_{0-12} of 6.15 mg · h/liter. The median amount secreted in the urine over 12 h was 19 mg/liter. In contrast to these low plasma concentrations, the median concentration of amikacin in ELF was 976.07 mg/liter, with no correlation between ELF concentrations and ventilator settings. Tracheal secretions were also collected at random and showed amikacin concentrations ranging from 1,517.5 mg/liter to 472 mg/liter at various time points from 1 to 24 h postdose. Two adverse events of nephrotoxicity and bronchospasm were considered possibly related to the study drug. In this study, pulmonary concentrations of amikacin were >10-fold higher than the CLSI breakpoint for *P. aeruginosa*, while serum concentrations were trivial. There were very wide variations in ELF amikacin concentrations in this study due to the small numbers and interpatient variability of critically ill patients, although the minimum concentrations were always maintained above the MIC for common VAP pathogens (186).

The above-discussed AFIS combination was explored in a phase I, placebo-controlled, dose escalation study in patients undergoing mechanical ventilation (187). An in-line vibrating-membrane nebulizer placed on the inspiratory arm was used to deliver the AFIS in this study. The nebulizer was placed 15 cm upstream of the Y piece, and the ventilator humidifier was left on during study drug administration. Patients with either VAP or ventilator-associated tracheobronchitis (VAT) were enrolled from a single intensive care unit (ICU) in Australia. Each patient received three ascending doses with one dose administered daily. On day 3, patients randomly received either placebo or the study drug. Three cohorts of patients were enrolled, with three patients per cohort. Doses of AFIS ranged from 100 and 40 mg to 500 and 200 mg of amikacin-fosfomycin, respectively. Systemic and tracheal concentrations of amikacin and fosfomycin were measured serially up to 24 h after dose 3. Eight adult male patients completed the study, seven of whom had VAP and one of whom had VAT. Five patients had monomicrobial Gram-negative infections, and all 8 were receiving concomitant systemic antibiotic therapy. Patient demographics were well balanced between the dosing cohorts, and the majority of patients were trauma victims requiring mechanical ventilator support. There were no adverse events observed throughout the study, and three patients, one from each dosing cohort, demonstrated clinical improvement and were extubated prior to the third dose of the study drug. Importantly, mean amikacin plasma C_{max} values never exceeded 1 mg/liter, even at the highest administered dose. Amikacin tracheal concentrations increased proportionally with a dose up to 300 mg, ranging from 6,103.33 to 11,617.50 μg/g from 100 to 400 mg, respectively. In contrast, the fosfomycin tracheal concentrations showed no relationship to dose. The mean plasma C_{max} did not exceed 0.5 mg/liter. The mean tracheal C_{max} reached an asymptote of ~6,000 μg/g after the 80-, 120-, and 160-mg doses. Based on the results of this study, the 300-mg–20-mg dose was selected for the above-mentioned phase II study.

Ciprofloxacin. The Pulmosphere DPI technology has also been applied to a zwitterionic betaine salt form of ciprofloxacin in a phase I study of healthy volunteers (188). Six male subjects were given a single dose of 32.5 mg of ciprofloxacin or placebo via a portable passive DPI. After inhalation, subjects were instructed to drink 240 ml of water in order to wash any powder remaining in the oral cavity into the gastrointestinal tract. Plethysmography was used to measure lung function, and serial blood and urine samples were collected up to 48 h postdose. Unique to the literature on inhaled antibiotics, this study utilized a physiologically based pharmacokinetic model in order to estimate the total and regional depositions of the ciprofloxacin DPI after inhaled admin-

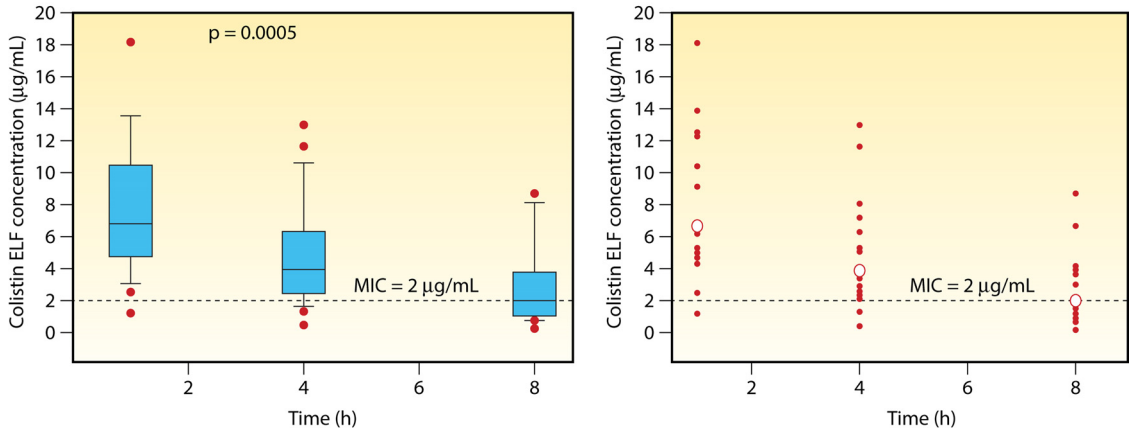


FIG 4 Colistin concentrations in ELF 1, 4, and 8 h after treatment with 80 mg of inhaled CMS. (Left) Median and interquartile range values; (right) individual concentrations. The dashed line represents the MIC of colistin for *A. baumannii* and *K. pneumoniae*. (Republished from reference 192 with kind permission from Springer Science+Business Media. © Copyright jointly held by Springer and ESICM 2012.)

istration. This model included the fraction of the dose deposited in the oral cavity and ingested as an oral dose, the fraction deposited in the trachea and bronchi, the fraction of the dose deposited in the deep alveolar space, and the half-life of mucociliary clearance. Previously simulated concentration-time profiles after intravenous and oral dosings of ciprofloxacin were also incorporated and normalized to the dose of inhaled ciprofloxacin used in this study. The model was used to fit the parameters of the three deposition fractions to the observed concentration-time profiles for each subject after inhalation. Maximum plasma concentrations of ciprofloxacin occurred 15 min after inhalation but never exceeded 0.065 mg/liter. The terminal systemic half-life after inhalation was 9.5 h. The geometric mean AUC in plasma after a single inhaled dose was 0.35 mg · h/liter. Complete urine collection data were available for only two subjects, for whom the amounts excreted in the urine over 48 h were 21.4 and 26.9% of the dose. Based on the pharmacokinetic model, the half-life for mucociliary clearance was ~10.8 h, and an average of 17.2% of the dose was deposited within the alveolar space. Overall, the ciprofloxacin DPI was well tolerated. Five of the six subjects complained of mild, transient dysgeusia after inhalation of the study drug, which resolved spontaneously. One subject experienced a marked reduction in FEV_1 values after inhalation of ciprofloxacin but did not report any subjective pulmonary complaints. There were no abnormal laboratory values observed in this study. The overall systemic absorption of inhaled ciprofloxacin in this study was substantially lower than the exposure seen after oral or intravenous dosing, despite the longer half-life observed in this study. This increased half-life can be attributed to the mucociliary clearance of inhaled ciprofloxacin and subsequent delayed absorption via the gastrointestinal tract.

Colistin. As discussed above, colistin is delivered as the prodrug formulation CMS, which requires *in vivo* conversion to the active compound colistin sulfate. This requirement for conversion makes achieving adequate drug concentrations challenging, especially within the pulmonary space. Studies on the intrapulmonary concentrations of colistin after i.v. administration in humans have reported conflicting results (189–191), while the animal studies discussed above, in which CMS or colistin was given via inhalation, showed higher pulmonary concentrations with lower systemic concentrations. In a human study, the pulmonary PK of CMS in patients with VAT in an ICU were assessed after a single inhalation. Patients received monotherapy with 80 mg inhaled CMS every 8 h via a vibrating-mesh nebulizer over 30 min for 7 days. The nebulizer was placed 15 cm from the Y piece in a nonhumidified ventilator circuit, and all patients had fixed ventilator settings in the assist control/volume control mode with a set respiratory rate, tidal volume, and peak-end expiratory pressure (PEEP). Twenty patients completed the study, 16 of whom achieved clinical cure. There were no significant changes in renal function in any patient. Median colistin concentrations in the ELF at 1, 4, and 8 h determined by BAL were 6.73 mg/liter, 3.9 mg/liter, and 2.0 mg/liter, respectively. The majority of the included patients (11 patients) had *Acinetobacter baumannii* cultured, with MICs ranging from 0.5 to 1 mg/liter, indicating that ELF colistin concentrations were maintained at or above the MIC up to 8 h after a single dose. As with previous studies including small cohorts of patients, ELF concentrations varied widely between patients, with coefficients of variation ranging from 56.4 to 89.4%. At the same time intervals, median colistin concentrations in serum were 1.2 mg/liter, 0.75 mg/liter, and 0.31 mg/liter, respectively. These concentrations translated into AUC_{0-8} values in ELF and serum of 29.8 mg · h/liter and 6.8 mg · h/liter, respectively (192). Figures 4 and 5 detail median and individual colistin concentrations in ELF and serum over time in relation to MIC breakpoints. Given the substantial proportion of patients (8/20) with ELF concentrations below 2 mg/liter at 8 h postadministration, higher initial doses may be needed for patients with suspected infections due to Gram-negative bacteria with higher MIC breakpoints, such as *P. aeruginosa* (breakpoint MIC of 4 mg/liter). A higher dose would also increase the AUC, as the PD index associated with efficacy for the polymyxins seems to be dependent on the AUC/MIC ratio (193). Given the low serum concentrations achieved in this study, a higher dose may still avoid systemic toxicity. Of note, this study used nonbroncoschopic BAL techniques, so different portions of the lungs were likely sampled in different patients. Also, these patients were critically ill and had VAT as opposed to bronchopneumonia. Finally, given the requirement for conversion from CMS to formed colistin, inhaled colistin sul-

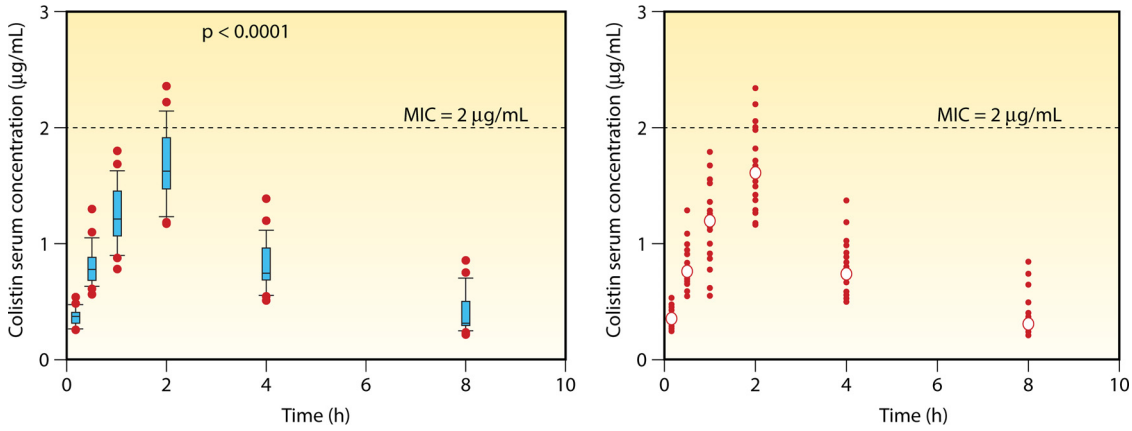


FIG 5 Colistin concentrations in serum 0.16, 0.5, 1, 2, 4, and 8 h after administration of 80 mg of inhaled CMS. (Left) Median and interquartile range values; (right) individual concentrations. The dashed line represents the MIC of colistin for *A. baumannii* and *K. pneumoniae*. (Republished from reference 192 with kind permission from Springer Science+Business Media. © Copyright jointly held by Springer and ESICM 2012.)

fate may also be used initially to achieve higher concentrations at the expense of increased adverse reactions, as discussed above.

Two studies have evaluated the safety and efficacy of CMS in a DPI in healthy volunteers and patients. It was well tolerated by healthy volunteers but achieved suboptimal pulmonary deposition compared to liquid nebulization. In patients, decreases in pulmonary function and severe coughing after inhalation were experienced. As discussed above, DPIs require good inspiratory effort from the patient in order to administer a sufficient dose. In these studies, a Twincer inhaler was used, which combines high dispersion effectiveness with high internal resistance. This generates a maximal fine-particle fraction with low inspiratory effort, therefore reducing the dependency for effectiveness on inspiratory flow rate and patient effort. In the first study, eight healthy volunteers inhaled 25 mg of dry-powder CMS in equal divided doses (194). Participants were instructed on the proper use of the inhaler and inhaled the 25-mg dose of CMS on day 1. On day 2, each subject swallowed 80 mg of CMS dissolved in 3 ml of normal saline on an empty stomach to evaluate the gastrointestinal absorption of colistin. Pulmonary function tests were performed on each subject at baseline and 5 and 30 min after inhalation. Serial blood sampling was performed after inhalation to 24 h postdose. After ingestion of CMS, blood sampling was performed at 1 and 3 h postdose. None of the subjects experienced any adverse events, and there were no clinically significant changes in lung function test results throughout the study. The mean plasma colistin C_{max} observed was 0.089 mg/liter, with a half-life of 2.75 h, equating to an AUC_{0-4} of 0.275 mg · h/liter. Interestingly, serum concentrations of colistin after oral ingestion remained below the limit of quantification (0.01 mg/liter), indicating that there is no meaningful contribution of gastrointestinally absorbed colistin after inhalation to total systemic exposure. These results confirm the minimal systemic absorption of colistin after inhalation of CMS and also reinforce the ability to inhale higher doses, in milligrams, via a DPI with the use of a Twincer inhaler.

A second study examined the use of a colistin DPI in both healthy volunteers and patients with CF (195). Importantly, this study utilized a dry-powder formulation of colistin sulfate and not CMS as in the above-described study. Six healthy volunteers and five patients with stable CF who were already being treated with daily nebulized colistin for at least 6 months were included. Patients were asked to stop taking nebulized colistin at least 3 days prior to the day of study drug administration. After the 3-day hiatus, patients were given 160 mg of colistin via a jet nebulizer on day 4, followed by serial blood sampling. On a second visit, after another 3-day hiatus from nebulized colistin, patients were asked to inhale 25 mg of colistin sulfate via a DPI, followed by serial blood sampling. Healthy volunteers were given only 25 mg of colistin via a DPI. The DPI used in this study was developed in-house by these authors in a previous study (196). Blood samples were drawn prior to and up to 4 h postdose. Pulmonary function tests were performed before and 3 h after inhalation.

CLINICAL OUTCOMES

The suboptimal outcomes achieved in treating Gram-negative LRTIs, despite maximal parenteral antibiotic therapy, combined with the rapid and unrelenting increase in bacterial resistance among these pathogens have fueled the demand for new antimicrobials and spurred the use of existing antibiotics in innovative ways, including alternate routes of administration. Although inhaled antibiotics have been used to treat patients with respiratory infections for decades, reports on efficacy and safety have been almost primarily restricted to patients with CF. Table 2 displays the currently available antibiotics specifically manufactured for inhaled use and their corresponding FDA-approved indications. The more pervasive use of inhaled antimicrobials in recent years has led to a promising increase in the amount of available data associated with this mode of administration in a myriad of different pulmonary pathophysiological conditions. Unfortunately, although the overall volume of data has increased, the quality of the clinical outcome studies evaluating inhaled antibiotics has not improved proportionally. The vast majority of clinical outcome studies that examine the use of inhaled antibiotics are retrospective and observational in nature, suffering from significant heterogeneity and a lack of rigor for patient selection, definition of clinical endpoints, and methodology for the evaluation of patients. The level of detail in which the following studies are discussed is relative to both the number and quality of studies in that specific area, and discussions are included as felt appropriate by the authors. Throughout this section, the microbiological terms

TABLE 2 Current commercially available inhaled antibiotic formulations

Antibiotic (trade name)	FDA indication
Aztreonam solution (Cayston)	Improvement of respiratory symptoms in patients ≥6 yr of age with CF, FEV_1 of >25% and <75%, and *P. aeruginosa* infection
Colistin dry powder (Colobreathe)	Not FDA approved; approved by the EMA for management of chronic pulmonary infections due to *P. aeruginosa* in patients ≥6 yr of age with CF
Tobramycin inhalation solution (Tobi)	Management of CF patients >6 yr of age with *P. aeruginosa* infection with FEV_1 of >25% and <75% on a 28-day alternating on/off schedule
Tobramycin inhalation powder (Tobi Podhaler)	Management of CF patients >6 yr of age with *P. aeruginosa* infection with FEV_1 of >25% and <80% on a 28-day alternating on/off schedule

bacterial colonization and infection are used to convey the presence of bacteria with no clinical signs or symptoms of infection and clinical signs or symptoms of infection due to the invasion of bacteria, respectively.

Non-CF Bronchiectasis

In contrast to pneumonia, the pathogens involved in bronchiectasis are found primarily in the sputum and bronchial mucosa. These pathogens release toxins that damage the mucosa and recruit neutrophils, leading to further insult. This injury prevents normal mucociliary clearance and exposes receptor molecules on the mucosal surface, allowing bacterial adherence and eventually allowing bronchial epithelial cell invasion by the respective infecting pathogen. These alterations also lead to increases in antimicrobial penetration, as the barriers to antibacterial drug movement are relatively permeable in the bronchial capillary endothelium, as it is devoid of zonula occludens.

While early reports of suppurative lung disease date back to the 19th century, primarily credited to Laennec (197), the contemporary defining characteristics of this lung disease are found upon computed tomography imaging. A bronchus with an internal diameter larger than the accompanying vessel or a bronchus that fails to taper at the periphery of the lung identifies subjects with bronchiectasis (198). Both genetic, particularly CF transmembrane conductance regulator gene mutations in CF patients, and environmental factors have been linked to bronchiectasis.

Bronchiectasis not owing to CF, is a "catchall" classification for a number of similar and possibly overlapping conditions that result in airway distortion and destruction. It is a condition that has increasing prevalence with age, and recognition of this disease has been steadily increasing (199). The pathogenesis is thought to arise as an exaggerated inflammatory response to lung tissue injury and/or infection (200). In this vicious-cycle hypothesis, a triggering event leads to an inflammatory response, which leads to structural damage to the airways and impaired mucociliary clearance. This milieu favors retained mucous and creates an environment that predisposes to bacterial colonization. This in turn promotes sustained inflammation, thus creating a vicious cycle leading to the disease state. Non-CF bronchiectasis represents a complex disease in which a multimodal approach to management targets the various factors responsible for the vicious cycle of disease.

Bronchiectasis is characterized by inflammation, dilation, infection, and sputum expectoration along with cartilage and epithelial destruction of the airway lining. While toxic exposures and postinfectious bronchiectasis are most common in developing countries, there are many etiologies of bronchiectasis in developed countries, including congenital diseases, bronchomalacia, and abnormalities resulting in impaired mucociliary clearance. In many cases, the true underlying cause is undetermined (201). The diagnosis of bronchiectasis is made largely based on radiographic data from computed tomography scans, along with the patient's background and clinical signs and symptoms. Patients with bronchiectasis have chronic low-level inflammation and low-grade infection, which can be exacerbated by superimposed viral or bacterial infections as well as other airway irritants. These patients often suffer from persistent cough, sputum expectoration, recurrent infections, and a large reliance on medical care, with a poor quality of life. In this way, management of these patients is similar to that of CF patients, consisting of antibiotics for exacerbations, immunizations, proper airway hygiene, and treatment of the underlying etiology if possible (202, 203). Patients with non-CF bronchiectasis have been shown to be infected predominantly with *P. aeruginosa*, *Haemophilus influenzae*, *Prevotella* spp., and *Veillonella* spp. (204). Chronic infection with *P. aeruginosa* is seen in up to 33% of patients with bronchiectasis and is associated with decreased lung function, poor quality of life, and more frequent hospitalizations. Additionally, organisms such as *Staphylococcus aureus* and nontuberculous mycobacteria have been isolated (205, 206). Given the many etiologies responsible for non-CF bronchiectasis along with the extensive flora of potential pathogens, treatment guidelines and algorithms should be adapted to the individual patient scenario. Additionally, antibiotics may be used as long-term therapy or for acute treatment of exacerbations. Along with the economic burden, the incidence of non-CF bronchiectasis continues to rise in the United States, with annual percentages increasing almost 9% every year from 2000 to 2007 (199, 207). Although non-CF bronchiectasis is seen across the whole population, the prevalence appears to be highest in individuals of advanced age and females.

Although CF is pathophysiologically responsible for the majority of cases of bronchiectasis, there are several other medical conditions that can result in this altered lung pathology (202). The encouraging results of treating CF bronchiectasis with inhaled aminoglycosides have led to their extrapolation to other similar disease states. To date, there have been four reported studies evaluating the effectiveness of inhaled tobramycin for non-CF bronchiectasis and chronic *P. aeruginosa* infection (41, 208–210). These studies have consistently demonstrated a significantly decreased respiratory burden of *P. aeruginosa*, similar to that in CF patients. Unlike CF patients, however, the use of inhaled tobramycin in cases of non-CF bronchiectasis has not led to sustained improvement of objective clinical measures such as pulmonary function test results.

As opposed to acute pulmonary infections, where the goal of antimicrobial therapy is to eradicate the infecting pathogen, the aim of treatment of chronic bronchial infections is to reduce the bacterial load and the coinciding inflammatory response. Additionally, chronic bronchial infections represent a different environment of infection, as bacteria are often growing on the surface of the airway epithelium rather than invading in a more virulent manner as in true pneumonia. This chronic airway colonization

often leads to the formation of biofilms, particularly in the case of *P. aeruginosa*. The high doses, prolonged penetration, and increased residence time of inhaled antimicrobials make them a more suitable agent for this particular type of infection. Treatment of CF bronchiectasis with inhaled antibiotics, especially tobramycin, has been shown to improve pulmonary function, decrease the sputum density of bacteria, and reduce the risk of exacerbation (211). Given the similarities in pathophysiologies of cases of CF and non-CF bronchiectasis, it stands to reason that similar results should be observed in this patient population. Chronic infection with *P. aeruginosa* has been associated with a deterioration of lung function, and inhaled antibiotic treatments of both acute and chronic infections have proven beneficial in this population, much like the CF counterpart (212–214). Inhaled antibiotics have been used in patients with bronchiectasis since the 1940s (215) and are currently recommended as treatment strategies in British and Spanish bronchiectasis guidelines (216, 217). A recent review detailed the utility of inhaled tobramycin in the treatment of patients specifically with non-CF bronchiectasis (218).

Tobramycin. Orriols et al. evaluated 17 patients who had failed oral antibiotic therapy for non-CF bronchiectasis at least once in the previous 90 days in a prospective pilot trial. All patients received either i.v. ceftazidime or tobramycin for 2 weeks, after which they were randomized to receive either the same drug given via inhalation for 12 months or symptomatic therapy only. Ceftazidime was given via inhalation through a jet nebulizer at a dose of 1 g every 12 h, and tobramycin was given as 100 mg every 12 h. The frequency and duration of hospitalization were lower in the inhalation treatment group, while bacterial eradication was not achieved in any patient (219).

In a second placebo-controlled crossover trial by the same group, 30 patients received 300 mg of inhaled tobramycin every 12 h in two 6-month cycles separated by a 1-month washout period. Again, fewer and shorter hospitalizations were observed in the treatment periods, along with a decrease in the sputum density of *P. aeruginosa*. Despite these outcomes, no differences were seen with regard to quality of life or systemic inflammatory markers (41).

In a blind, active-control study, the effect of the addition of a twice-daily tobramycin inhalation solution to a 14-day outpatient course of oral ciprofloxacin was examined in patients with non-CF bronchiectasis who were chronically infected with *P. aeruginosa* and suffering from an acute exacerbation. A jet nebulizer was utilized to administer 300 mg of tobramycin twice daily, and oral ciprofloxacin was given at a dose of 750 mg twice daily. A total of 43 subjects completed the study, and there was no statistically significant difference in cure rates at the day 21 test-of-cure assessment, despite a 20.4% numerically higher cure rate in the ciprofloxacin-only group. Clinical success was defined as a reduction in sputum volume, improvement in purulence, and decreased cough and/or dyspnea, as assessed by the investigator. A nonsignificant difference in *P. aeruginosa* eradication from sputum was seen in subjects receiving tobramycin and ciprofloxacin (34.6% versus 18.5%). There was no difference in the emergence of resistance, while microbiological and clinical outcomes showed greater concordance in the tobramycin-plus-ciprofloxacin group (80% versus 40.9%). Wheezing was reported more commonly in the tobramycin arm, as expected, while more patients in the ciprofloxacin-only group withdrew from the study due to adverse events (18.5% versus 11.5%). There was no observed difference in lung function between the groups, as measured by FEV_1, while twice as many subjects in the ciprofloxacin-only group required hospitalization for worsening symptoms. This study follows suit with previous studies indicating that inhaled antibiotic therapy decreases bacterial density and improves eradication but fails to provide improved clinical outcomes. While evidence for patients with CF has shown that clinical success is often associated with a reduction in bacterial density, the studies of non-CF bronchiectasis to date have not shown this same finding. Of note, this study did not assess time to exacerbation or time to next hospitalization, which could potentially coincide with improved bacterial load reduction (208).

An additional phase II, multicenter, placebo-controlled, randomized, double-blind trial was conducted by Barker et al. (209). As opposed to the two studies mentioned above, which used an i.v. formulation of tobramycin in a nebulizer, this study utilized 300 mg of a tobramycin inhalation solution every 12 h. The tobramycin inhalation solution was given daily for 28 days along with placebo. Compared to placebo, tobramycin decreased the bacterial density of *P. aeruginosa* in the sputum and increased clinical improvement, as assessed by the investigators.

In a similar open-label trial, 41 patients received three treatment cycles with a tobramycin inhalation solution for 14 days on followed by 14 days off. After the three cycles, significant improvements in the subjective symptom severity score and quality of life were seen (210).

Gentamicin. In 65 patients who received either 80 mg of inhaled gentamicin or placebo over a year-long period, decreased exacerbations, improved quality of life, and no severe adverse events were observed for the gentamicin group (220). In a separate study, 28 patients with bronchiectasis and mucus hypersecretion received 40 mg of gentamicin inhaled twice daily for 3 days compared to placebo. Sputum myeloperoxidase (MPO) levels were assessed to determine whether gentamicin prevents MPO-induced airway injury. Compared to placebo, MPO levels along with the daily volume of sputum production were significantly reduced in patients receiving gentamicin. Additionally, improvements in the 6-min walking test, the subjective Borg scale, and self-sputum assessment were seen only in the gentamicin group (221).

Ciprofloxacin. In a phase II trial that enrolled 124 patients, inhalation of 32.5 mg dry-power ciprofloxacin twice daily for 28 days via a DPI achieved significant reductions in sputum bacterial loads and was well tolerated. Pathogen eradication was achieved more often in the ciprofloxacin group than in the placebo group, and reports of bronchospasm were minimal (222). Additionally, treatment with 150 mg of inhaled dual-release liposomal ciprofloxacin for 28 days followed by 28 antibiotic-free days showed significant antimicrobial efficacy against *P. aeruginosa* in 42 patients infected with this pathogen at baseline and delayed the time to first pulmonary exacerbation by a median of 76 days. Fewer pulmonary adverse events were observed with inhaled liposomal ciprofloxacin than with placebo (223).

Colistin. In the first controlled clinical trial to use an adaptive aerosol delivery device, patients with non-CF bronchiectasis and chronic *P. aeruginosa* infection were randomized to receive either inhaled colistin or placebo after receiving systemic antibiotics for an acute exacerbation. Of the patients with documented adherence to treatment, the time to next exacerbation significantly improved (224).

Studies have shown that up to half of patients with chronic

obstructive pulmonary disease (COPD) have associated lower lobe bronchiectasis, and this leads to higher rates of microbial colonization and severe exacerbations (225). As such, Steinfort and Steinfort examined the effect of long-term inhaled colistin added to standard treatment regimens in patients with COPD and bronchiectasis. Colistin was given via inhalation as 30 mg daily to 18 patients over a mean treatment duration of 41 months. All of the patients had *P. aeruginosa* or *Stenotrophomonas maltophilia* isolated from their sputum. Only 3 patients (16.6%) showed improvement in FEV_1 over the course of the study, although quality-of-life measures were significantly better after the addition of inhaled colistin to the standard-of-care regimen. No emergence of resistance to colistin was observed, although only 3 patients showed eradication of bacteria. No adverse effects were reported despite the lengthy duration of treatment (226).

COPD often leads to chronic bronchial bacterial infection and colonization, and up to 33% of patients with bronchiectasis are chronically colonized with *P. aeruginosa* (227). The goals for these patients are much like those for CF patients: to prevent exacerbations and reduce the number and frequency of hospitalizations. A study examined the clinical and microbiological outcomes in adult patients with non-CF bronchiectasis or COPD and chronic *P. aeruginosa* colonization who received inhaled tobramycin or colistin alone or in combination via a jet nebulizer as outpatients. Both drugs were given twice daily, and patients received inhaled antibiotics for a minimum of 12 weeks. The doses of inhaled tobramycin and colistin used were either 100 or 200 mg and either 1 million or 2 million IU, respectively. In total, 81 patients were evaluated, the majority of whom received inhaled tobramycin followed by colistin followed by the combination. The primary finding in this study was that therapy with colistin showed better efficacy in eradicating *P. aeruginosa* and less development of resistance than did tobramycin, although there were no differences in clinical outcomes, including the number of hospitalizations. This study suffered from several limitations, including ambiguous inclusion criteria, observational design, differences in dosages and adjunctive antibiotic use that were not accounted for, and results that conflict with many previously reported results, including a treatment-emergent resistance rate of 48% for tobramycin. Most importantly, the outcomes for patients with bronchiectasis and those with COPD were reported together (228).

Aztreonam. Two large, phase III clinical trials evaluating the efficacy of inhaled aztreonam in this patient population have been conducted. Unfortunately, both of these trials failed to show a clinical benefit, and the rates of adverse events were higher in the aztreonam group than in the placebo group (229).

Summary of inhaled antibiotics for non-CF bronchiectasis. Taking the results of these studies together, a meta-analysis evaluated randomized controlled trials of adults with stable non-CF bronchiectasis given inhaled antimicrobials for at least 4 weeks. Eight studies encompassing 590 patients and utilizing amikacin, aztreonam, ciprofloxacin, colistin, gentamicin, or tobramycin were included. All studies administered the agent via a jet nebulizer, with the exception of ciprofloxacin, for which a DPI was used. In terms of sputum load of *P. aeruginosa*, inhaled antibiotics produced a significantly greater reduction in bacterial loads than in control groups and were associated with a 4-fold-higher chance of achieving complete bacterial eradication. Inhaled antibiotics were also shown to significantly reduce the risk of acute exacerbations compared to controls, although the meta-analysis failed to show a benefit in the incidence of hospitalizations. There was also no statistically significant difference in quality-of-life scores between the two groups. The rate of bronchospasm was 10% in the inhaled-antibiotic group, compared to 2.3% in the controls, and was higher with aminoglycosides than with colistin or ciprofloxacin. Despite heterogeneity in the testing methodology, the meta-analysis did not show a statistically significant difference in the emergence of bacterial resistance in the inhalation treatment group compared to the controls. This meta-analysis lends some support to the use of inhaled antibiotics for the treatment of chronic bacterial infections in the airways of patients with stable non-CF bronchiectasis (230). There was significant heterogeneity in this analysis, especially surrounding the duration of inhaled therapy. Additional studies are needed to delineate the ideal drug, dose, frequency, and duration for these patients.

A more recent meta-analysis specifically examined the clinical utility of prolonged inhaled antibiotics for patients with non-CF bronchiectasis (231). The promise of long-term oral antibiotic therapy with macrolides such as azithromycin in this population (212) has led to the discussion of longer courses of inhaled therapies, using either a continuous or rotating schedule. This analysis included all of the same studies as those in the analysis described above and found similar results, i.e., reductions in the bacterial density of *P. aeruginosa* in the sputum and increased eradication with treatment. Again, no difference in the emergence of resistance was observed. In contrast to the above-described analysis, this group determined that patients in the inhaled-antibiotic groups required additional antibiotic therapy less often. There were also fewer exacerbations in the inhaled-antibiotic group. The incidences of wheezing and bronchospasm were higher in the treatment groups. This group did not perform an analysis of hospitalizations due to the modest sample size and heterogeneity. Again, inhaled antibiotics did not improve lung function or quality of life.

Two Cochrane reviews of the use of prolonged antibiotics for non-CF bronchiectasis in children and adults have been completed, with the most recent update being in August 2015 (232, 233). The more current review included 18 randomized trials, 6 of which reported the use of inhaled antibiotics, examining the use of prolonged antibiotic therapy ($\geq$4 weeks) in 1,157 patients. Unfortunately, the outcomes associated with inhaled antibiotics were not analyzed separately. The overall quality of evidence was moderate, although the analysis was limited due to the heterogeneity of outcomes reported in the trials. Overall, significant effects in favor of prolonged antibiotics were observed for the treatment of bronchiectasis. A nonsignificant reduction in hospitalizations was also seen, while a significantly higher rate of drug resistance was observed in the intervention arm. There was no difference in adverse events. The authors of this study stress the need for appropriate patient selection for prolonged antibiotic therapy and call for improvements of the shortcomings of current trials in future studies, including study duration, bacterial colonization versus infection, and drug resistance at the individual and community levels.

The vast majority of data presented in this section for the treatment of non-CF bronchiectasis with inhaled antibiotics lies with tobramycin. These studies most often administered tobramycin at 300 mg every 12 h via a jet nebulizer as adjunct therapy or monotherapy either long term or in an on/off cycle, similar to the way in which it is utilized in CF patients. Other inhaled agents are beginning to be explored in this population, including ciprofloxacin via

a DPI. The theoretical benefit of inhaled antibiotics in this population is largely extrapolated from data for CF patients, and their use is recommended in at least two national guidelines. The results of the included studies show consistently reduced bacterial densities in the sputum of patients with non-CF bronchiectasis and a positive effect on subjective quality-of-life measurements. With the exception of aztreonam, pulmonary adverse events were rarely reported, even after prolonged use (>3 years).

Unfortunately, unlike for patients with CF, few of the trials discussed above showed an improvement in objective lung function as measured by FEV_1. Patients with non-CF bronchiectasis may be more difficult to treat than CF bronchiectasis patients, as other studies evaluating inhaled antibiotics have also failed to show an improvement in FEV_1, which may be due in part to non-CF bronchiectasis being precipitated by a fixed airflow obstruction in many cases (222–224). A limitation to the studies examining inhaled antimicrobials for non-CF bronchiectasis is a lack of information regarding the pathophysiology of the disease. The vast majority of the patients in the trials conducted in the United States were smokers. Patients with non-CF bronchiectasis are often older and a much more heterogeneous population, so different dosages and dosing strategies may also need to be considered for these patients. Additionally, the heterogeneity in dosing regimens and clinical endpoints makes interpretation and extrapolation of these results challenging. Despite encouraging outcomes regarding reductions in sputum bacterial density and microbial eradication, inconsistent and/or a lack of results with regard to quality-of-life improvement, a reduced need for systemic antibiotics, and amelioration of objective pulmonary function create pause when applying these findings to all patients with non-CF bronchiectasis. Additionally, the heterogeneity in the non-CF bronchiectasis population compared to patients with CF leaves many questions unanswered as to the ideal patient for whom to prescribe this therapy and whether the underlying etiology has an effect on outcomes. Future directions for this population include studies utilizing liposomal formulations of inhaled antibiotics, given the prevalence of biofilms and the need for sputum penetration of the upper airways in these patients. Additionally, cohesive outcome definitions and consistency in the methods of observing these patients will help the generalizability of results from forthcoming studies. In the future, validated scoring systems such as the bronchiectasis severity index may assist in determining which patients stand to benefit the most from inhaled antibiotic therapy (234).

Based on the currently available evidence, there are at least two scenarios in which inhaled antibiotics can provide benefit to patients with non-CF bronchiectasis. The first scenario is that of patients experiencing an acute exacerbation who currently have or previously had a Gram-negative organism, particularly *P. aeruginosa*, isolated from a sputum culture. This is particularly true for patients who have a fluoroquinolone-resistant *P. aeruginosa* strain isolated and have no other oral antibiotic options but do not require i.v. antibiotic therapy based on the severity of their illness. Inhaled antibiotic monotherapy represents an attractive selection in this scenario, as the patient is able to leave the hospital without a central venous catheter, home health care, or routine laboratory monitoring. Inhaled antibiotics may also be used in addition to systemic therapy in this scenario for those patients who require dual therapy based on the severity of their illness. In either case, the chosen inhaled agent to be used should be based on the availability of the appropriate inhalation solution that is specifically formulated for aerosol delivery and the associated proper delivery device (Table 2). Until site-specific respiratory microbiological breakpoints that take into account the concentrations achievable in the sputum and lungs with inhaled antibiotics are widely available, resistance to the chosen inhaled agent reported upon *in vitro* susceptibility testing should not deter the use of that agent. Concentrations above the maximum MIC for that bug-drug combination can be easily achieved by inhalation, and symptomatic improvement often occurs in this patient population despite *in vitro* resistance to the inhaled agent. The duration of treatment of acute exacerbations of non-CF bronchiectasis should be up to 14 to 21 days, depending on the clinical response of the patient. The second scenario is that of patients with non-CF bronchiectasis who experience frequent exacerbations of bronchiectasis per year requiring antibiotic therapy or those with exacerbations causing significant morbidity, regardless of frequency. Patients who meet these criteria and have had documented respiratory cultures positive for Gram-negative organisms, especially *P. aeruginosa*, should receive either chronic, continuous inhaled antibiotic therapy or therapy in a 28-day on/off cycle as for patients with CF. Again, the choice of agent should be based primarily on feasibility and having the appropriately formulated compound and delivery device and less on *in vitro* susceptibility reports. As discussed above, it is important to remember that the goal of antimicrobial therapy in cases of non-CF bronchiectasis is not to eradicate the infecting pathogen but to reduce the bacterial load and the coinciding inflammatory response. The aim of treatment of cases of non-CF bronchiectasis with inhaled antibiotics should be to improve pulmonary function, decrease the density of bacteria in sputum, and reduce the risk of a future exacerbation. Although complete pathogen eradication may not occur during or after treatment with inhaled antimicrobials, the emergence of resistant bacterial pathogens was rare in the available studies. Therefore, a lack of sterilization should not preclude the use of inhaled therapy for this disease state, as the benefits of this route of administration stretch beyond simple microbiological endpoints.

Ventilator-Associated Tracheobronchitis

This section focuses on nosocomially acquired tracheobronchitis in patients undergoing mechanical ventilation via an endotracheal tube. Although acute exacerbations of chronic bronchitis and acute bronchitis can occur in patients with COPD, these infections are even less well defined than VAT and have significantly different underlying pathophysiologies.

VAT can be most easily defined as an LRTI involving the conducting zone of the lung, i.e., the tracheobronchial tree, while sparing the gas exchange zone, or the lung parenchyma.

VAT represents a wide, largely undefined spectrum of diseases with many ambiguous clinical definitions (235, 236). Nosocomial LRTIs occur more commonly in patients undergoing mechanical ventilation due to the ability of the endotracheal tube to bypass natural host lung defenses, permit the leakage of bacteria from the upper airway and oro-/nasopharynx directly into the lower airway, prevent the removal of bacteria into the esophagus due to the balloon cuff, and damage the ciliated epithelium of the trachea. Additionally, the endotracheal tube is an ideal environment for the formation of biofilms. Arguably, the most widely accepted definition of VAT includes the signs and symptoms of an LRTI with an absence of radiographic evidence of such. Based on the

limited existing literature, the incidence of VAT ranges from 2.7 to 10%, with common inciting pathogens similar to those for VAP, including *P. aeruginosa*, *A. baumannii*, and methicillin-resistant *S. aureus* (MRSA). Much like VAP, VAT has been associated with an increased length of stay in an ICU and a longer duration of mechanical ventilation. The primary risk factor for VAT appears to be colonization of the endotracheal tube and lower airway with nosocomially acquired pathogens within the nasopharynx. In contrast to VAP, innate host defenses may succeed in defeating the overwhelming infectious process and stemming the tide of full-blown VAP from VAT. However, VAT may still persist due to the tracheobronchial colonization acquired during this process. Given that pulmonary infiltrates may not be seen or may be difficult to see, especially in early VAP, distinguishing between VAP and VAT is extremely challenging (237). Additionally, the inherent imprecision of chest radiographs, particularly the portable versions, in patients who are mechanically ventilated would require routine chest computed tomography scans in order to more conclusively differentiate VAT from VAP. From a clinical standpoint, it is reasonable to believe that VAT exists given postmortem studies demonstrating high bacterial loads in patients without histopathological changes indicative of pneumonia (238–240). An algorithm for the diagnosis of VAT has been proposed, in which the diagnostic criteria for VAP put forth by the American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) are modified slightly (241). These guidelines also regard VAT as a cause of LRTI and an alternate diagnosis in patients with questionable VAP. These experts regard VAT as a step in the dynamic equilibrium from colonization to pneumonia. In this definition of VAT, the patient would have the same clinical signs and symptoms as those associated with VAP. The criteria for microbiological diagnosis based on an endotracheal aspirate would remain the same. The difference would come in the lack of a new or progressive infiltrate upon chest imaging and a BAL fluid sample (if BAL is performed) with $<10^4$ CFU/ml of bacteria. This definition seems to make sense given the proposed lack of lower respiratory pathophysiology associated with VAT. VAT may also be thought of as an initial disease on the early progression pathway to VAP. In this sense, VAT could be used as a marker for early treatment initiation in order to prevent full-blown VAP. This could potentially be achieved by utilizing serial surveillance endotracheal aspirate cultures and initiating antibiotic therapy before the onset of symptoms. Initiation of treatment during VAT may be prudent given previous findings that showed increases in lower respiratory tract colonization over the duration of mechanical ventilation, with a peak occurring ~2 days before the emergence of clinical signs and symptoms of VAP (242, 243). This window of microbial influx before symptoms appear could represent VAT and a viable window in which to begin therapy with antimicrobials, although this requires further research, as at least one study has shown a lack of a protective effect against subsequent VAP for antimicrobial treatment in patients with VAT (244). In the future, it may be possible to improve the diagnosis of VAT by utilizing the standard methodology for diagnosing VAP. For instance, during BAL fluid sampling, the first aliquot of saline is often disregarded as representing the upper airway and bronchial region and is normally not analyzed for culture for the diagnosis of VAP. Comparison of the bacteriology of this first aliquot to those of subsequent aliquots could help differentiate VAT from VAP and allow improved patient selection for inhaled antimicrobials.

Gentamicin. The first reported study to investigate the therapeutic effect of inhaled antibiotics in mechanically ventilated patients was conducted by Palmer et al. in 1998 (42). These authors sought to confirm the adequacy of the delivery of gentamicin and amikacin to the airway and defined appropriate measures of response. In this study, 6 patients received either 80 mg radiolabeled gentamicin every 8 h or 400 mg amikacin every 8 or 12 h for 2 to 3 weeks. Inspiratory and expiratory filters captured drug particles for measurement of deposited fractions. Patients with an existing tracheostomy tube and purulent tracheobronchial secretions but no radiographic evidence of pneumonia were enrolled prospectively. All patients included in this study were male, and four of the six had *P. aeruginosa* isolated from tracheobronchial cultures either alone or with another Gram-negative organism. Tracheal aspirate volumes, antibiotic concentrations in tracheal aspirates, and levels of inflammatory markers (tumor necrosis factor alpha [TNF-α], interleukin-1β [IL-1β], soluble intracellular adhesion molecule 1, and human leukocyte elastase) were measured for all patients. The mean amount of nebulized drug deposited in the lungs was 21.9%, while 9.5% was found on the ventilator or tracheostomy tube. The mean sputum concentrations on day 5 30 min to 1 h after inhalation were 1,179 mg/liter and 5,353 mg/liter for gentamicin and amikacin, respectively. Serum concentrations were below the quantifiable limit in all but one patient, who had underlying renal dysfunction. The administration of inhaled aminoglycosides decreased the volume of tracheobronchial secretions by ~50% in all patients over the course of the study. Weekly microbiological cultures showed eradication of *P. aeruginosa* from >70% of patients, and Gram stains for almost 80% of patients showed no Gram-negative bacilli. Despite this, three patients had an isolate cultured that was resistant to the administered antibiotic during therapy. After antibiotic pressure was removed, susceptible organisms were cultured after the end of antibiotic therapy. There were no significant decreases in the levels of any of the inflammatory markers studied. This study demonstrated the feasibility of administering inhaled antibiotics to mechanically ventilated patients with tracheostomy tubes and provided the methodological foundation for future studies in this area.

In a study focused on the prevention of Gram-negative LRTIs in patients with a tracheostomy tube, 80 mg of gentamicin was administered via a plastic catheter introduced deeply into the trachea three times daily for an average of ~3 days per patient. Endotracheally administered gentamicin significantly reduced the frequency of purulent secretions and positive tracheal aspirate cultures compared to placebo, although 56% of patients given gentamicin still had positive cultures, including those with organisms susceptible to gentamicin. Systemic antibiotics were given more frequently for presumed nosocomial pneumonia for patients in the placebo group, and more deaths due to pulmonary infection were observed in the placebo group (25% versus 8.65%; the *P* value was nonsignificant [NS]). Concentrations of gentamicin were measured in bronchial secretions and plasma and were determined to be 230 mg/liter and 2.7 mg/liter, respectively, although the precise timing of these measurements was unclear (39). As this study was conducted by using endotracheally instilled gentamicin, it is not known how these results would compare to inhaled therapy for prophylaxis.

In one of the most well-conducted studies on VAT, Palmer et al. completed a randomized, double-blind, placebo-controlled trial in critically ill patients requiring mechanical ventilation for at

least 72 h (245). In this study, VAT was defined by the volume of secretions produced. Patients who produced ≥2 ml during a 4-h period with organisms seen on a Gram stain were randomized to receive inhaled antibiotics or placebo. If the Gram stain showed Gram-negative organisms, gentamicin was administered as 80 mg every 8 h via a jet nebulizer for 14 days in order to attempt to cure VAT and prevent progression to VAP. Patients with Gram-positive organisms on a Gram stain were given inhaled vancomycin. Forty-three patients completed the study, with 19 receiving inhaled antibiotics and 24 receiving placebo. When measured over the 14-day study period, clinical signs and symptoms of VAP decreased from 73.6% on day 1 to 35.7% on day 14 in the treatment group, compared to 75% and 78.6%, respectively, in the placebo group. After controlling for age, patients in the treatment group were 77% less likely to demonstrate clinical signs and symptoms of VAP than those in the placebo group. Patients in the treatment group also had a significantly higher reduction in bacterial growth, as expected, while no emergence of resistance was observed for any of the 19 patients on inhaled antibiotics. Mortality rates were similar between the groups, while more patients in the treatment group were able to be weaned from the ventilator. Importantly, a significantly higher number of patients in the placebo group were started on systemic antibiotics during the study period than in the treatment group. Therefore, even in patients with overlapping VAT and VAP, the blind treating physicians viewed the inhaled placebo group as clinically worsening more often. The most important limitation to this study was the lack of consideration for clinical signs and symptoms in the diagnosis of VAT, which was based solely on the volume of sputum production. In this trial, only 11 of the 43 patients included had only VAT as defined by the inclusion criteria, while the remaining patients had signs and symptoms consistent with VAP. Even so, in the 5 patients randomized to receive treatment from this group of 11 patients, none progressed to VAP (245).

Polymyxins. The most recent study on polymyxins, a short report, detailed the effect of monotherapy with inhaled colistin for the treatment of VAT due to colistin-only-susceptible Gram-negative pathogens. Patients with diagnosed VAT as defined by the definition mentioned above who were not receiving systemic antibiotics received 1 million U of inhaled colistin three times daily for 1 week using a vibrating-mesh nebulizer. Only 12 patients were included, and the most commonly isolated organism was *P. aeruginosa*, followed by *A. baumannii*. Clinical cure was achieved in 9/12 patients, and microbiological response and eradication were achieved in 8/9 cured patients, 5 of whom showed complete eradication. There was no VAT-related mortality in this study, and inhaled colistin was well tolerated. This study provides encouraging results on the effectiveness of inhaled therapy in patients with VAT who were not receiving concomitant systemic antimicrobials and with almost panresistant Gram-negative organisms (246).

In a case series detailing patients with diagnosed VAT for whom VAP was excluded and systemic antibiotics were not given, VAT was defined as the presence of new purulent secretions after at least 48 h of mechanical ventilation, with no new infiltrates on a chest radiograph. Colistin was administered as CMS in 625,000 U every 12 h over 15 min via a jet nebulizer for 7 days. Twenty patients were included, 17 of whom had MDR *P. aeruginosa* cultured from respiratory secretions. Ninety-five percent of the 20 patients had negative tracheal aspirate cultures at day 7, and the volume of secretions significantly decreased. Two patients developed mild bronchospasm, which did not require discontinuation of treatment. However, two patients also developed acute kidney injury. Only 1 patient went on to develop VAP in this study (247).

A second case series described the outcomes for three patients with nosocomial pneumonia or tracheobronchitis due to MDR *P. aeruginosa* who were treated with inhaled colistin in addition to systemic therapy. All cases reported successful clinical outcomes, with no adverse events (248). A small case series also documented favorable outcomes of inhaled colistin in two children with VAT due to *A. baumannii* and *P. aeruginosa* (249).

A third case series reported the outcomes for 19 patients meeting criteria for pneumonia (14) or tracheobronchitis (5) who were treated with inhaled polymyxin B over a 3-year period (5). The majority of patients with pneumonia had received i.v. polymyxin B without improvement, while patients with tracheobronchitis received inhaled polymyxin B alone. Importantly, bacterial isolates were not tested for susceptibility to polymyxin B. The dose of polymyxin B was 500,000 U given twice daily through a "conventional inhaler" along with pretreatment with a β-agonist. The majority of patients also received other concomitant systemic antibiotics. Most patients (84%) had MDR *P. aeruginosa* infection and received an average of 14 days of inhaled polymyxin B. Four patients reported cough and/or bronchospasm. Ten patients (53%) achieved investigator-assessed cure, and 8 achieved improvement in their clinical status, including all cases of tracheobronchitis (250).

Summary of inhaled antibiotics for VAT. The collection of studies included in this section primarily evaluated the use of 80 mg inhaled gentamicin every 8 h for patients with VAT. These studies include one fairly high-quality, randomized, double-blind, placebo-controlled trial by Palmer et al. (245) showing a significantly decreased progression to VAP after treatment of patients with VAT with inhaled antibiotics. Overall, there are very limited data with regard to inhaled antibiotics in patients with VAT, while systemic antibiotics seemed to provide both microbiological and clinical benefits in this population. None of these studies reported adverse events associated with inhalation therapy, and more recent, high-quality studies do not demonstrate the emergence of bacterial resistance.

In a recent meta-analysis, the frequency, outcome, and treatment of VAT were reviewed and analyzed. Seventeen articles including data on 7,056 patients were examined. The incidence of VAT was determined to be 11.5% (386/3,362). There was significant heterogeneity in the methods of establishing a microbiological diagnosis of VAT, although the majority of studies utilized endotracheal aspirate cultures. The most common pathogen causing VAT was *P. aeruginosa* (27%), followed by *Acinetobacter* spp. (18%). There were no studies evaluating inhaled antibiotics for the prevention of VAT. Overall, antimicrobial therapy (systemic antibiotic with or without an inhaled antibiotic) was not found to be associated with lower rates of mortality than those associated with placebo or no treatment. The majority of studies (3/4 studies) showed that antimicrobial treatment did not decrease the duration of mechanical ventilation or length of stay in an ICU, although two randomized controlled trials showed that the frequency of progression to VAP was lower in patients receiving antimicrobial therapy for VAT (251). Nseir and colleagues completed several well-designed trials with patients with VAT indicating that systemic antibiotic therapy improves outcomes and re-

duces the progression to VAP, although these studies did not examine inhaled therapies (252). Despite the limited number of reported studies evaluating the use of inhaled antibiotics for VAT, given the localized pathophysiology, it is sensible to conclude that inhaled agents may be equally as effective as systemic agents with regard to clinical and microbiological outcomes. Even with the heterogeneity in diagnostic criteria and treatment modalities reported in studies of inhaled antibiotics in patients with VAT, a general trend toward improved outcomes and reduced progression to VAP seems to be present. Further well-controlled studies are needed in order to ascertain the true benefit of antimicrobials in this patient population, particularly with regard to inhaled agents.

Based on the currently available evidence, inhaled antibiotics have an important role in the treatment of VAT. Inhaled antibiotic therapy may be used as monotherapy in patients who exhibit clinical signs and symptoms of VAT without radiographic evidence of pneumonia, have a Gram-negative isolate cultured from sputum or tracheobronchial secretions, have symptoms localized to the respiratory tract, and are not systemically ill or hemodynamically unstable. In patients with VAT who have a greater severity of illness at onset and require systemic antimicrobial therapy, inhaled antibiotics should be added as adjunct therapy. For patients who are treated with systemic antibiotics alone for VAT and progress to VAP, inhaled antibiotics should be added to the treatment regimen in order to improve microbiological eradication from the upper airway and ET tube. Inhaled antibiotics should be given for the equivalent recommended duration of systemic therapy for patients with Gram-negative nosocomial pneumonia and should be dependent on the type of organism, with *P. aeruginosa* and other nonfermenting Gram-negative pathogens requiring a longer course of therapy. The choice of the inhaled agent should depend on the availability of the agent that is specifically designed for inhaled administration along with the appropriate delivery device, with less consideration given to *in vitro* microbiological susceptibilities.

Nosocomial Pneumonia

Nosocomial pneumonia is the leading cause of death from hospital-acquired infection (23). Aspiration, inhalation, hematogenous spread, and direct inoculation are mechanisms by which pathogenic bacteria can access the lower respiratory tract. Although the mechanisms are identical to those for community-acquired pneumonia, patients subjected to a health care environment are at a higher risk of exposure to many of these factors. Microaspiration is believed to be the most common mechanism for the development of pneumonia, and patients within the health care system are more likely to have exposure to and colonization of the oropharynx and gastrointestinal tract by pathogenic organisms (253). The endotracheal tube in an intubated patient is an important risk factor for pneumonia, providing a direct route past the defenses of the upper airway in addition to impairing mucociliary clearance and pooling of secretions around the endotracheal tube itself. Pulmonary infections are among the frequently acquired infections in the health care system and have been divided into three categories based on timing and risk factors contributing to the infection: health care-associated pneumonia, hospital-acquired pneumonia, and VAP.

Gram-negative bacteria have become of specific interest owing to the emerging resistance among particular pathogens, including *P. aeruginosa*, *A. baumannii*, *Enterobacter* spp., and *Klebsiella* spp. Multidrug-resistant organisms become more frequent with factors including recent or prolonged hospitalization, recent antibiotic use, or residence in a nursing home or long-term-care facility (254). Unfortunately, an increasing trend of multidrug resistance has become all too commonplace among many institutions throughout the United States and the world (255). In the most severe infections, there is clear evidence that an appropriate choice of empirical antibiotics is associated with improved outcomes (256). Experts have laid out recommendations to prevent the further progression of multidrug resistance by focusing on appropriate empirical therapy, optimization of antimicrobial pharmacodynamics to overcome resistance, limiting inappropriate antimicrobial exposure, and surveillance of drug-resistant pathogens with early patient isolation to prevent the dissemination of these organisms (28, 257).

In patients with nosocomial pneumonia, administration of antimicrobials directly to the respiratory tract is associated with improved treatment success, with no differences in toxicity (258, 259). These data support the addition of inhaled antibiotics as an adjunct to standard treatment for these patients, and more recent data presented below have also concurred with these findings. The majority of these data are for patients with MDR Gram-negative infections, as they were reported after the introduction of ATS/IDSA guidelines for the treatment of hospital-acquired and ventilator-associated pneumonia, which recommend inhaled therapy only in these cases (241). There are a paucity of data for the use of inhaled agents as monotherapy or in cases of non-MDR infections.

Ventilator-associated pneumonia. Advances within the field of medicine have extended human life expectancy and allowed clinicians to rescue the lives of patients with diseases that in the not-so-distant past would have been fatal. These advances have not come without consequences. In the case of respiratory infections, the advent of mechanical ventilation has revolutionized the care of patients with chronic and acute lung diseases. This artificial airway has become a perfect environment for the growth and persistence of certain microbial species and has spawned what we now consider one of the most common and difficult types of infections to treat: VAP. VAP is defined as pneumonia occurring >48 h after endotracheal intubation and initiation of mechanical ventilation (24). Ventilator-associated pneumonia develops in upwards of 30% of patients who undergo mechanical ventilation for >48 h and increases the risk of all-cause mortality up to 2.5 times, with an attributable mortality rate of up to 1.5% (260–262). The actual attributable mortality rate for VAP is difficult to determine due to the challenges of firmly establishing a diagnosis and variances in diagnostic criteria, and thus, a wide range of estimates have been reported (263–265). VAP has also been associated with an increased length of stay in an ICU and an increased duration of mechanical ventilation, along with $39,828-higher mean hospitalization costs than for patients without VAP in 2012 (266, 267).

The causative pathogenic microorganisms for VAP may vary based on the patient population, duration of hospitalization, duration of mechanical ventilation, and diagnostic method used (24). Gram-negative organisms are commonly isolated as the causative pathogens among patients diagnosed with VAP (24). The most common Gram-negative organisms isolated include *P. aeruginosa*, *E. coli*, *Proteus* spp., and *Enterobacter* spp. Isolation of Gram-positive organisms in patients diagnosed with VAP has also

been reported, with *S. aureus* being isolated in 20% of cases (24). Early-onset VAP (defined as onset 3 to 7 days after mechanical ventilation) has been reported to be caused by *H. influenzae*, *S. pneumoniae*, methicillin-susceptible *S. aureus*, and susceptible *Enterobacteriaceae* (23, 265, 268). *P. aeruginosa*, *Acinetobacter* species, MRSA, and MDR Gram-negative organisms are isolated more frequently in patients with late-onset VAP (>7 days after mechanical ventilation) (23, 265, 268). A proposed reason for the difference in the distribution patterns of causative pathogens is the widespread use of antibiotics in patients diagnosed with late-onset VAP (268).

Gram-negative pathogens causing VAP, in particular *P. aeruginosa*, grow well in hypoxic environments such as mucus and possess an elaborate quorum-sensing system to allow growth into a hardy biofilm (269). These bacteria use alginate and other exopolysaccharides to localize themselves within mucopurulent masses in the lungs and sputum. These biofilm modes of growth hinder the activity of antibiotics by preventing penetration via electrostatic interactions and possessing a slow-growing quiescent phenotype that is not easily killed by antimicrobials, particularly cell wall-active agents. Additionally, as subinhibitory concentrations of antibiotics have been shown to induce biofilm formation and proliferation (270, 271), administering systemic antibiotics to patients with VAP could potentially exacerbate the problem. In addition to facilitating drug deposition in the lungs, the ET tube itself is an important target for inhaled antimicrobials, as this is often a reservoir for microbial biofilm formation (272, 273). The inhalation of antibiotics and subsequent coating of the inner lumen of the ET tube by the antimicrobial may prevent biofilm formation. The high concentrations achieved in the proximal airway close to the inhalation device along with the small particle sizes of inhaled agents may also promote improved biofilm penetration. In addition, the virulence factors produced by biofilm-forming *P. aeruginosa* have been shown to trigger the release of antibiotics such as amikacin from within liposomal formulations of the inhaled product, thereby increasing their localized activity within this environment (274).

Administration of inhaled antimicrobials in mechanically ventilated patients with nosocomial pneumonia. The advent of endotracheal intubation has provided easy access to the respiratory tract to administer antimicrobials. Despite concerns about the development of antimicrobial resistance and adverse effects expressed by the Centers for Disease Control and Prevention (275), increasing amounts of data are supporting the use of inhaled antibiotics for the prevention and treatment of VAP. A meta-analysis of existing literature observed significantly decreased rates of ICU-acquired pneumonia in patients receiving prophylactic antibiotics administered via the respiratory tract (276). In spite of the overall dearth of reported data describing and supporting its use, inhaled antibiotics are becoming a more frequent therapeutic option for the treatment of VAP due to the ever-increasing rates of bacterial resistance and the decreasing efficacy of antimicrobials administered systemically.

Although the increased desire to explore nontraditional ways to administer antibiotics to improve patient outcomes is encouraging, delivery of inhaled antibiotics to mechanically ventilated patients represents a particularly difficult challenge given the added complexities of the artificial respiratory system. In addition to the particle and delivery issues discussed above in this review, the presence of mechanical ventilation also introduces the added variable of humidity. Humidity within the ventilator circuit has been shown to increase aerosol loss and decrease drug delivery up to 40%. Along with humidity, the density of the inhaled gas and ventilator parameters also influence the amount of drug delivered to the lungs (76, 79, 277). Traditionally, nebulizers have been the device of choice for delivering inhaled agents to ventilated patients, although their efficiency in this scenario has been questioned (94, 95, 278, 279). In one of the few *in vitro*/*in vivo* studies performed in the field of inhaled antimicrobials, Miller and colleagues demonstrated the influence of different ventilator settings and nebulizer techniques on antibiotic concentrations achieved in tracheal secretions (125). In one study using ultrasonic nebulization, volume control ventilation produced higher nebulizer efficiency than did pressure control ventilation, while peak-end expiratory pressure (PEEP) and inspiratory flow had no effect, although prolongation of the inspiratory time increased nebulizer output (127, 280). Also, the efficiency of jet and ultrasonic nebulizers can be improved with the use of a spacer on the inspiratory arm of the ventilator circuit (94, 281). An excellent review detailing the variables associated with optimal inhaled-drug delivery to patients undergoing mechanical ventilation was recently reported (278). Currently, there are no direct comparative studies of types of nebulizers in patients on mechanical ventilation, although the current preference among experts is the vibrating-mesh nebulizer over the ultrasonic nebulizer due to hygiene concerns, drug heating during aerosolization, and the extra labor required with the ultrasonic nebulizer.

In brief, the most important considerations for inhaled delivery of antibiotics during mechanical ventilation are the ventilator settings. The ventilator should be set to volume control as opposed to pressure control, with a tidal volume of ≥500 ml to increase lung deposition. The inspiration-to-expiration ratio should be increased to acquire a long inspiratory time. In patients with acute respiratory distress syndrome, the tidal volume can be kept to 6 ml/kg at the expense of potentially decreased drug deposition, as described above. The inspiratory flow rate should be kept under 80 liters/min and ideally set at 40 liters/min, and nebulization should be synchronized with inspiratory flow. Most commercially available nebulizers are not breath synchronized and should therefore be used with a ventilator-integrated aerosolization system. Although a clear consensus on the ideal location has not been reached, the nebulizer is most often placed 10 to 15 cm from the Y piece on the inspiratory limb for continuous nebulization and as close as possible to the patient in a breath-synchronized mode. Heated humidity has been shown to decrease the amount of delivered drug, and therefore, heated humidifiers should be switched off during aerosolization. A helium-oxygen driver gas mixture has been shown to improve lung deposition over air or oxygen in animals, although human studies regarding this are lacking (278). Table 3 provides suggested steps to optimize the delivery of inhaled antibiotics via a jet nebulizer in mechanically ventilated patients.

A survey regarding the practice of and knowledge and beliefs about aerosol therapy during mechanical ventilation polled 854 European ICU physicians. Almost all (99%) respondents reported using aerosol therapy during mechanical ventilation, although only about 30% reported using inhaled antibiotics on more than 5 patients per year. The majority of these ICU physicians reported utilizing a jet nebulizer most commonly, and the most frequently inhaled antibiotic was colistin (59%), followed by tobramycin

TABLE 3 Steps to optimize inhaled drug delivery during mechanical ventilation

Step	Instruction(s)
1	Review inhaled-antibiotic order and assess need for pretreatment bronchodilator
2	Clear excess secretions from airway, if present
3	Load drug in nebulizer to manufacturer-recommended specifications
4	Position nebulizer on inspiratory line 10–15 cm from Y piece
5	Use continuous flow during nebulizer operation and remove heat-moisture exchanger from circuit
6	Set gas flow to nebulizer according to the manufacturer's specifications, and adjust ventilator limits to compensate for added flow
7	Run nebulizer until it begins to sputter
8	Remove nebulizer from circuit, rinse with sterile water, and run dry
9	Reconnect heat-moisture exchanger, and return ventilator to previous settings
10	Monitor patient for adverse events
11	Assess outcome of treatment

(31%). Regrettably, 77% of these clinicians reported never changing the ventilation settings during nebulization. Only 22% reported discontinuing humidification, and only ~33% reported ever changing the protective expiratory limb filter, despite reports of serious consequences associated with this practice (282). In addition, most respondents failed to answer specific questions regarding optimal droplet size and specific nebulizer performance. Almost all (90%) of the physicians considered aerosol therapy during mechanical ventilation to be of some interest, while 72% believed that inhaled antimicrobial therapy can improve the effectiveness of pneumonia treatment.

A follow-up study by the same group utilized a cross-sectional point prevalence study over 14 days in 81 ICUs in 22 countries including 2,808 patients (283). A total of 678 (24%) patients received at least one inhaled medication during the study period, about half of whom were patients who were intubated. Antibiotics were delivered to intubated patients via jet, ultrasonic, and vibrating-mesh nebulizers in 62%, 29%, and 9% of cases, respectively. Unfortunately, ventilator settings were changed in only 30% of inhaled antibiotic administrations. The heated humidifier was turned off in 59% of administrations, and the nebulizer was placed upstream of the inspiratory limb of the circuit in only 9% of aerosol antibiotic administrations. A ventilator-integrated, breath-actuated nebulization system was available in 60% of cases, and a filter was placed on the expiratory limb in 66% of administrations, although this filter was not changed in relation to nebulization in 88% of cases. No ventilator dysfunction was documented over the study period. Antibiotics were given to only 31 patients involved in the survey at only 14 of the 81 centers. Colistin was the most frequently used inhaled antibiotic (79%), and the most common indication was nosocomial pneumonia (67%). Inhaled antibiotics were utilized 19% of the time for tracheobronchitis and 6% of the time for prophylaxis. Bronchospasm was reported three times in this study, all associated with inhaled colistin.

These surveys highlight the strong subjective interest in and perceived benefit of inhaled antibiotics but report poor uptake in their utilization. They also display an overall lack of knowledge regarding important characteristics involved in antibiotic administration and the suboptimal and often potentially dangerous implementation of inhaled therapy in mechanically ventilated patients (38). In the future, guidelines introduced by well-respected critical care and infectious disease societies may help boost interest in inhaled antibiotic therapy and translate and simplify the bench-to-bedside knowledge gaps in the optimal use of inhaled antibiotics in patients undergoing mechanical ventilation.

Treatment with inhaled colistin monotherapy. In the largest, most well-designed cohort to date, 28 patients with VAP due to MDR *A. baumannii* or *P. aeruginosa* were treated with inhaled colistin monotherapy in a prospective, observational study (284). These patients were compared to those with MDR VAP treated with inhaled colistin along with an i.v. aminoglycoside ($n = 15$) and to patients with susceptible VAP treated with standard therapy ($n = 122$). Patients in the susceptible VAP group received an i.v. β-lactam for 14 days plus or minus an aminoglycoside for 3 days, while patients in the MDR VAP group received inhaled colistin as 5 million IU every 8 h for 7 to 19 days plus or minus an aminoglycoside for 3 days. Nebulization was performed via a vibrating-plate nebulizer positioned 10 cm proximal to the Y piece over 60 min, with removal of the heat-moisture exchanger. Of note, all patients in the MDR VAP group received inappropriate initial antibiotic therapy, as the majority of the *A. baumannii* and *P. aeruginosa* isolates were susceptible only to colistin and aminoglycosides. In the susceptible VAP group, 84% of patients received appropriate initial therapy. Demographic characteristics were similar between the groups, with the exception of increased durations of mechanical ventilation and ICU stay and previous administration of antibiotics in the MDR VAP group. The mean duration of inhaled colistin was 12 days (range, 7 to 19 days), and the overall clinical cure rate was 67% in the MDR VAP group, compared to 66% in the susceptible group. In the 28 patients receiving inhaled colistin monotherapy, the cure rate at the end of treatment was 68%, compared to 67% in patients receiving both inhaled colistin and i.v. aminoglycosides ($P = 0.94$). Clinical pulmonary infection scores, treatment failure, and all-cause ICU mortality were not statistically different between the MDR and susceptible groups. Interestingly, among the 16 patients in the MDR VAP group with persistent or recurrent VAP due to *P. aeruginosa*, four strains regained susceptibility to β-lactams. In two patients treated with inhaled colistin who had a recurrence of VAP, the colistin MIC increased from 0.75 mg/liter and 1.5 mg/liter to 3 mg/liter. Serum peak and trough concentrations of colistin were also measured on days 2 and 3 in 9 patients receiving inhaled colistin monotherapy and 7 patients receiving inhaled colistin combined with i.v. aminoglycosides. Peak and trough concentrations on days 2 and 3 were ~2 mg/liter and 1 mg/liter, respectively, and were not different between the groups. Serum creatinine levels increased to >1.5 times the baseline value in 12% of patients in the MDR VAP group, compared to 8% in the susceptible group ($P = 0.47$). This study represents the largest cohort of patients receiving inhaled colistin monotherapy for MDR VAP to date. The rate of clinical cure observed in this study for patients with MDR VAP also receiving i.v. aminoglycosides was comparable to that for patients with susceptible VAP receiving i.v. β-lactams, despite no patients in the MDR group receiving appropriate initial antibiotic therapy. In two patients receiving inhaled colistin, one of whom also received an i.v. aminoglycoside, the MIC for *P. aeruginosa* increased from 0.75 and 1.5 mg/liter to 3 mg/liter. Despite almost therapeutic serum concentrations, there was not an increased incidence of nephrotoxicity in the inhaled-colistin

group. The dose of inhaled colistin in this report was significantly higher than those used in previous studies, and the nebulization procedure was optimized during ventilation as recommended, which may have contributed to the outcomes seen in this analysis.

A brief report also detailed another center's experience utilizing inhaled colistin in 21 patients with pneumonia due to MDR *A. baumannii* (17) or *P. aeruginosa* (4). Only 3 of the 21 patients had VAP, and the type of pneumonia in the other 18 patients was not reported. Inhaled colistin was given as 1 million IU four times daily in the majority of patients for a median of 14 days. No patients received i.v. colistin or any other antibiotic active against their pneumonia pathogen. A favorable response was achieved in 85% of patients, while 57% had both favorable clinical and favorable microbiological outcomes. The vast majority (18/21) of patients in this study had a favorable microbiological outcome, and 61% had documented eradication. The crude mortality rate was 46.7%, with an attributable mortality rate of 14.3%. One patient suffered from bronchospasm, but no other adverse events related to inhaled colistin were reported (285).

A small case series of 5 patients detailed the outcomes of monotherapy with inhaled colistin for MDR Gram-negative nosocomial pneumonia. Three of the five patients had VAP, while the other two were classified only as having nosocomial pneumonia. The most common pathogen in 3 of the 5 patients was *A. baumannii*, and most (4/5) patients received 1 million IU every 8 h for 6 to 11 days. Intravenous antibiotics were given concurrently, although the isolated pathogens were always resistant to these agents. Cure or improvement in pneumonia was achieved in 4 of 5 patients, and the fifth patient expired during the study. In the only patient with follow-up cultures available, *A. baumannii* was still isolated in bronchial secretions at day 5. No adverse events were experienced by any patients in this study (286).

An expert review by Falagas et al. summarized the previously available literature regarding inhaled antibiotics as monotherapy for pneumonia. This review included 7 studies comprising 63 patients, 40 of whom suffered from nosocomial pneumonia. Colistin was the most common inhaled antibiotic utilized, and clinical cure was achieved in 86% of patients. The authors of this analysis conclude that inhaled therapy alone should not be excluded as an option in certain patient situations, especially when there are concerns regarding penetration into the lungs or the toxicity of systemic agents, and that more data are needed regarding this mode of therapy (287).

Treatment with inhaled adjunctive therapy. As a word of caution, combination therapy with inhaled and systemic antimicrobials must be approached with attentiveness in some cases and until further data are available. For example, the polycationic structure of azithromycin uses self-promoted uptake to enter *P. aeruginosa* cells via displacement of elemental dications. This same mechanism is utilized by aminoglycosides, and the high lung concentrations achieved after inhalation may cause drug-drug interactions not normally seen between these agents when used systemically in combination. Concomitant administration of these two agents has been shown to decrease antibacterial activity and negatively affect clinical outcomes with inhaled tobramycin (288). Given the large numbers of patients with acute and chronic Gram-negative LRTIs who use azithromycin for its anti-inflammatory properties, this could potentially pose an issue for inhaled therapy.

(i) Aminoglycosides. A retrospective observational study was conducted to assess the efficacy and safety of inhaled aminoglycosides as adjunctive therapy in surgical ICU patients with Gram-negative VAP. Patients received either tobramycin or amikacin via nebulization synchronized with a ventilator. All 22 patients included in this study received both inhaled and systemic antibiotics, the majority of whom (16 patients) received inhaled tobramycin for an average of 7.3 days. The most common pathogen responsible for VAP was *P. aeruginosa* (54%), and 7 patients had a recurrence of VAP. Only 1 patient had emergence of a resistant organism upon recurrence of VAP. Three patients died in this study. No adverse events were reported in this evaluation, and all patients were weaned from mechanical ventilation. Notably, 56% of patients in this study had been treated for a prior episode of VAP before inclusion in this analysis (289).

A single-center cohort study compared the clinical outcomes of 93 patients treated with i.v. antibiotics and adjunctive inhaled antibiotics versus patients who did not receive inhaled antibiotics for VAP (290). The 19 patients in the group that received adjunctive inhaled antibiotics received 150 mg colistin inhaled twice daily or 300 mg tobramycin inhaled twice daily. VAP was defined as the presence of a new or progressive pulmonary infiltrate and two of the following: temperature of $>$38.3°C or $<$36°C, leukocyte count of $>$12,000 leukocytes/ml or $<$4,000 leukocytes/ml, or purulent tracheal secretions. Microbiological confirmation of VAP was confirmed by BAL fluid culture with growth of *P. aeruginosa* or *A. baumannii*, defined as $>10^4$ CFU/ml. The nebulizer was positioned 30 cm from the endotracheal tube on the inspiratory limb of the ventilator circuit, and humidification was discontinued during delivery of the aerosol. The 74 patients in the group that received a noninhaled antibiotic had shorter durations of mechanical ventilation (18.9 $\pm$ 15.9 days versus 38.4 $\pm$ 32.4 days; $P < 0.001$), ICU stay (37.5 $\pm$ 42.5 days versus 56.3 $\pm$ 33.4 days; $P = 0.001$), and hospital stay (39.0 $\pm$ 42.5 days versus 58.3 $\pm$ 33.4 days; $P = 0.001$). The incidences of microbiologically confirmed recurrent VAP were similar between both study groups (10.5% in the group that received an inhaled antibiotic versus 14.9% in the group that received a noninhaled antibiotic; $P = 0.48$). Thirty-day mortality was less frequent among patients in the group that received an inhaled antibiotic than among those in the group that received a noninhaled antibiotic (0% versus 17.6%; $P = 0.063$). This is one of the only studies to show improved outcomes in patients not receiving inhaled antimicrobials, although the discordant numbers being compared in this study preclude strong inferences being made from these results.

A randomized, double-blind pilot study in 10 patients compared the safeties and efficacies of i.v. and inhaled tobramycin (291). Patients with clinical symptoms of VAP or a new pulmonary infiltrate on a chest radiograph after 96 h of intubation were eligible for the study. Patients with BAL fluid yielding $\geq 10^4$ CFU/ml of *P. aeruginosa* or *Acinetobacter* spp. sensitive to tobramycin were included in the study. The group administered inhaled tobramycin received i.v. placebo every 24 h plus 300 mg inhaled tobramycin every 12 h with an i.v. β-lactam antibiotic (piperacillin-tazobactam or imipenem-cilastatin). The group administered i.v. tobramycin received i.v. tobramycin plus placebo nebulization plus either i.v. piperacillin-tazobactam or imipenem-cilastatin. Patients were treated for 14 days in both study groups. Nebulization was synchronized with inspiration with a flow rate of $>$6 liters/min, and the nebulizer was fitted 30 cm from the ET tube on the inspiratory limb. Upon extubation, inhaled tobramycin or placebo was administered via a jet nebulizer for the du-

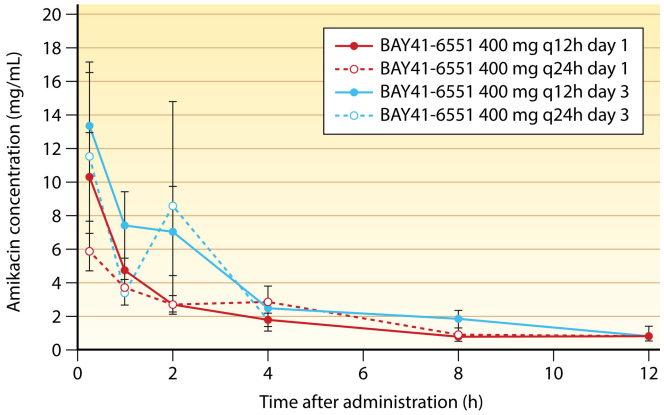


FIG 6 Mean amikacin concentrations and standard deviations in tracheal aspirates over time on days 1 and 3. q12h, every 12 h. (Republished from reference 292 with kind permission from Springer Science+Business Media. © Copyright jointly held by Springer and ESICM 2011.)

ration of the treatment period. Cure was defined as extubation within the study period. Patients who were still intubated were consider cured if their multiple-organ-dysfunction score (MODS) was improving, they were afebrile, and pulmonary infiltrates as well as physical signs of pneumonia had resolved. All patients in the group receiving inhaled tobramycin were cured at 28 days, whereas three patients out of five patients in the group receiving i.v. tobramycin were cured. Patients in the group receiving inhaled tobramycin had more ventilator-free days than did those in the group receiving i.v. tobramycin (24 ± 3 days versus 14 ± 13 days; $P = 0.12$).

The above-discussed drug-device combination of BAY41-6551 has been studied in a multicenter, randomized, placebo-controlled, double-blind, phase II trial in mechanically ventilated patients with Gram-negative nosocomial pneumonia. Patients were included if they had VAP or hospital-acquired or health care-associated pneumonia; a specific stratification by type of pneumonia was not provided. The study drug, amikacin, or placebo was delivered as 400 mg every 12 or 24 h via the pulmonary drug delivery system (PDDS) for 7 to 14 days in addition to standard systemic therapy, as determined by the patients' treating physician. In this phase II study, the primary endpoint was the proportion of patients achieving an amikacin concentration in tracheal aspirates of ≥25 times an MIC of 256 mg/liter and an AUC/MIC ratio of ≥100. *P. aeruginosa* was the most commonly isolated pathogen in 43.6% of the 55 patients enrolled. The study drug was given for ~6 days in all 3 groups. The primary endpoint was achieved for 50% (6/12) of patients in the group receiving amikacin every 12 h and in only 16.7% (3/18) of patients in the group receiving amikacin every 24 h. Figure 6 displays the concentration-time profile of amikacin in tracheal aspirates on day 1 and day 3. In further describing the PK, concentrations peaked 15 to 60 min after inhalation and showed a time-dependent decline, with higher concentrations being observed on day 3 than on day 1. Although these authors assumed a worst-case MIC of 256 mg/liter, the C_{max} and AUC achieved on day 3 with 400 mg every 12 h were substantial, at 16,212 mg/liter and 61,908 mg · h/liter, respectively, and would provide more-than-adequate exposure for pathogens within the susceptible range. Despite the high pulmonary concentrations, the C_{max} achieved in serum on day 3 was 3.16 mg/liter at 1 h postinhalation. The clinical cure rate in the group receiving the drug every 12 h compared to placebo was 93.8% versus 87.5% ($P = 0.467$). In the placebo group, systemic antibiotics were added or escalated more often in the first 7 days of the study than in the treatment groups. Interestingly, there were no differences seen in microbiological eradication between the treatment and placebo groups (68.8% versus 62.5%; $P > 0.999$). Of note, cultures used to diagnose pneumonia were often high-quality bronchoscopy specimens, whereas sputum was often used to assess microbiological cure, as patients were extubated at the time of assessment. Mild bronchospasms were reported by two patients given BAY41-6551. In this study, peak amikacin concentrations were 800 times higher in tracheal aspirates than in plasma and 4,000 times higher than the concentrations achieved in the lung after i.v. administration (292). Given the extremely high concentrations achieved, a lower dose given once daily may be suitable for susceptible pathogens. Ideally, this study should be repeated with ELF concentrations being measured.

A retrospective study examined the clinical and microbiological outcomes for patients receiving adjunctive inhaled antibiotics for VAP due to nonfermenting Gram-negative bacilli. All patients in this study were admitted to the ICU, and antibiotics were delivered via a jet nebulizer. Forty-nine patients were included, and the most commonly isolated organism was *P. aeruginosa*. Inhaled tobramycin was the most common adjunctive therapy followed by amikacin and CMS, at doses of 300 mg, 1,000 mg, and 150 mg every 12 h, respectively. Nearly all (98%) patients received concomitant systemic antimicrobial therapy. Clinical success was achieved in 73% of patients, and microbiological success was documented via repeat culture in 70% of the episodes of VAP. Notably, in the 20 episodes of VAP that experienced treatment failure with systemic monotherapy, clinical success was subsequently achieved in 85% of these episodes after the addition of inhaled antibiotics. There were no adverse events reported, and six patients died as a result of their VAP (293).

Finally, a descriptive study of patients with active cancer and Gram-negative VAP evaluated the use of inhaled or intravenous aminoglycosides or colistin (294). Sixteen patients who received inhaled aminoglycosides or colistin were compared to 16 patients who received the same agents systemically alone. A tobramycin inhalation solution was used to administer 300 mg of inhaled tobramycin every 12 h. Inhaled amikacin, gentamicin, and colistin were prepared from commercially available parenteral preparations, and the dosing regimens given were 100 mg every 8 h or 300 mg every 12 h, 100 mg every 8 h, and 100 mg every 8 h, respectively, via a jet nebulizer. Clinical response was classified as complete or partial resolution (fever defervescence, decreased suctioning requirements, and resolution of symptoms and signs of pneumonia), improved ventilator parameters and laboratory findings (improved blood gas levels, normalization of the white blood cell count, and/or receding pulmonary infiltrates on a chest radiograph at the end of therapy), or failure, which was defined as a worsening of the clinical and ventilator parameters and/or progression of contiguous or noncontiguous consolidation upon radiography at the end of therapy. Microbiological eradication was defined as eradication of the causative organism in patients with an available follow-up culture. There were no significant differences in baseline demographics between patients receiving inhaled and those receiving i.v. aminoglycosides or colistin. The majority of patients in both groups had solid-organ cancer, while

>30% of patients in each group had lung cancer. The median clinical pulmonary infection score was >6, and the median duration of mechanical ventilation was >20 days for both groups. Over two-thirds (69%) of patients were infected with *P. aeruginosa*, and the median durations of antibiotic therapy were 10 and 11 days in the groups receiving i.v. and inhaled antibiotics, respectively. Fourteen of the 16 patients in the group administered an inhaled antibiotic received aminoglycosides, 8 of whom received tobramycin, 4 of whom received amikacin, and 2 of whom received gentamicin. All patients in the group administered an inhaled antibiotic received concomitant systemic antibiotics. Only 13 patients in the group receiving inhaled antibiotics and 9 patients in the i.v. treatment group were evaluated for clinical response for unclear reasons. Of these patients, 100% in the group treated with inhaled antibiotics had complete clinical resolution, compared to 55% in the i.v. treatment group ($P < 0.01$). Additionally, in the 13 and 12 patients for whom follow-up cultures were available in the inhalation and i.v. treatment groups, 77% and 8% achieved bacterial eradication, respectively ($P < 0.0006$). In a subsequent logistic regression adjusting for renal dysfunction, concomitant antibiotic use, corticosteroid use, lung cancer, radiation therapy, comorbidities, clinical pulmonary infection score, infecting pathogen, and duration of critical-care-unit stay, the likelihoods of complete clinical resolution and bacterial eradication were significantly greater in patients treated with inhaled antibiotics. No pulmonary adverse events were observed in the inhalation treatment group, and more patients in the i.v. treatment group developed renal dysfunction (31% versus 0%; $P \leq 0.04$). This study provides additional data for immunocompromised patients indicating that adjunctive inhaled antibiotics may be more effective than i.v. antibiotics alone. Notably, adverse events, including bronchospasm, were not observed despite the use of parenteral formulations of many of the inhaled agents.

(ii) Polymyxins. Colistin has been by far the most widely used inhaled antibiotic for nosocomial Gram-negative LRTIs. The majority of use has come from Europe in patients with MDR or colistin-only-susceptible pathogens. Almost all of the reported data focus on patients with VAP, and inhaled colistin is most often combined with i.v. colistin and/or another broad-spectrum systemic Gram-negative agent. Table 4 summarizes the reports of inhaled colistin as an adjunct therapy for Gram-negative nosocomial pneumonia.

A prospective study enrolled 60 patients treated with VAP due to MDR Gram-negative bacteria in Greece who were treated with inhaled CMS. Colistin was delivered as 3 million IU three times daily via nebulization and was given with systemic antibiotics in 57/60 patients. The most common pathogen causing VAP was *A. baumannii* in 37/60 patients, followed by *P. aeruginosa* in 12 cases. The mean duration of inhaled colistin was 16.4 days (range, 5 to 49 days), and clinical improvement of VAP was seen in 83.3% of patients. No adverse events were reported in this study. The overall morality rate was 25%, and only one patient had a recurrence of VAP due to *S. maltophilia*. This study supports the safety of inhaled colistin and adds to the data supporting it as adjunctive treatment. There were no data regarding microbiological clearance in this study, but overall, the majority of patients had a favorable outcome, although no comparator group was included in this study (295).

An open-label, randomized, controlled study investigated the safety and benefits of CMS as adjunctive therapy in adult patients with Gram-negative VAP (296). Patients were randomized to receive systemic antibiotics plus inhaled sterile normal saline ($n = 49$) or systemic antibiotics plus inhaled CMS (equivalent to 75 mg of colistin) every 12 h until the end of systemic VAP treatment ($n = 51$). The choice and duration of systemic therapy were determined by the treating physician. Inhaled CMS was administered via a jet or ultrasonic nebulizer for 10 min or until the nebulized solution container was empty. Microbiological cultures of a respiratory specimen aspirated from the endotracheal tube were collected on day 3 of treatment and every 7 days thereafter. The demographic characteristics and comorbidities at baseline were similar between the groups, with mean acute physiology and chronic health evaluation (II) scores (APACHE II scores) of 19.7 and 18.5 for the CMS and placebo groups, respectively. The most common infecting pathogen was *A. baumannii*, in 69.6% and 61.2% of patients in the CMS and placebo groups, respectively, and the most frequent systemic antibiotic used was imipenem-cilastatin or meropenem, followed by CMS. Inhaled therapy was administered for a significantly longer duration on average in the placebo group than in the CMS group (11.8 versus 9.5 days; $P = 0.005$). Favorable clinical outcomes at day 28 (defined as complete resolution of all signs and symptoms of pneumonia and improvement or lack of progression of all abnormalities on a chest radiograph) were similar between the two groups (51% in the CMS group versus 53.1% in the normal saline group; $P = 0.84$). Favorable microbiological outcomes (defined as eradication or presumed eradication) were greater in patients who received CMS than in those who received normal saline (60.9% versus 38.2%; $P = 0.03$). Bronchospasm was experienced by 7.8% of CMS-treated patients, compared to 2% of placebo-treated patients ($P = 0.36$). Renal impairment was observed in 25.5% and 22.4% of patients in the CMS and placebo groups, respectively ($P = 0.82$). Unfortunately, the decision to evaluate clinical outcomes at 28 days, over 2 weeks after the end of the mean duration of therapy, precludes any conclusions about the effect of adjunctive CMS on the time to clinical cure or cure at the end of therapy. As demonstrated in animal studies, the addition of inhaled antibacterials to standard systemic therapy consistently leads to improved microbiological eradication, likely due to the supratherapeutic concentrations achieved and the ability of locally administered agents to penetrate biofilms.

A well-designed retrospective cohort study examined patients with VAP due to colistin-only-susceptible *A. baumannii*, *P. aeruginosa*, or *K. pneumoniae* who received colistin for ≥72 h (297). Patients treated with inhaled and i.v. colistin or i.v. colistin were matched in a 1:1 ratio based on age (±10 years), simplified acute physiology score (II) (SAPS II) at ICU admission (±5 points), and sequential organ failure assessment (SOFA) scores (±2 points) on the day when colistin was started. Colistin was administered intravenously at daily per-kilogram doses of ~100,000 IU every 8 to 12 h and inhaled at 3 million IU divided three times daily via a jet or ultrasonic nebulizer. The primary outcome of the study was clinical cure, defined as the resolution of all signs and symptoms of pneumonia and improvement or lack of progression of all chest radiograph abnormalities at the end of treatment and judged by blind investigators. A total of 208 patients were included, with 104 receiving inhaled and i.v. colistin and 104 receiving i.v. colistin. There were no significant differences in demographics between the two patient populations, although more patients in the groups that received inhaled and i.v. colistin were infected with *A. bau-*

TABLE 4 Summary of reports of inhaled colistin as adjunct therapy for Gram-negative nosocomial pneumonia

Reference	Study design	Patient population	Regimen for comparator group	Inhaled regimen	Duration(s) (days)[a]	Outcome(s)	Toxicity	Description
289	Prospective observational	60 patients with MDR Gram-negative VAP	None	3 million IU every 8 h + systemic antibiotics	16.4 ± 10.9	83.3% clinical improvement	None reported	No comparator group; microbiological data not provided
290	Randomized, placebo controlled	100 patients with Gram-negative VAP	Placebo inhalation + systemic antibiotics	75 mg every 12 h + systemic antibiotics	9.5 ± 4.6	No difference in clinical outcome; improved microbiological outcome with inhaled colistin	7.8% bronchospasm	Clinical and microbiological outcomes evaluated at 28 days
291	Retrospective matched	208 patients with colistin-only-susceptible Gram-negative VAP	i.v. colistin	3 million IU every 8 h + i.v. colistin	7 (5–14)	Improved clinical cure in inhaled-antibiotic group; no significant difference in microbiological cure	None reported	Concomitant systemic antibiotics not described
293	Retrospective	43 patients with MDR *A. baumannii* VAP	i.v. colistin	75 mg every 12 h + i.v. colistin	14	No difference in clinical or microbiological outcome at day 5 or end of therapy	None reported	87% of patients with severe sepsis/septic shock at baseline
294	Retrospective	20 ICU patients with MDR *P. aeruginosa* nosocomial pneumonia	i.v. colistin alone, inhaled colistin alone	2 million IU every 8 h + i.v. colistin	19.3 (3–46) for i.v. + inhaled antibiotics, 27.2 (6–96) for inhaled antibiotic alone	78% favorable response with i.v. vs 100% for inhaled antibiotic alone and 40% for i.v. antibiotic alone; no patients achieved microbiological eradication	None reported	56% of patients on i.v. and inhaled colistin also had extrapulmonary infection
295	Retrospective	45 patients with MDR *A. baumannii* VAP	None	Mean dose of 4.29 million IU + systemic antibiotics	10.29	57.8% favorable clinical outcome; 37.8% microbiological eradication	None reported	Only 60% had follow-up cultures available
296	Retrospective	96 patients with MDR Gram-negative nosocomial pneumonia	i.v. colistin	75 or 150 mg every 12 h + systemic antibiotics	11 (7–16.25)	No significant difference in clinical cure, microbiological eradication, or mortality	None reported	Clinical cure rate higher in group administered inhaled antibiotic when only patients with high-quality respiratory cultures were evaluated
297	Prospective observational	8 patients with MDR *P. aeruginosa* VAP	None	500,000 IU every 8 h + i.v. colistin	15.9	70% clinical cure	None reported	Most patients were coinfected with a Gram-positive pathogen
298	Retrospective matched	86 patients with MDR Gram-negative VAP	i.v. colistin	1 million IU every 12 h + i.v. colistin	13 (5–56)[b]	Significantly improved clinical cure in group administered inhaled antibiotic; no difference in microbiological eradication	None reported	No detail provided on concomitant systemic antibiotics
302	Retrospective	49 patients with Gram-negative pneumonia	None	500,000 IU every 6 h + systemic antibiotics	12 ± 8	93% microbiological eradication	None reported	Parenteral formulation used for inhalation
303	Retrospective	121 patients with Gram-negative VAP	i.v. colistin	2.1 million IU per day + systemic antibiotics	16.9 ± 9.8	Significantly improved clinical cure in group administered inhaled antibiotic; no difference in mortality	None reported	Significantly more patients in group administered i.v. antibiotic only with colistin-only-susceptible organisms; use of inhaled colistin was independent predictor of clinical cure
304	Retrospective	8 patients with MDR Gram-negative pneumonia	None	1.5 million–6 million IU every 6–8 h + systemic antibiotics	10.5 (3–32)	88% clinical improvement or cure; 80% bacterial eradication	None reported	No uniform inhaled dosing strategy or duration

[a] Presented as means, means ± standard deviations, medians (interquartile ranges [underlined]), or means (ranges) unless otherwise specified.
[b] Presented as median (range).

mannii and fewer were infected with *K. pneumoniae* than in the group that received i.v. colistin (69.2% versus 53.8% [$P = 0.02$] and 8% versus 20% [$P = 0.01$], respectively). More than 80% of patients in both groups received inadequate initial antibiotic therapy, with no difference between the groups. The median durations of colistin treatment were 7 and 10 days in the group receiving inhaled plus i.v. colistin compared to the group receiving i.v. colistin, respectively, which did not differ statistically. The rate of clinical cure was statistically significantly better in the group receiving inhaled plus i.v. colistin than in the group receiving i.v. colistin (69.2% versus 54.8%; $P = 0.03$). The rate of microbiological cure (clearance of the infecting pathogen from posttreatment respiratory cultures) in the group receiving inhaled plus i.v. colistin group was 13.4% higher than that in the group receiving i.v. colistin group, although this did not reach significance ($P = 0.08$). There were no significant differences in ICU mortality or nephrotoxicity during colistin therapy between the groups. These authors also performed a logistic regression analysis, which indicated that trauma-related admissions and inhaled and i.v. colistin therapy were independent predictors of clinical cure. Conversely, higher SAPS II and SOFA scores, septic shock at the onset of VAP, and acute kidney injury during colistin therapy were independently associated with clinical failure. Although the isolates in this study were determined to be susceptible to colistin only, these authors did not report whether the patients in either group received concomitant systemic therapy with other agents. It is implied that patients in both groups received i.v. colistin alone with or without inhaled colistin, which can no longer be recommended given the difficulty in achieving adequate PK/PD indices and the documented regrowth and emergence of resistance with monotherapy (298). Emergence of colistin-resistant Gram-negative isolates was not explicitly reported in this study. The lack of difference in mortality rates with improved clinical cure rates in this study and others argue that VAP has less of an impact on overall ICU survival than do age, comorbidities, severity of illness, and other factors. Even so, the improved clinical and microbiological outcomes without increased toxicity in this study warrant further exploration in larger, controlled trials.

In a study looking specifically at critically ill adult patients with VAP due to MDR *A. baumannii*, patients received either i.v. colistin only or i.v. and inhaled colistin and were evaluated retrospectively (299). The primary outcomes consisted of both clinical and microbiological responses to therapy on day 5 and at the end of therapy. Clinical success was defined as resolution of signs and symptoms of VAP with no need for additional antibiotic therapy, and microbiological clearance was defined as eradication of *A. baumannii* upon follow-up culture. Colistin was administered i.v. as either 2.5 mg/kg every 12 h (maximum of 300 mg) or 2.5 mg/kg every 6 h (maximum of 600 mg), and inhaled colistin was given at 75 mg twice daily via a nebulizer of an undefined type. Forty-three patients were included in the study, 29 of whom received i.v. and inhaled colistin and 15 of whom received i.v. colistin alone. There were no significant differences in demographics between the two populations, and ~30% of patients in each group received a concomitant aminoglycoside. There were no differences in clinical outcomes at day 5 (44% for i.v. colistin only versus 35% for i.v. and inhaled colistin; $P = 0.75$) or at the end of colistin therapy (38% for i.v. colistin only versus 14% for i.v. and inhaled colistin; $P = 0.13$) between the two groups. There was also no difference in microbiological clearance between i.v. colistin only and i.v. plus inhaled colistin (69% versus 76%; $P = 0.73$). Finally, nephrotoxicity and mortality rates also did not differ significantly between the groups, although the proportion of patients with nephrotoxicity was 23% higher in the group that received i.v. and inhaled colistin. These authors do not report the doses of i.v. colistin administered in patients receiving i.v. and inhaled colistin compared to those receiving i.v. colistin alone. Overall, patients in this study who received the higher dose of i.v. colistin had a lower rate of clinical cure (7% versus 30%; $P = 0.25$) and a higher rate of mortality (67% versus 45%; $P = 0.18$). All patients were treated for 14 days, and no patients were coinfected with any other pathogens. Importantly, no colistin-resistant *A. baumannii* organisms were isolated during this study. Given the retrospective nature of this study and the high percentage (87%) of patients with severe sepsis and/or septic shock on admission, the decreased cure rates in the groups receiving high-dose i.v. colistin and i.v. plus inhaled colistin are likely a reflection of selection bias on the part of the treating physician.

In a 2-year retrospective case series of 20 ICU patients who developed nosocomial pneumonia due to MDR *P. aeruginosa*, the outcomes of colistin therapy were reported for i.v. colistin alone, inhaled colistin alone, or i.v. and inhaled colistin. Only 5 of the 20 patients included had VAP, while the other 15 had undefined nosocomial pneumonia. In this series, colistin was given as 2 million IU three times daily via nebulization. In total, 9 patients received both i.v. and inhaled colistin, and 6 received inhaled colistin alone, while 5 received i.v. colistin only. All patients received colistin in combination with a β-lactam, most commonly piperacillin-tazobactam or meropenem. The groups were comparable with respect to baseline demographics and severity of illness at baseline, although 56% of the group receiving i.v. and inhaled colistin had an extrapulmonary source of infection. Treatment was administered for an average of 19 to 27 days between the groups. Follow-up cultures were available for 19 of 20 patients, and none of them achieved microbiological eradication. In contrast, the clinical response was deemed favorable in 100% and 78% of patients on inhaled colistin only and i.v. and inhaled colistin, respectively. In the group receiving i.v. colistin only, the response was favorable in only 40% of patients (2/5 patients), and all 5 patients in this group died. Only 3 of the other 15 patients died during the study. Nephrotoxicity was difficult to establish in this study, as many patients had baseline renal dysfunction, although patients on only inhaled colistin experienced less toxicity than did those in the other two groups (300). Although this study included a very small number of patients, in general, the patients receiving inhaled colistin either alone or with i.v. colistin tended to have improved outcomes over those of patients receiving i.v. colistin alone. The addition of antibiotics other than colistin to these patients' therapy makes interpretation of these results difficult.

A retrospective analysis of the efficacy of inhaled colistin included 45 patients requiring mechanical ventilation in an ICU who were diagnosed with MDR *A. baumannii* VAP (301). VAP was diagnosed and microbiologically confirmed by positive cultures from either bronchial secretions or BAL fluid samples. Patients received i.v. antimicrobial regimens in addition to inhaled colistin, predominantly a carbapenem. The mean daily dose of inhaled colistin was 4.29 million ± 0.82 million IU, with a mean administration duration of 10.29 days. A favorable microbiological outcome (defined as eradication) was noted for 17 patients (37.8%), and 26 patients (57.8%) had a favorable clinical outcome

(defined as clinical cure or improvement) without evidence of severe adverse effects. The all-cause mortality rate was 42.2%.

Another large retrospective study of critically ill patients with nosocomial pneumonia who received i.v. colistin alone or in combination with inhaled colistin evaluated the incidence of clinical cure. This was a multicenter trial in which two sites used jet nebulizers and one used a vibrating-mesh nebulizer. The type of pneumonia was not specifically reported, and inhaled colistin was dosed at either 75 or 150 mg every 12 h depending on the study site. Fifty-one patients received i.v. colistin only, and 44 received both i.v. and inhaled colistin. The most commonly isolated pathogens were *A. baumannii* and *P. aeruginosa*, and all isolates were susceptible to colistin, although the majority of patients received additional systemic agents, most commonly a carbapenem or tigecycline. The mean durations of i.v. colistin were 11.2 and 12.2 days in the groups receiving i.v. colistin only and i.v. and inhaled colistin, respectively. The median duration of inhaled colistin was 11 days. Less than half (39.2%) of patients administered only i.v. therapy achieved a clinical cure, compared to 54.5% in the group administered i.v. and inhaled therapy, although this was not significantly different. There were also no differences in microbiological eradication in this study. The mortality rate was ~16% lower in the group administered i.v. and inhaled colistin ($P = 0.106$). The mortality rate due to pneumonia was >30% lower in the group administered i.v. and inhaled colistin, although this was not significantly different (70.4% versus 40%; $P = 0.055$). When patients with bronchoscopic BAL fluid cultures were evaluated separately from those with nonbronchoscopic BAL fluid or tracheal aspirate cultures, there was a statistically significant difference in clinical cure, with better outcomes in the group administered i.v. and inhaled colistin (57.1% versus 31.3%; $P = 0.033$) (302).

A prospective observational case series included mechanically ventilated patients treated with colistin for MDR *P. aeruginosa* pneumonia (303). Ten patients were included, 8 of whom had VAP. Tracheobronchial secretions or BAL fluid specimens were utilized for microbiological diagnosis of VAP. All 10 patients received i.v. CMS at 3 million IU every 8 h, and patients diagnosed with VAP received inhaled colistin at 500,000 IU every 8 h. Inhaled colistin was continued even after the discontinuation of i.v. therapy until eradication of the pathogen was confirmed in two consecutive cultures, for a median duration of 15.9 days. Clinical cure (defined as a resolution of clinical signs and symptoms, including fever and leukocytosis, and improvement of chest radiographs) or improvement was reported for 7 of the 10 patients. No adverse effects attributable to colistin were observed. Most patients in this analysis were also coinfected with a Gram-positive pathogen.

In one of the more well-designed retrospective studies evaluating the efficacy and safety of inhaled antibiotics, Kofteridis et al. compared i.v. colistin alone to i.v. and inhaled colistin in patients with VAP due to MDR Gram-negative pathogens (304). This study matched patients with monomicrobial VAP at a 1:1 ratio to receive either combination therapy or monotherapy. Inhaled colistin was delivered as 1 million IU twice daily; the type of nebulizer used was not mentioned. Forty-three patients in each group were evaluated, and the primary pathogen responsible for VAP was *A. baumannii* (77%). All strains were colistin susceptible, and the median durations of therapy were 10 days for the group administered i.v. colistin only and 13 days for the combination group. There were no statistically significant differences in clinical or microbiological cure, mortality, or adverse events between the groups. The clinical cure rate in the combination group was almost double that in the group that received i.v. colistin alone (54% versus 32.5%; $P = 0.05$). Interestingly, twice as many patients in the combination group had a recurrence of their VAP, while the mortality rate was almost half that of the group that received i.v. therapy alone (23% versus 42%; $P = 0.06$). In a logistic regression model, combination therapy was not identified as an independent predictor of clinical cure. No adverse events related to inhaled colistin were reported, although 8 patients in each group developed nephrotoxicity. This study demonstrates that although no statistically significant differences were found, patients on combination therapy tended to experience less morbidity and mortality. However, several limitations were noted in this study (305, 306), including a lack of information regarding the timing of initiation of colistin therapy, the absence of data on the concurrent use of other antibiotics, and the omission of the total colistin dose administered in both study groups. Of note, the results of this study showed that inhaled colistin had no impact on bacterial growth or microbiological eradication, a finding in contrast to data from previous studies. This study differed from others, as the primary pathogen was *A. baumannii* and a lower dose of inhaled colistin was employed. Repeat respiratory cultures were not dictated, as this study was retrospective (304). Despite common hesitations regarding the efficacy of the polymyxins, and colistin in particular, reported studies show a clinical efficacy of >50% despite a high baseline severity of illness. This response rate is similar to those reported in previous studies examining the use of piperacillin, imipenem-cilastatin, and ciprofloxacin for *P. aeruginosa* pneumonia (307). Given the good clinical efficacy in Gram-negative LRTIs when given systemically, administration of colistin via inhalation should maximize effectiveness by providing increased concentrations locally and minimize toxicity. This is especially true for difficult-to-treat pathogens for which the polymyxins remain the only viable treatment option.

Berlana et al. described 80 patients treated with colistin for infections due to *A. baumannii* (86%) and *P. aeruginosa* (14%). Forty-nine of these patients suffered from pneumonia, and they all received inhaled colistin for a mean duration of 12 days. The type of pneumonia was not categorized in this study. Twelve patients infected with *A. baumannii* also received i.v. colistin, while no patients infected with *P. aeruginosa* received parenteral colistin, although 85% of patients received some concomitant antibiotic. The doses of inhaled colistin varied widely in this study, although the majority (79%) of patients received 0.5 million IU every 6 h. For the 40 patients with pretreatment respiratory cultures, 37 of them were negative upon repeat culture at the end of treatment. Clinical cure was not evaluated in this study, and the overall mortality rate among all patients was 18%, although this included patients with other foci of infection in addition to pneumonia. Nephrotoxicity was specifically examined in this study and was not observed in any patients with evaluable data. Of note, this study used the parenteral form of CMS for inhalation therapy via a nebulizer (308).

A similar retrospective cohort study compared i.v. colistin ($n = 43$) to inhaled colistin plus i.v. colistin ($n = 78$) in patients with VAP (309). The most common pathogen responsible for VAP was *A. baumannii*, in >70% of patients in each group. The mean daily dose of inhaled colistin was 2.1 million IU, and therapy was started within 4 days of administration of i.v. colistin in >90%

of patients. Eighteen patients in the group administered i.v. and inhaled colistin also received another concomitant systemic antibiotic, compared to 5 in the group administered i.v. colistin only. Of note, there were significantly more patients with colistin-only-susceptible pathogens in the group that received i.v. colistin only (72.1% versus 47.4%; $P = 0.009$). A greater incidence of clinical cure among the group administered inhaled plus i.v. colistin than in the group administered i.v. colistin only was observed (79.5% versus 60.5%; $P = 0.025$). This association remained true in the group of patients for which colistin was the only microbiologically active antimicrobial (76.7% versus 57.9%; $P = 0.049$). Importantly, upon multivariate analysis, administration of inhaled colistin was the only independent predictor for the cure of VAP (odds ratio [OR], 2.53; 95% confidence interval [CI], 1.11 to 5.76). There was no difference in all-cause or in-hospital mortality rates between the two groups (39.7% for the combination group versus 44.2% for the monotherapy group; $P = 0.92$). By multivariate analysis, a higher APACHE II score (OR, 1.12; 95% CI, 1.04 to 1.20), the presence of a malignancy (OR, 4.11; 95% CI, 1.18 to 14.23), and a lower daily dosage of i.v. colistin (OR per million international units, 0.81; 95% CI, 0.68 to 0.96) were all significant predicators of mortality. Although adverse events were not specifically examined in this study, no events associated with inhaled colistin were reported.

A small retrospective study examined the use of inhaled colistin for eight patients with MDR Gram-negative pneumonias, 88% of which were due to *A. baumannii*. Six of the eight patients included had VAP. The dose of inhaled colistin ranged from 1.5 million to 6 million IU divided every 6 to 8 h for a mean duration of 10.5 days, and 7/8 patients received concomitant i.v. colistin or another systemic agent. Seven of the eight patients achieved improvement or cure in their pneumonia by the end of treatment, and 4/5 evaluable patients had bacterial eradication. No superinfection with Gram-positive organisms or emergence of colistin-resistant Gram-negative organisms was detected. There were no reported adverse events related to inhaled colistin (310).

A small case series of two patients with HIV documented clinical success in the prevention of recurrence of *P. aeruginosa* pneumonia using inhaled colistin for long durations, up to 17 months, without adverse effects (311).

Finally, a recent systematic review and meta-analysis examined the reported evidence regarding the efficacy and safety of adjunct inhaled colistin with i.v. colistin versus i.v. colistin alone in patients with nosocomial pneumonia due to MDR Gram-negative pathogens. This review included some of the studies mentioned in this section plus an additional two not discussed here (312, 313). In this analysis, clinical outcome was defined as the clinical response rate, classified as clinical cure (resolution of presenting signs and symptoms of infection at the end of treatment) or improvement (partial resolution of present signs and symptoms). Microbiological outcome was defined as eradication of the pathogen in culture specimens at the end of hospitalization. The quality of the included studies was evaluated on the Newcastle-Ottawa scale, with point values of 7 to 8 and 5 to 6 indicating very good and good studies, respectively. In total, 299 studies were identified in the initial search, although only 9 were included, comprising 672 patients. All included studies were published from 2010 to 2014, only one was prospective, all but two were performed in Europe, and five of the nine studies evaluated only VAP. In the groups that received i.v. and inhaled colistin, i.v. colistin was given for an average of 15.2 days at a mean dose of 6.3 million IU/day, compared to 12.5 days at a dose of 6.5 million IU/day in the groups that received i.v. colistin only. The daily dose of inhaled colistin was 2 million to 4 million IU divided twice or three times. Four studies reported concomitant antibiotic use during administration of colistin. The results from eight studies were available for evaluation of clinical response rates and showed significantly better clinical improvement and clinical cure in the groups that received i.v. plus inhaled colistin than in the groups that received i.v. colistin alone (odds ratio, 1.81; 95% CI, 1.30 to 2.53; $P = 0.0005$). Importantly, this outcome did not show evidence of statistical heterogeneity based on the inconsistency index ($I^2 = 21\%$). In a subgroup analysis comparing i.v. and inhaled colistin to i.v. colistin alone, patients receiving i.v. and inhaled therapy tended to have a higher clinical cure rate, although this was not statistically significant (odds ratio, 1.69; 95% CI, 0.98 to 2.91; $P = 0.06$). Six studies commented on microbiological eradication and showed higher rates of eradication in the groups that received i.v. and inhaled colistin (odds ratio, 1.66; 95% CI, 1.11 to 2.49; $P = 0.01$). All nine included studies were available to assess mortality, which again showed a significantly lower all-cause ICU or hospital mortality rate in groups that received i.v. and inhaled colistin (odds ratio, 0.69; 95% CI, 0.50 to 0.95; $P = 0.02$), without statistical heterogeneity ($I^2 = 0\%$). Nephrotoxicity was the only safety outcome analyzed in this review, and no significant difference between the groups was observed. All nine studies showed very-good-quality scores on the Newcastle-Ottawa scale, and statistical evaluations indicated no evidence of publication bias. This systematic review and meta-analysis demonstrates a favorable impact of i.v. and inhaled colistin on clinical improvement, clinical cure, microbiological eradication, and mortality compared to i.v. colistin alone, without increased toxicity in the combination group (314). It is important to remember that although statistical heterogeneity was not observed when data from all nine studies were combined, there are still contradictory conclusions between individual studies owing to differences in sample size, selection bias, pathogen distribution, and delivery device. The consistent dosing of inhaled colistin among all nine studies is in concordance with existing literature and supports the anecdotal data discussed above.

In the only study examining the use of inhaled polymyxin B for the treatment of MDR Gram-negative LRTIs, 25 critically ill patients who received i.v. and/or inhaled polymyxin B along with other systemic antimicrobials were evaluated retrospectively. Almost all patients (92%) were admitted to the ICU, and 88% were mechanically ventilated. Inhaled polymyxin B was most frequently given as 2.5 mg/kg/day divided every 6 h and was used in eight patients, most often in combination with a carbapenem. *A. baumannii* was the most frequently isolated pathogen in 55% of patients, and all cultured Gram-negative isolates were susceptible to polymyxin B. The overall end-of-treatment mortality rate was 21%, while a favorable clinical response was reported for 76% of patients. No differences in outcomes between patients receiving i.v. and those receiving inhaled polymyxin B were observed (76% versus 67%; $P = 0.63$). Respiratory-related adverse events were not reported in this study, although there were two instances of neurotoxicity reported, but the route of polymyxin B administration in these cases was unclear. The lack of standardization of therapy and a control group, along with small numbers, limit the ability to draw firm conclusions from this study. Although the lack

of reported pulmonary adverse events in this study is encouraging, further studies examining the use specifically of inhaled polymyxin B as an adjunct therapy for Gram-negative LRTIs are urgently needed (315).

(iii) β-Lactams. In a 3-year prospective study of 25 mechanically ventilated patients with *Enterobacteriaceae* or *P. aeruginosa* pneumonia, i.v. and inhaled cefotaxime, ceftazidime, or tobramycin were administered along with selective digestive decontamination with polymyxin B, tobramycin, and amphotericin. The inhaled dose was given as half of the i.v. dose four times daily. Antibiotics were continued until tracheal aspirate cultures were sterile for two consecutive samples. Eradication was achieved in 96% of patients within 9 days after the start of treatment, and only two patients experienced a relapse. No emergence of resistant organisms was observed (316).

A prospective, randomized, phase II trial was designed to assess the efficacy and safety of inhaled ceftazidime and amikacin in patients with VAP due to *P. aeruginosa*. Forty patients were included in this study, 20 of whom received inhaled ceftazidime and amikacin and 20 of whom received i.v. ceftazidime, amikacin, or ciprofloxacin, depending on susceptibilities. Inhaled ceftazidime was administered via a vibrating-plate nebulizer at 15 mg/kg 8 times per day, and amikacin was administered at 25 mg/kg once daily. Notably, patients with extrapulmonary infections and those who received >24 h of antibiotics active against *P. aeruginosa* were excluded. In this study, the extrapulmonary depositions of ceftazidime and amikacin were 37% each. Cure was assessed on day 9 of therapy and was achieved in 70% and 55% of the patients in the groups administered inhaled and i.v. therapy, respectively ($P = 0.33$). There were also no differences in the recurrence of VAP, length of stay, or duration of ventilation between groups. There was no emergence of resistance in the group that received inhaled therapy, and the 4 patients who initially had a *P. aeruginosa* strain intermediate to ceftazidime or amikacin had successful bacterial eradication at day 9. Patients in the group that received inhaled therapy had more rapid microbiological clearance, with 94% of patients having negative BAL fluid cultures on day 3, compared to 40% in the i.v. treatment group. No episodes of bronchospasm were observed, although one patient was excluded from the study after nebulization-dependent alveolar derecruitment. Three adverse events related to obstruction of the expiratory filter, which led to sudden cardiac arrest in one patient, were reported. Plasma concentrations were measured in both groups after 4 days of therapy, which showed significantly lower plasma concentrations of both drugs in the group that received inhaled therapy. The ceftazidime trough concentration in plasma was ~4-fold lower in the group that received inhaled therapy, and the amikacin peak concentration in plasma was ~5-fold lower, while trough concentrations were similar. Overall, the group administered inhaled therapy achieved more rapid bacterial eradication with similar clinical outcome results despite not receiving systemic therapy. Systemic concentrations were significantly lower, although several adverse events were reported in the group that received inhaled therapy. Notably, patients were treated for only 8 days in either group during this study (317).

In one case report of a patient with severe nosocomial pneumonia due to *P. aeruginosa*, i.v. imipenem-cilastatin was given for 10 days, without improvement in clinical signs and symptoms and gas exchange. After day 10, inhaled imipenem-cilastatin was added as 50 mg every 6 h over 30 min via a jet nebulizer connected to the ventilator circuit. After 48 h of inhaled therapy, the patient showed a marked decrease in tracheobronchial secretions and white blood cell counts. The patient's fever resolved, and after 5 days of inhaled therapy, he was able to be weaned off the ventilator without any adverse events (318).

A short report detailed the use of inhaled ampicillin-sulbactam in 20 mechanically ventilated patients with *A. baumannii* pneumonia. Ten patients received i.v. ampicillin-sulbactam alone, compared to 10 who received both i.v. and inhaled ampicillin-sulbactam dosed at 3 g every 8 h. After 2 to 3 days of therapy, patients who received both i.v. and inhaled ampicillin-sulbactam had reductions in viable counts of bacteria to $<10^2$ CFU/ml, compared to no reduction in CFU per milliliter for those who received i.v. therapy alone (319).

(iv) Summary of inhaled antibiotics for treatment of VAP. Compared to the two disease states discussed above in this review, there is a wealth of reported data evaluating the use of inhaled antibiotics in the treatment of Gram-negative nosocomial pneumonia. The bulk of these data is for patients with VAP; the largest collection of studies utilized inhaled colistin and the majority of reports are from institutions in Europe. The most commonly administered regimen for inhaled colistin is 3 million IU every 8 to 12 h given via a jet nebulizer. There are also several studies using aminoglycoside antibiotics via the inhaled route and only minimal data associated with β-lactam use. The vast majority of the studies included in this section are retrospective and observational in nature, and there are very few high-quality, randomized, placebo-controlled studies in this field. Even so, the data taken together consistently show positive clinical and microbiological outcomes with inhaled therapy along with a lack of any serious pulmonary toxicity. For those studies in which a comparator group was included, inhaled antibiotics as adjunct therapy often display improved clinical and microbiological outcomes over systemic therapy alone.

The PDDS used for inhalation of BAY41-6551 is an exciting improvement over currently available delivery systems, especially for patients undergoing mechanical ventilation. This drug-device combination is currently being evaluated as adjunct therapy in two randomized, placebo-controlled, phase III clinical trials, INHALE 1 and INHALE 2 (ClinicalTrials registration numbers NCT01799993 and NCT00805168, respectively). These results will represent the first controlled trial utilizing a drug-device combination specifically formulated for inhaled administration of an antimicrobial agent in mechanically ventilated patients with Gram-negative nosocomial pneumonia and will help to definitively determine the place for this route of administration in therapy.

Data from the currently available literature are insufficient to support the routine use of inhaled antibiotics as monotherapy in patients with Gram-negative nosocomial pneumonia. For patients who are not critically ill, have no extrapulmonary signs or symptoms of infection, and have monomicrobial pneumonia due to a Gram-negative pathogen, monotherapy with an inhaled antibiotic is a reasonable therapeutic selection. Inhaled antibiotics should be utilized as adjunct therapy in patients with nosocomial pneumonia, particularly VAP, due to Gram-negative organisms, especially *P. aeruginosa* and other nonfermenters such as *A. baumannii*. Specifically, inhaled antibiotics should be started empirically along with systemic therapy in patients with a history of or who are at high risk for infections by MDR Gram-negative patho-

gens given the mortality associated with delayed appropriate antibiotic therapy in these patients. In this case, the inhaled agent may represent the only microbiologically active agent despite *in vitro* resistance, as discussed above. Inhaled antibiotics are essential in order to penetrate the biofilm formed within the ET tube during mechanical ventilation and to reach the lung parenchyma in sufficient concentrations in these patients suffering from an illness for which the morbidity and mortality rates are extremely high.

Although a variety of agents have been studied for inhaled administration in cases of nosocomial pneumonia, the choice of the agent should depend on which appropriately formulated inhalation solution is available, along with the most effective delivery device, especially for those patients who are mechanically ventilated. It is essential that inhaled therapy is administered with optimized ventilator settings and given by a trained professional with experience in delivering these agents, as discussed above (Table 3). Inhaled antibiotics should be given as long as the patient is intubated and for the same duration as that for systemic therapy. If patients are extubated prior to the end of systemic therapy, continuing to administer the inhaled agent via nebulization or a DPI is reasonable. If serious pulmonary adverse events occur, the inhaled agent should be discontinued.

Inhaled antibiotics for prevention Gram-negative LRTIs. Since the upper respiratory tract is rapidly colonized after hospitalization for acute illness and given that nosocomial pneumonias are often preceded by upper airway colonization, it is reasonable to assume that prevention of airway colonization via inhaled antimicrobials may help prevent hospital-acquired Gram-negative LRTIs.

(i) Polymyxins. In a population of critical-care patients where MDR Gram-negative organisms are endemic, a single-center, open-label, randomized trial was conducted to evaluate the impact of prophylactic inhaled colistin on the incidence of VAP (320). Adult patients mechanically ventilated for >48 h without signs and symptoms of active respiratory infection were included. Patients received either CMS or placebo three times daily for 10 days of ventilation or until extubation, whichever was sooner. A jet nebulizer was used, and CMS was delivered as 500,000 U. The primary outcome was the incidence of VAP at 30 days, with additional secondary outcomes including the incidence of VAT, length of stay in the ICU, mortality, number of days without systemic antibiotic exposure, and emergence of colistin-resistant bacteria. A total of 84 patients received inhaled colistin for a median of 10 days, compared to 84 who received placebo for a median of 9 days. Inhaled prophylaxis was initiated within a median of 8 h following intubation in both groups, and the majority of patients (76.2% in the colistin group versus 71.4% in the placebo group; $P = 0.60$) received systemic antibiotics during the 10-day prophylaxis period, with no differences in the numbers or types of antibiotics received. The overall incidence of VAP in the colistin group was 16.7%, compared to 29.8% in the placebo group ($P = 0.07$), although the incidence of VAP due to Gram-negative organisms was lower in the colistin group (10.7% versus 17.9%; $P = 0.03$). Interestingly, the incidence of VAP in patients receiving systemic antibiotic therapy for treatment of Gram-negative organisms was also lower in the colistin group (14.1% versus 31.7%; $P = 0.03$). Among patients who developed VAP, the subsequent ICU mortality rate was lower for the patients receiving inhaled colistin (7.1% versus 44%; $P = 0.03$). There were no differences in the incidence of VAT, hospital mortality, length of stay, or number of days without systemic antibiotics between the groups. No colistin-resistant isolates emerged during the study, and there were no differences in the incidences of adverse events, including bronchospasm, between the groups. This study demonstrates a trend toward improved outcomes for patients receiving VAP prophylaxis with inhaled colistin. It is difficult to determine the true effect of prophylaxis, as patients continued the study even if they developed VAP and were treated for an active infection. Of note, the dose of inhaled colistin in this study is roughly half of what is normally used for the treatment of VAP. Although there was no observed difference in the incidences of VAP between the groups, the patients receiving inhaled colistin as prophylaxis demonstrated a lower rate of VAP than what is normally expected, despite the fact that the median duration of ventilation was >10 days in both groups (12 versus 13.5 days; $P = 0.26$).

In a small case series of five patients colonized with *P. aeruginosa*, 1 million IU of colistin inhaled every 8 h reduced microbial growth and eventually sterilized cultures by day 6 (321). A meta-analysis from 2006 evaluating the effect of antibiotic administration via the respiratory tract on the prevention of ICU-acquired pneumonia revealed a decrease in the incidence of ICU-acquired pneumonia in patients receiving prophylaxis. Although this analysis combined both instillation and nebulization routes of administration, these data support the potential for reducing the incidence of pneumonia in particularly vulnerable critically ill patients (276).

Inhaled polymyxin B was administered in a prospective study designed to evaluate its efficacy at preventing upper respiratory tract colonization with *P. aeruginosa*. Randomly selected patients were given polymyxin B via a hand atomizer at a dose of 2.5 mg/kg/day divided every 4 h within 24 h of admission. The polymyxin B solution was atomized and sprayed into the posterior pharynx, tracheostomy tube, or endotracheal tube as applicable. Fifty-eight patients were included in this study, 33 of whom received polymyxin B and 25 of whom were controls. Only 7 of the 33 polymyxin B-treated patients became colonized, compared to 17 of the 25 controls. There were no significant demographic differences between the groups. Administration of polymyxin B showed the greatest reduction in colonization for patients admitted to the ICU for 1 week or more. The vast majority of patients in both groups (88 and 76%) required systemic antibiotics during their ICU stay, although only 4 patients developed pneumonia during the study. Six patients developed renal failure, although detectable plasma concentrations of polymyxin B were found in only one of these patients. No other toxicities were observed (322). A follow-up study by the same group examined the effect of this therapy on the incidence of Gram-negative pneumonia. In this study, either saline or polymyxin B was given via inhalation in on/off cycles of 8 weeks. Approximately half (49% and 53%, respectively) of the patients in the saline and polymyxin B groups received systemic antibiotics throughout the study. Only 1.6% of patients receiving polymyxin B had upper airway colonization by organisms susceptible to polymyxin B, compared to 9.7% of patients in the placebo period. There was no difference in the numbers of patients acquiring pneumonia between the placebo and polymyxin B cycles (17 versus 18 patients), although the majority of these cases were due to Gram-positive organisms or Gram-negative organisms intrinsically resistant to polymyxin B (i.e., *Proteus* spp.). There was also no difference in mortality rates between

cycles (12.2 versus 12%) (40). Unfortunately, the results of this study along with the results of the study by Feeley et al. in 1975 (7) significantly diminished the enthusiasm around inhaled antibiotics for years afterwards. Intratracheal colistin, as opposed to inhaled colistin, has also been shown to reduce the incidence of nosocomial pneumonia by 10 to 25% in critically ill patients undergoing mechanical ventilation. Emergence of colistin-resistant organisms was not observed in this study (323).

(ii) β-Lactams. A randomized, double-blind, placebo-controlled study evaluated the safety and efficacy of inhaled ceftazidime versus placebo for VAP prevention in 40 critically ill trauma patients (43). Adult patients admitted to the trauma ICU who were expected to undergo mechanical ventilation for at least 7 days were randomized to receive either ceftazidime at 250 mg every 12 h or placebo for 7 days. Study treatment was initiated within 48 h of hospital admission and continued until VAP developed or until day 7. Inhaled ceftazidime was administered via a jet nebulizer with optimized ventilator settings by a respiratory therapist. In this study, BAL was performed pretreatment and once posttreatment during days 4 to 7 for analysis of both inflammatory markers (TNF-α, IL-1β, IL-6, and IL-8) and ceftazidime concentrations. Blood samples were also collected during the time of BAL. The primary outcomes of this study were the incidences of VAP at days 7 and 14 and at the end of the ICU stay. There were no significant differences between the ceftazidime ($n = 20$) and placebo ($n = 20$) groups at baseline. The mean numbers of doses in the ceftazidime and placebo groups were 12.5 and 11.7, respectively. No significant difference in the number of patients who developed VAP at day 7 was noted ($P = 0.22$). There was a statistically significant reduction in the incidence of VAP among patients who received inhaled ceftazidime at day 14 ($P = 0.021$) and throughout their ICU stay ($P = 0.022$). For the patients who developed VAP, >85% of patients in each group received systemic antibiotics prior to developing VAP. This seems to imply that these patients had extrapulmonary foci of infection prior to developing VAP, but the reason for this systemic usage is not described by these authors. In the patients who developed VAP ($n = 35$), the most common (26%) infecting organism was *P. aeruginosa*. Notably, 100% of these *Pseudomonas* isolates were susceptible to ceftazidime. There was no difference in the duration of mechanical ventilation, ICU length of stay, total antibiotic therapy, and mortality between the two study groups. Ceftazidime concentrations were detectable in the BAL fluid of 16 of 19 patients, with a median concentration of 56 mg/liter (range, 2.1 to 443.9 mg/liter). Interestingly, 3 of these 16 patients had BAL fluid concentrations that were lower than the 8-mg/liter breakpoint for ceftazidime, although none of these 3 patients went on to develop VAP. Serum concentrations of ceftazidime were undetectable in 17 of 18 patients. Additionally, BAL fluid concentrations of TNF-α, IL-1β, and IL-8 were significantly decreased in the ceftazidime group compared to the placebo group, in which they were significantly increased from baseline concentrations. The 73% reduction in the incidence of VAP at day 14 in this study utilizing a β-lactam antibiotic for VAP prevention in mechanically ventilated ICU patients provides key evidence for the role of inhaled agents in decreasing the morbidity and mortality burdens associated with this serious infection. The lack of emergence of resistance and adverse events supports the need to validate these findings in a larger patient population utilizing only inhaled therapy without systemic antimicrobials.

A similar prospective, double-blind study compared the incidence of VAP at 14 days and 30 days among 105 patients who received inhaled ceftazidime to that among those who received placebo (324). Patients were included if they were admitted to the ICU and considered to be at high risk (>25%) for VAP. Ceftazidime was delivered as 250 mg every 12 h for 7 days or until the patient was extubated. Again, respiratory therapists administered inhaled ceftazidime via a jet nebulizer with optimized ventilator settings. Fifty-three patients received ceftazidime, and 52 received placebo, and the overall rates of VAP at 14 and 30 days were 43% and 50%, respectively. There were no significant differences between the groups at baseline, including the median number of doses of the study drug (14 in the ceftazidime group versus 13 in the placebo group; $P = 0.79$). In this report, the difference in the incidences of VAP between the ceftazidime group and the placebo group was not statistically different at 14 days (40% versus 46%; $P = 0.50$) or at 30 days (49% versus 50%; $P = 0.90$). Four of the six most common infecting pathogens in this study were Gram-positive organisms and therefore not covered by ceftazidime, and these authors failed to describe whether systemic antibiotic therapy was utilized. There were no significant differences in secondary outcomes between the two groups, including mortality, duration of mechanical ventilation, and length of stay. The reason for the conflicting results between this study and the above-described study are not clear, although the study described above included fewer patients, and the incidence of VAP in the placebo group was higher than that in this study, possibly providing a larger margin for effect. Also, 17% of the patients in this study received fewer than six doses of the study drug. In this study, two patients developed VAP due to ceftazidime-resistant *P. aeruginosa*. Adverse effects were not described.

(iii) Summary of inhaled antibiotics for prevention of VAP. The efficacy data for the use of inhaled antibiotics for the prevention of VAP are encouraging but contradictory and remain to be definitively proven in future studies. Both inhaled colistin at 500,000 IU three times daily and ceftazidime at 250 mg every 12 h have demonstrated reduced incidences of Gram-negative VAP compared to placebo in patients undergoing mechanical ventilation. Given the lack of an appropriately formulated compound of inhaled ceftazidime, this agent cannot be recommended, although it is likely that other appropriately formulated agents would see similar success. There were no serious adverse events reported in these studies, and the emergence of resistant Gram-negative organisms was exceedingly rare. Therefore, patients undergoing mechanical ventilation who are at high risk for VAP may benefit from inhaled antibiotic prophylaxis, especially considering the benefit-to-risk ratio of the morbidity and mortality associated with the development of VAP. The most appropriate patient for inhaled antibiotic prophylaxis needs to be carefully identified, and VAP risk scoring systems such as those utilized in the above-described studies may be useful tools. Clinicians considering implementing this strategy should pay very close attention to changes in their institution's microbial flora and be vigilant about monitoring for increases in resistance among Gram-negative respiratory pathogens, especially in the units where this therapy is being used.

SAFETY

In general, testing and proving the safety of inhaled antibiotics are much more difficult than for other routes of administration in *in vitro*, preclinical, and clinical studies (325). In addition, current

pharmaceutical toxicology studies judge safety margins based on target organ toxicity measured as a function of the drug exposure (AUC) in the organ relative to plasma. As discussed above, an ideal inhaled antibiotic should produce as little systemic exposure as possible, therefore making only local pulmonary toxicity studies relevant, for which there is no standard method. In addition, animal studies may serve as a poor model, as rodent upper airways are extremely sensitive to insult from inhaled particles, and changes are not usually predictive of irritancy in humans. The most concerning adverse effects that one might expect with inhaled therapy, such as macrophage accumulation, cellular infiltration, epithelial degeneration, necrosis, fibrosis, and hyperplasia, are not able to be routinely monitored in humans in a practical manner.

Two potential concerns with regard to the administration of inhaled antibiotics are the development of antibiotic resistance and the adverse effects sustained when administering the medication. There have been two primary arguments supporting the concern of resistance development. First, there has been discussion of an antibiotic gradient occurring due to the increased deposition of inhaled antimicrobials at the proximal airway compared to the lower concentrations in the deeper airway (326). This concept should no longer be a concern for several reasons. For one, newer, more efficient delivery devices have been developed, and a better understanding of the particle characteristics and inhalation techniques required to deliver adequate drug concentrations to the distal airways has been attained. Additionally, a more thorough comprehension of PK/PD parameters and mathematical modeling allowed the introduction of the concept of the mutant prevention concentration (MPC), that is, the capacity to severely restrict the selection of resistant mutants during antibiotic treatment. This MPC is usually severalfold higher than the MIC and requires higher exposures to be achieved. The gradient of drug concentrations in the lungs is not the cause of resistance development; it is actually the theoretical concern of subinhibitory concentrations in the deep airways. In fact, administration of antimicrobials via inhalation deposits drug into the lungs at concentrations exponentially higher than would be possible with i.v. administration, as has been demonstrated by both animal and human data presented here. Therefore, aerosolization of antibiotics is actually more likely to achieve concentrations above both the MIC and MPC of most intrapulmonary organisms, while systemic administration of the same agent would achieve high concentrations in the plasma but low concentrations in ELF, creating a gradient. Consequently, systemic administration theoretically increases the chances of subinhibitory concentrations in the lungs and failure to reach the MPC (327). Administering antimicrobials via inhalation may actually help prevent resistance as opposed to inducing it. Finally, the data supporting increased resistance have come almost solely from patients with CF who had been treated with inhaled antibiotics over extremely long periods of time (326). Even in the most commonly quoted study supporting an increased prevalence of resistance, pulmonary function improved throughout the study, even in patients with isolates for which the MIC of tobramycin exceeded 128 mg/liter, and at 6 months, there was no increase in the rate of superinfection with tobramycin-resistant Gram-negative organisms.

Furthermore, in a recent randomized, placebo-controlled trial in critically ill patients by Palmer and Smaldone, inhaled antibiotics eradicated 96% of organisms, compared to 9% with placebo, and new drug resistance was not observed (328). On the contrary, the level of resistance to systemic antimicrobials was significantly higher in the placebo group. In fact, randomized clinical trials have not demonstrated the emergence of drug-resistant organisms, even in CF patients (329, 330). A recent phase III trial investigating long-term inhaled aztreonam for CF patients with *P. aeruginosa* infection found no changes in the MIC_{50} of aztreonam in any of the isolates. On the contrary, these authors found increases in susceptibility to tobramycin, suggesting that alternative therapies may actually preserve susceptibilities to first-line therapies (331). Although the use of inhaled antimicrobials for the prevention of VAP fell out of favor due to the landmark study by Feeley et al. (7), Klick and colleagues performed a similar study, alternating polymyxin administration with placebo, and did not see an increase in pseudomonal resistance (40), although in both cases, the drugs were sprayed into the posterior pharynx and instilled into the ET tube. Of note, the study by Feeley et al. isolated polymyxin-resistant organisms, although these organisms were *Flavobacteria* and *Enterococcus* isolates, which are not common respiratory pathogens. Similar results were observed by Brown et al. after implementing inhaled polymyxin B prophylaxis to control an ICU outbreak of *P. aeruginosa* (332). More recent investigations have found that inhaled tobramycin, ceftazidime, and colistin were effective at preventing VAP without the emergence of resistance (276). It is reasonable to assume once again that high concentrations administered over shorter, defined courses are actually likely to prevent resistant colonies from forming when used for VAP, VAT, and non-CF bronchiectasis.

Another theoretical concern is the deposition of antibiotics in the environment during inhalation. Nebulizers, in particular, can allow aerosolized particles to escape and be deposited in the patient's environment and may result in the inadvertent exposure of others to these particles. This may lead to bronchospasm or alterations in the microbial flora of healthy individuals, among other effects. Education of patients and their caregivers regarding the proper technique for inhaled administration is paramount to achieving effective deposition and preventing a loss of medication into the environment. In addition, employees who administer aerosolized antibiotics in the inpatient setting need to be protected and educated on precautionary practices for their use. Neither the National Institute for Occupational Safety and Health (NIOSH) nor the Occupation Safety and Health Administration (OSHA) considers inhaled antibiotics hazardous drugs and has not developed guidelines for the safe handling of these medications. Despite this, aerosolized antibiotics may pose an occupational hazard to respiratory therapists and other health care workers who administer them, through unintentional inhalation and exposure to escaped aerosols. In a recent survey of health care workers who administer aerosolized medications, only 52% of respondents reported receiving training on the safe handling of inhaled antibiotics. In addition, >80% of respondents reported not always using eye/face protection or a respirator when administering these agents, citing that these measures were not in the institutional protocol (333). Appropriate controls, including standard procedures for personal protective equipment, should be put in place within institutions that administer inhaled antibiotics in order to minimize the likelihood of unnecessary exposure to health care workers who administer them.

Continuous in-line nebulizers have been shown to complicate mechanical ventilatory support, although these nebulizers are

now rarely used, and more is understood on how to optimally deliver nebulizations during mechanical ventilation (334). Additionally, nebulizer reservoirs can act as a cesspool for water-loving Gram-negative organisms, and therefore, contaminated nebulizer reservoirs can produce deleterious effects, especially in mechanically ventilated patients (335). Proper cleaning of nebulizers used on multiple patients in the inpatient setting is essential, as is true for any nondisposable hospital equipment.

As discussed above repeatedly, the appropriate formulation of drug must be used for inhalation. The use of parenteral formulations for inhaled therapy has been linked to fatal respiratory distress syndrome and hypotension (336, 337). Inhalation of certain formulations of antimicrobial products can be associated with increased rates of adverse events. Specifically, the presence of sulfites in products has been shown to lead to bronchoconstriction (338). This is another reason why only products specifically formulated for inhalation should be used for inhalation antimicrobial therapy whenever possible. For example, in a randomized, double-blind study of CF patients, measurements of lung function were significantly decreased after inhalation of colistin sulfate compared to CMS (45). Colistin sulfate has also demonstrated a higher rate of bronchoconstriction than CMS (339). Polymyxin B has led to bronchoconstriction when administered via inhalation, potentially due to histamine release upon inhalation (340, 341). Neurotoxicity was previously associated with polymyxins but is now largely irrelevant with new formulations and modes of administration (342). The risk of nephrotoxicity is likely to be minimal given the limited systemic absorption after inhalation, as detailed in this review. In addition, newer, more advanced techniques for both devices and particles for drug delivery, as discussed above, have decreased the side effects associated with inhaling these agents, provided that they are used correctly in the appropriate setting.

LIMITATIONS OF INHALED ANTIMICROBIALS

The penetration of inhaled antibiotics into the lung parenchyma of patients with infected lungs is largely unknown, as the majority of investigations reporting intrapulmonary concentrations via this route were completed in phase I trials in healthy volunteers. Existing data for phase II studies of patients with VAP show wide interpatient variability in pulmonary concentrations after inhaled delivery.

In some studies, systemic absorption after inhalation reached plasma concentrations high enough to cause toxicity, primarily in animals and patients with diseased lungs. In contrast, the low systemic concentrations achieved after inhalation in most cases mean that inhaled antibiotics should probably not be used alone in critically ill patients with Gram-negative LRTIs or those with an extrapulmonary focus of infection.

Inhalation formulations are expensive and often require the assistance and time of knowledgeable respiratory therapists, particularly when used on a patient undergoing mechanical ventilation. Cost issues related to commercially prepared inhalation solutions may lead to pressure to use extemporaneously prepared solutions, often containing preservatives and with a lack of data supporting pulmonary deposition, particle size, and efficacy. In addition, the cost associated with more modern, more efficient delivery devices leads to the use of older nebulizers that are used repeatedly so that cost can be averaged over time, which may in turn negatively affect efficacy.

DISCUSSION

The prevalence and magnitude of bacterial resistance among Gram-negative pathogens will continue to increase, along with the yearly rates of morbidity and mortality related to antibiotic-resistant infections (29). This increase in bacterial resistance will continue to affect the ability to achieve optimal clinical outcomes with the current approaches in antimicrobial therapy. Despite advances in our knowledge and understanding of human infections and the overwhelming wealth of data available on treatment of patients with respiratory infections, pneumonia continues to remain the leading cause of death from infectious diseases worldwide. Compounding this issue is the frighteningly low number of antibiotics in development for the treatment of Gram-negative respiratory infections. To combat these issues, we must explore novel ways to optimize our existing antimicrobial arsenal. Although the concept of delivering antibiotics directly to the lungs via inhalation has been around for decades, recent advances in particle engineering, delivery devices, administration techniques, and our understanding of PK and PD allow the improved use of this route of administration. Antibiotics delivered directly to the site of infection are able to circumvent the layers of tissue and body fluids that separate the drug in the bloodstream from the drug in the lung and capitalize on maximizing efficacy while minimizing systemic toxicity.

Appropriate formulations of antibiotics and delivery devices must be used when administering these agents via the inhalation route to patients with Gram-negative LRTIs. Particle size and engineering characteristics are of the upmost importance for inhaled antibiotics in order to provide adequate pulmonary deposition to the lower airway and lung parenchyma. The MMAD should be between 1 and 5 μm, and spray-drying is currently the most effective manufacturing technique. Liposomal formulations can help improve residence time within the desired site of action and further decrease systemic distribution after inhalation. As clinicians, the choice of the appropriate delivery device is crucial to maximize the delivered dose and improve patient compliance. Jet nebulizers have been the most widely used device but do not allow for consistency in regard to particle size. Vibrating-mesh or -plate nebulizers are the most reliable devices at present until newer technologies such as the PDDS prove themselves definitively in phase III trials. Reliable *in vitro* PK and PD models are lacking in the field of inhaled antibiotics but will need to be improved in order to continue to advance this route of administration and support regulatory drug approval in this area. Clinical microbiological breakpoints need to become site specific and take into consideration the high concentrations of drug achievable within the lungs after inhalation administration. The reported animal and human PK studies evaluating inhaled antibiotics support the concept of being able to achieve high pulmonary concentrations along with low systemic absorption after inhalation. Finally, the appropriate process of inhaled drug delivery in patients undergoing mechanical ventilation is vitally important, and certain steps must be followed in order to provide optimal drug delivery to these patients.

Antibiotics delivered via inhalation have consistently demonstrated intrapulmonary concentrations severalfold higher than those achieved after parenteral administration in both animal models and human patients. Importantly, in diseased animal lungs with altered pathophysiology and ventilation-perfusion mismatch, they have demonstrated a significantly improved abil-

ity to penetrate areas of bronchopneumonia, resulting in better microbiological eradication. In clinical studies, adverse events associated with the use of inhaled antibacterials have been exceedingly rare and mild, even when these agents are given over the course of several years. Importantly, the theoretical concern of the emergence of resistant pathogens after administering inhaled antibiotics has not come to fruition in modern studies.

The majority of data regarding the treatment of Gram-negative LRTIs with inhaled antibiotics as monotherapy or adjunctive therapy demonstrate improved microbiological and clinical outcomes, although almost all of the literature consists of anecdotal reports and retrospective, uncontrolled case series. In the treatment of non-CF bronchiectasis, inhaled antibiotics alone or in addition to systemic agents clearly increase the eradication of bacteria from sputum. Additionally, inhaled antibiotics often also produce better quality-of-life outcomes but rarely seem to influence standard objective markers of lung function, including FEV_1. The administration of systemic antibiotics to patients with VAT has been shown to improve the morbidity and mortality of this disease and decrease the progression to VAP. Data for the use of inhaled antibiotics for VAT seem to mimic these results and may be a viable option, even as monotherapy, to both treat this infection and decrease the incidence and mortality associated with VAP. The use of inhaled antibiotics for the prevention of nosocomial pneumonia, especially in mechanically ventilated patients, is controversial but holds promise for future studies given the signals of improved outcomes, overall lack of toxicity, and absence of emergence of resistance seen throughout current studies. Similarly, in the treatment of Gram-negative nosocomial pneumonia, inhaled antibiotics as monotherapy or adjunct therapy have not been shown to lead to worse clinical or microbiological outcomes and have not produced significant toxicities or adverse events. In fact, many of the studies reviewed here reported improved microbiological eradication and clinical cure albeit with often erratic definitions of primary and secondary outcomes. Given the morbidity and mortality associated with nosocomial pneumonia, particularly VAP, the addition of inhaled antibiotics as adjunct therapy seems to be warranted in this scenario.

The majority of the studies included in this review did not contain a comparator group and allowed heterogeneous administration of concomitant systemic antimicrobials. Additionally, the dose, frequency, and duration of inhaled therapy were often not stated and varied widely among reports. Most of the studies included in this review were observational in nature, and the decision to add inhaled antimicrobials often depended on the susceptibility of the organism and the patient's severity of illness. Unfortunately, many studies regularly do not report the specific drug formulation used for inhalation or the exact type of device and specifications used. This information is vitally important and must be included in future studies in order to improve the understanding of the ideal clinical situations in which inhaled antibiotics can help maximize effects.

The many complex variables associated with the inhalation administration of antibiotics to patients with Gram-negative respiratory LRTIs have been extensively reviewed here. Inhaled antibiotics can provide benefit to many patients with a variety of pulmonary infectious complications; however, continued research in this area remains necessary. The current role for this therapy is as monotherapy or adjunct therapy in specific patients with non-CF bronchiectasis, VAT, or nosocomial pneumonia due to Gram-negative pathogens, as outlined throughout this review. Advances in the treatment of respiratory infections will undoubtedly include the more widespread use of inhaled antimicrobials based on the foundational principles laid out in this review.

FUTURE DIRECTIONS

The renewed interest in inhaled antibiotics is an exciting frontier but also one fraught with challenges. In order to give clinicians the confidence to use inhaled antimicrobials in patients with Gram-negative LRTIs, further studies assessing the efficacy and safety of these agents are needed. Guidelines regarding the appropriate and optimal use of inhaled antibiotics in targeted patient populations should be introduced by societies such as the IDSA, the ATS, and the Society of Critical Care Medicine in order to improve upon existing practices and expand the knowledge base of clinicians around the world for this exciting field. We encourage pharmaceutical companies that manufacture antibiotics to explore the inhalation route. Governmental initiatives such as the Generating Antibiotic Incentives Now (GAIN) Act should help fund the development of these novel administration routes given the limited number of new systemic agents being developed and brought to market. Public-private partnerships such as the Innovative Medicines Initiative, including the New Drugs for Bad Bugs and Combatting Bacterial Resistance in Europe programs, are also important models for moving drug development forward in areas of unmet medical need (343, 344). This increased development may allow us to preserve our antibiotic armamentarium by utilizing available agents in ways that maximize their efficacy and safety. Additionally, clinical trials evaluating systemic antibiotics in the treatment of pneumonia should allow the utilization of inhaled antibiotics in order to establish their role in a controlled clinical trial environment. Finally, more of the currently available antibiotics that are active against Gram-negative pathogens need to be explored for their utility as inhaled agents. This has recently been accomplished with meropenem, cefepime, and piperacillin-tazobactam, showing MMADs within the ideal range, although parenteral formulations were used in this evaluation (345).

One of the most challenging aspects of developing and using inhaled antimicrobials is the drug delivery device. As discussed above, there can be wide variability between devices and between manufacturers of the same device. Also, the devices available are able to deposit only a small portion of the dose into the lung periphery. Advanced devices such as the PDDS are needed to improve the delivery of these agents and in turn decrease the waste associated with inhalation, which should drive down costs and improve engineering capabilities as bioavailability increases.

The recent advancements in our understanding of the PK/PD aspects of antimicrobials have revolutionized the way in which we study and use antimicrobials in the clinical arena. From *in vitro* pharmacodynamic models to Monte Carlo simulations, we are now able to extrapolate results from small populations receiving an antibiotic to larger simulated populations. This gives us improved knowledge on PK/PD alterations that may occur and how best to achieve the index of antibacterial efficacy in special patient populations. Unfortunately, these same PK/PD advancements have not occurred in the realm of inhaled antibacterials. Future studies will need to focus on how the PK of inhaled agents changes in specific patient populations, with special consideration toward patients with pneumonia and ventilation-perfusion mismatches.

In addition to the above-mentioned INHALE 1 and 2 trials,

there are currently six trials evaluating inhaled antibiotics listed on ClinicalTrials.gov. Four of these six trials are evaluating Gram-negative antibacterials, one of which is a phase I PK study and the other three of which are for patients with pneumonia. The results of these trials will be instrumental in addressing some of the many remaining unanswered questions regarding inhaled antibiotics: Which patients should receive inhaled therapy? Should it be added to the standard of care or administered as monotherapy? Should inhaled antibiotics be used only for MDR pathogens or for all Gram-negative LRTIs? What are the appropriate dose, frequency, and duration of inhaled antibiotics?

We hope that we have provided some insight into these lingering uncertainties based on the currently available data, and we look forward to future progress made within this field.

ACKNOWLEDGMENTS

There was no financial support for this work. We thank Melinda Soriano for her contribution to this work.

REFERENCES

1. **Dessanges JF.** 2001. A history of nebulization. J Aerosol Med **14:**65–71. http://dx.doi.org/10.1089/08942680152007918.
2. **Aiache J-M.** 1990. Aerosol therapy in France. J Aerosol Med **3:**85–120. http://dx.doi.org/10.1089/jam.1990.3.85.
3. **Tiffeneau R, Brun E.** 1947. Sur le diamètre des particules dans les aérosols: titre inconnu. Sem Hop Paris **1947**(27):5.
4. **Mearns MB, Hunt GH, Rushworth R.** 1972. Bacterial flora of respiratory tract in patients with cystic fibrosis, 1950-71. Arch Dis Child **47:**902–907. http://dx.doi.org/10.1136/adc.47.256.902.
5. **Farber JE, Ross J.** 1950. The use of aerosol penicillin and streptomycin in bronchopulmonary infections. Calif Med **73:**214–217.
6. **Kuhn RJ.** 2001. Formulation of aerosolized therapeutics. Chest **120:**94S–98S. http://dx.doi.org/10.1378/chest.120.3_suppl.94S.
7. **Feeley TW, Du Moulin GC, Hedley-Whyte J, Bushnell LS, Gilbert JP, Feingold DS.** 1975. Aerosol polymyxin and pneumonia in seriously ill patients. N Engl J Med **293:**471–475. http://dx.doi.org/10.1056/NEJM197509042931003.
8. **Xu J, Murphy SL, Kochanek KD, Bastian BA.** 2016. Deaths: final data for 2013. Natl Vital Stat Rep **64:**1–119. http://www.cdc.gov/nchs/data/nvsr/nvsr64/nvsr64_02.pdf. Accessed 1 July 2015.
9. **Mandell LA, Wunderink RG.** 2015. Pneumonia, chapter 153. *In* Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J (ed), Harrison's principles of internal medicine, 19th ed. McGraw-Hill, New York, NY.
10. **Rodvold KA, George JM, Yoo L.** 2011. Penetration of anti-infective agents into pulmonary epithelial lining fluid: focus on antibacterial agents. Clin Pharmacokinet **50:**637–664. http://dx.doi.org/10.2165/11594090-000000000-00000.
11. **Sylvester JT, Shimoda LA, Aaronson PI, Ward JP.** 2012. Hypoxic pulmonary vasoconstriction. Physiol Rev **92:**367–520. http://dx.doi.org/10.1152/physrev.00041.2010.
12. **Densen P, Mandell GL.** 1980. Phagocyte strategy vs. microbial tactics. Rev Infect Dis **2:**817–838. http://dx.doi.org/10.1093/clinids/2.5.817.
13. **Krieg DP, Helmke RJ, German VF, Mangos JA.** 1988. Resistance of mucoid Pseudomonas aeruginosa to nonopsonic phagocytosis by alveolar macrophages in vitro. Infect Immun **56:**3173–3179.
14. **Stiver HG, Zachidniak K, Speert DP.** 1988. Inhibition of polymorphonuclear leukocyte chemotaxis by the mucoid exopolysaccharide of Pseudomonas aeruginosa. Clin Invest Med **11:**247–252.
15. **Baldwin DR, Honeybourne D, Wise R.** 1992. Pulmonary disposition of antimicrobial agents: methodological considerations. Antimicrob Agents Chemother **36:**1171–1175. http://dx.doi.org/10.1128/AAC.36.6.1171.
16. **Wang F, Daugherty B, Keise LL, Wei Z, Foley JP, Savani RC, Koval M.** 2003. Heterogeneity of claudin expression by alveolar epithelial cells. Am J Respir Cell Mol Biol **29:**62–70. http://dx.doi.org/10.1165/rcmb.2002-0180OC.
17. **Campbell L, Abulrob AN, Kandalaft LE, Plummer S, Hollins AJ, Gibbs A, Gumbleton M.** 2003. Constitutive expression of p-glycoprotein in normal lung alveolar epithelium and functionality in primary alveolar epithelial cultures. J Pharmacol Exp Ther **304:**441–452. http://dx.doi.org/10.1124/jpet.102.042994.
18. **Michot JM, Seral C, Van Bambeke F, Mingeot-Leclercq MP, Tulkens PM.** 2005. Influence of efflux transporters on the accumulation and efflux of four quinolones (ciprofloxacin, levofloxacin, garenoxacin, and moxifloxacin) in J774 macrophages. Antimicrob Agents Chemother **49:**2429–2437. http://dx.doi.org/10.1128/AAC.49.6.2429-2437.2005.
19. **Hall MJ, DeFrances CJ, Williams SN, Golosinskiy A, Schwartzman A.** 2010. National Hospital Discharge Survey: 2007 summary. Natl Health Stat Rep **2010:**1–20, 24.
20. **Arancibia F, Bauer TT, Ewig S, Mensa J, Gonzalez J, Niederman MS, Torres A.** 2002. Community-acquired pneumonia due to Gram-negative bacteria and Pseudomonas aeruginosa: incidence, risk, and prognosis. Arch Intern Med **162:**1849–1858. http://dx.doi.org/10.1001/archinte.162.16.1849.
21. **Quartin AA, Scerpella EG, Puttagunta S, Kett DH.** 2013. A comparison of microbiology and demographics among patients with healthcare-associated, hospital-acquired, and ventilator-associated pneumonia: a retrospective analysis of 1184 patients from a large, international study. BMC Infect Dis **13:**561. http://dx.doi.org/10.1186/1471-2334-13-561.
22. **Jones RN.** 2010. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clin Infect Dis **51**(Suppl 1):S81–S87. http://dx.doi.org/10.1086/653053.
23. **American Thoracic Society, Infectious Diseases Society of America.** 2005. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med **171:**388–416. http://dx.doi.org/10.1164/rccm.200405-644ST.
24. **Chastre J, Fagon JY.** 2002. Ventilator-associated pneumonia. Am J Respir Crit Care Med **165:**867–903. http://dx.doi.org/10.1164/ajrccm.165.7.2105078.
25. **Gaynes R, Edwards JR, National Nosocomial Infections Surveillance System.** 2005. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis **41:**848–854. http://dx.doi.org/10.1086/432803.
26. **Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F.** 2007. Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect **13:**560–578. http://dx.doi.org/10.1111/j.1469-0691.2007.01681.x.
27. **Chastre J, Wolff M, Fagon JY, Chevret S, Thomas F, Wermert D, Clementi E, Gonzalez J, Jusserand D, Asfar P, Perrin D, Fieux F, Aubas S, PneumA Trial Group.** 2003. Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. JAMA **290:**2588–2598. http://dx.doi.org/10.1001/jama.290.19.2588.
28. **Infectious Diseases Society of America, Spellberg B, Blaser M, Guidos RJ, Boucher HW, Bradley JS, Eisenstein BI, Gerding D, Lynfield R, Reller LB, Rex J, Schwartz D, Septimus E, Tenover FC, Gilbert DN.** 2011. Combating antimicrobial resistance: policy recommendations to save lives. Clin Infect Dis **52**(Suppl 5):S397–S428. http://dx.doi.org/10.1093/cid/cir153.
29. **Centers for Disease Control and Prevention.** 2013. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf. Accessed 26 January 2015.
30. **Hede K.** 2014. Antibiotic resistance: an infectious arms race. Nature **509:**S2–S3. http://dx.doi.org/10.1038/509S2a.
31. **Safdar N, Dezfulian C, Collard HR, Saint S.** 2005. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. Crit Care Med **33:**2184–2193. http://dx.doi.org/10.1097/01.CCM.0000181731.53912.D9.
32. **Ambrose PG, Bhavnani SM, Ellis-Grosse EJ, Drusano GL.** 2010. Pharmacokinetic-pharmacodynamic considerations in the design of hospital-acquired or ventilator-associated bacterial pneumonia studies: look before you leap! Clin Infect Dis **51**(Suppl 1):S103–S110. http://dx.doi.org/10.1086/653057.
33. **Hagerman JK, Hancock KE, Klepser ME.** 2006. Aerosolised antibiotics: a critical appraisal of their use. Expert Opin Drug Deliv **3:**71–86. http://dx.doi.org/10.1517/17425247.3.1.71.
34. **Le J, Ashley ED, Neuhauser MM, Brown J, Gentry C, Klepser ME, Marr AM, Schiller D, Schwiesow JN, Tice S, VandenBussche HL,**

Wood GC, Society of Infectious Diseases Pharmacists Aerosolized Antimicrobials Task Force. 2010. Consensus summary of aerosolized antimicrobial agents: application of guideline criteria. Insights from the Society of Infectious Diseases Pharmacists. Pharmacotherapy **30:**562–584. http://dx.doi.org/10.1592/phco.30.6.562.
35. **Wood GC.** 2011. Aerosolized antibiotics for treating hospital-acquired and ventilator-associated pneumonia. Expert Rev Anti Infect Ther **9:**993–1000. http://dx.doi.org/10.1586/eri.11.126.
36. **Patton JS.** 1996. Mechanisms of macromolecule absorption by the lungs. Adv Drug Deliv Rev **19:**3–36.
37. **Patton JS, Fishburn CS, Weers JG.** 2004. The lungs as a portal of entry for systemic drug delivery. Proc Am Thorac Soc **1:**338–344. http://dx.doi.org/10.1513/pats.200409-049TA.
38. **Ehrmann S, Roche-Campo F, Sferrazza Papa GF, Isabey D, Brochard L, Apiou-Sbirlea G.** 2013. Aerosol therapy during mechanical ventilation: an international survey. Intensive Care Med **39:**1048–1056. http://dx.doi.org/10.1007/s00134-013-2872-5.
39. **Klastersky J, Huysmans E, Weerts D, Hensgens C, Daneau D.** 1974. Endotracheally administered gentamicin for the prevention of infections of the respiratory tract in patients with tracheostomy: a double-blind study. Chest **65:**650–654. http://dx.doi.org/10.1378/chest.65.6.650.
40. **Klick JM, du Moulin GC, Hedley-Whyte J, Teres D, Bushnell LS, Feingold DS.** 1975. Prevention of gram-negative bacillary pneumonia using polymyxin aerosol as prophylaxis. II. Effect on the incidence of pneumonia in seriously ill patients. J Clin Invest **55:**514–519.
41. **Drobnic ME, Sune P, Montoro JB, Ferrer A, Orriols R.** 2005. Inhaled tobramycin in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection with Pseudomonas aeruginosa. Ann Pharmacother **39:**39–44.
42. **Palmer LB, Smaldone GC, Simon SR, O'Riordan TG, Cuccia A.** 1998. Aerosolized antibiotics in mechanically ventilated patients: delivery and response. Crit Care Med **26:**31–39. http://dx.doi.org/10.1097/00003246-199801000-00013.
43. **Wood GC, Boucher BA, Croce MA, Hanes SD, Herring VL, Fabian TC.** 2002. Aerosolized ceftazidime for prevention of ventilator-associated pneumonia and drug effects on the proinflammatory response in critically ill trauma patients. Pharmacotherapy **22:**972–982. http://dx.doi.org/10.1592/phco.22.12.972.33596.
44. **Dalhoff A.** 2014. Pharmacokinetics and pharmacodynamics of aerosolized antibacterial agents in chronically infected cystic fibrosis patients. Clin Microbiol Rev **27:**753–782. http://dx.doi.org/10.1128/CMR.00022-14.
45. **Westerman EM, Le Brun PP, Touw DJ, Frijlink HW, Heijerman HG.** 2004. Effect of nebulized colistin sulphate and colistin sulphomethate on lung function in patients with cystic fibrosis: a pilot study. J Cyst Fibros **3:**23–28. http://dx.doi.org/10.1016/j.jcf.2003.12.005.
46. **Patton JS, Byron PR.** 2007. Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov **6:**67–74. http://dx.doi.org/10.1038/nrd2153.
47. **Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD.** 1992. Allometric relationships of cell numbers and size in the mammalian lung. Am J Respir Cell Mol Biol **6:**235–243. http://dx.doi.org/10.1165/ajrcmb/6.2.235.
48. **Laube BL, Jashnani R, Dalby RN, Zeitlin PL.** 2000. Targeting aerosol deposition in patients with cystic fibrosis: effects of alterations in particle size and inspiratory flow rate. Chest **118:**1069–1076. http://dx.doi.org/10.1378/chest.118.4.1069.
49. **Kuhn RJ.** 2002. Pharmaceutical considerations in aerosol drug delivery. Pharmacotherapy **22:**80S–85S. http://dx.doi.org/10.1592/phco.22.6.80S.33907.
50. **Brain JD, Valberg PA.** 1979. Deposition of aerosol in the respiratory tract. Am Rev Respir Dis **120:**1325–1373.
51. **Backman P, Adelmann H, Petersson G, Jones CB.** 2014. Advances in inhaled technologies: understanding the therapeutic challenge, predicting clinical performance, and designing the optimal inhaled product. Clin Pharmacol Ther **95:**509–520. http://dx.doi.org/10.1038/clpt.2014.27.
52. **Byron PR.** 1986. Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. J Pharm Sci **75:**433–438. http://dx.doi.org/10.1002/jps.2600750502.
53. **Li X, Vogt FG, Hayes D, Jr, Mansour HM.** 2014. Physicochemical characterization and aerosol dispersion performance of organic solution advanced spray-dried microparticulate/nanoparticulate antibiotic dry powders of tobramycin and azithromycin for pulmonary inhalation aerosol delivery. Eur J Pharm Sci **52:**191–205. http://dx.doi.org/10.1016/j.ejps.2013.10.016.
54. **Haynes A, Nakamura J, Heng C, Heuerding S, Thompson G, Malcolmson RJ.** 2010. Aerosol performance of tobramycin inhalation powder. Respir Drug Deliv **3:**701–706.
55. **Geller DE, Weers J, Heuerding S.** 2011. Development of an inhaled dry-powder formulation of tobramycin using PulmoSphere technology. J Aerosol Med Pulm Drug Deliv **24:**175–182. http://dx.doi.org/10.1089/jamp.2010.0855.
56. **Miller DP, Tan T, Nakamura J, Malcolmson R, Tarara T, Weers J.** 30 June 2015. Physical characterization of tobramycin inhalation powder. I. Rational design of a stable engineered-particle formulation for delivery to the lungs. Mol Pharm http://dx.doi.org/10.1021/acs.molpharmaceut.5b00147.
57. **d'Angelo I, Conte C, La Rotonda MI, Miro A, Quaglia F, Ungaro F.** 2014. Improving the efficacy of inhaled drugs in cystic fibrosis: challenges and emerging drug delivery strategies. Adv Drug Deliv Rev **75:**92–111. http://dx.doi.org/10.1016/j.addr.2014.05.008.
58. **Mansour HM, Rhee YS, Wu X.** 2009. Nanomedicine in pulmonary delivery. Int J Nanomedicine **4:**299–319.
59. **Moreno-Sastre M, Pastor M, Salomon CJ, Esquisabel A, Pedraz JL.** 22 July 2015. Pulmonary drug delivery: a review on nanocarriers for antibacterial chemotherapy. J Antimicrob Chemother http://dx.doi.org/10.1093/jac/dkv192.
60. **Zhou QT, Leung SS, Tang P, Parumasivam T, Loh ZH, Chan HK.** 2015. Inhaled formulations and pulmonary drug delivery systems for respiratory infections. Adv Drug Deliv Rev **85:**83–99. http://dx.doi.org/10.1016/j.addr.2014.10.022.
61. **Stockmann C, Roberts JK, Yellepeddi VK, Sherwin CM.** 2015. Clinical pharmacokinetics of inhaled antimicrobials. Clin Pharmacokinet **54:**473–492. http://dx.doi.org/10.1007/s40262-015-0250-x.
62. **Kelly C, Jefferies C, Cryan SA.** 2011. Targeted liposomal drug delivery to monocytes and macrophages. J Drug Deliv **2011:**727241. http://dx.doi.org/10.1155/2011/727241.
63. **Sackner MA, Hirsch J, Epstein S.** 1975. Effect of cuffed endotracheal tubes on tracheal mucous velocity. Chest **68:**774–777. http://dx.doi.org/10.1378/chest.68.6.774.
64. **Husain S, Capitano B, Corcoran T, Studer SM, Crespo M, Johnson B, Pilewski JM, Shutt K, Pakstis DL, Zhang S, Carey ME, Paterson DL, McCurry KR, Venkataramanan R.** 2010. Intrapulmonary disposition of amphotericin B after aerosolized delivery of amphotericin B lipid complex (Abelcet; ABLC) in lung transplant recipients. Transplantation **90:**1215–1219. http://dx.doi.org/10.1097/TP.0b013e3181f995ea.
65. **Montgomery AB, Pitlick WH, Nardella P, Tracewell WG, Ramsey BW.** 2000. Sputum concentrations and systemic pharmacokinetics of aerosolized tobramycin (Tobi) in diseased lungs. Respir Drug Deliv **1:**19–24.
66. **Dolovich MB, Dhand R.** 2011. Aerosol drug delivery: developments in device design and clinical use. Lancet **377:**1032–1045. http://dx.doi.org/10.1016/S0140-6736(10)60926-9.
67. **Hickey AJ.** 2013. Back to the future: inhaled drug products. J Pharm Sci **102:**1165–1172. http://dx.doi.org/10.1002/jps.23465.
68. **Patton JS, Brain JD, Davies LA, Fiegel J, Gumbleton M, Kim KJ, Sakagami M, Vanbever R, Ehrhardt C.** 2010. The particle has landed—characterizing the fate of inhaled pharmaceuticals. J Aerosol Med Pulm Drug Deliv **23**(Suppl 2):S71–S87. http://dx.doi.org/10.1089/jamp.2010.0836.
69. **Hess D, Fisher D, Williams P, Pooler S, Kacmarek RM.** 1996. Medication nebulizer performance. Effects of diluent volume, nebulizer flow, and nebulizer brand. Chest **110:**498–505.
70. **Coates AL, MacNeish CF, Meisner D, Kelemen S, Thibert R, MacDonald J, Vadas E.** 1997. The choice of jet nebulizer, nebulizing flow, and addition of albuterol affects the output of tobramycin aerosols. Chest **111:**1206–1212. http://dx.doi.org/10.1378/chest.111.5.1206.
71. **Vecellio L, Abdelrahim ME, Montharu J, Galle J, Diot P, Dubus JC.** 2011. Disposable versus reusable jet nebulizers for cystic fibrosis treatment with tobramycin. J Cyst Fibros **10:**86–92. http://dx.doi.org/10.1016/j.jcf.2010.10.004.
72. **O'Riordan TG.** 2002. Formulations and nebulizer performance. Respir Care **47:**1305–1312; discussion 1312–1313.
73. **Loffert DT, Ikle D, Nelson HS.** 1994. A comparison of commercial jet nebulizers. Chest **106:**1788–1792. http://dx.doi.org/10.1378/chest.106.6.1788.

74. **Waldrep JC, Keyhani K, Black M, Knight V.** 1994. Operating characteristics of 18 different continuous-flow jet nebulizers with beclomethasone dipropionate liposome aerosol. Chest **105:**106–110. http://dx.doi.org/10.1378/chest.105.1.106.
75. **Daniels T, Mills N, Whitaker P.** 2013. Nebuliser systems for drug delivery in cystic fibrosis. Cochrane Database Syst Rev **4:**CD007639. http://dx.doi.org/10.1002/14651858.CD007639.pub2.
76. **Goode ML, Fink JB, Dhand R, Tobin MJ.** 2001. Improvement in aerosol delivery with helium-oxygen mixtures during mechanical ventilation. Am J Respir Crit Care Med **163:**109–114. http://dx.doi.org/10.1164/ajrccm.163.1.2003025.
77. **Dhand R.** 2002. Nebulizers that use a vibrating mesh or plate with multiple apertures to generate aerosol. Respir Care **47:**1406–1416; discussion 1416–1418.
78. **Geller DE, Rosenfeld M, Waltz DA, Wilmott RW, AeroDose TOBI Study Group.** 2003. Efficiency of pulmonary administration of tobramycin solution for inhalation in cystic fibrosis using an improved drug delivery system. Chest **123:**28–36. http://dx.doi.org/10.1378/chest.123.1.28.
79. **Dhand R.** 2008. Aerosol delivery during mechanical ventilation: from basic techniques to new devices. J Aerosol Med Pulm Drug Deliv **21:**45–60. http://dx.doi.org/10.1089/jamp.2007.0663.
80. **Haddrell AE, Davies JF, Miles RE, Reid JP, Dailey LA, Murnane D.** 2014. Dynamics of aerosol size during inhalation: hygroscopic growth of commercial nebulizer formulations. Int J Pharm **463:**50–61. http://dx.doi.org/10.1016/j.ijpharm.2013.12.048.
81. **Heyder J.** 2004. Deposition of inhaled particles in the human respiratory tract and consequences for regional targeting in respiratory drug delivery. Proc Am Thorac Soc **1:**315–320. http://dx.doi.org/10.1513/pats.200409-046TA.
82. **Mainz JG, Schadlich K, Schien C, Michl R, Schelhorn-Neise P, Koitschev A, Koitschev C, Keller PM, Riethmuller J, Wiedemann B, Beck JF.** 2014. Sinonasal inhalation of tobramycin vibrating aerosol in cystic fibrosis patients with upper airway Pseudomonas aeruginosa colonization: results of a randomized, double-blind, placebo-controlled pilot study. Drug Des Devel Ther **8:**209–217. http://dx.doi.org/10.2147/DDDT.S54064.
83. **Kesser KC, Geller DE.** 2009. New aerosol delivery devices for cystic fibrosis. Respir Care **54:**754–767; discussion 767–768. http://dx.doi.org/10.4187/002013209790983250.
84. **Tiddens HA, Bos AC, Mouton JW, Devadason S, Janssens HM.** 2014. Inhaled antibiotics: dry or wet? Eur Respir J **44:**1308–1318. http://dx.doi.org/10.1183/09031936.00090314.
85. **Tiddens H.** 2004. Inhaled antibiotics. Pediatr Pulmonol Suppl **26:**92–94.
86. **Harrison MJ, McCarthy M, Fleming C, Hickey C, Shortt C, Eustace JA, Murphy DM, Plant BJ.** 2014. Inhaled versus nebulised tobramycin: a real world comparison in adult cystic fibrosis (CF). J Cyst Fibros **13:**692–698. http://dx.doi.org/10.1016/j.jcf.2014.04.004.
87. **Konstan MW, Flume PA, Kappler M, Chiron R, Higgins M, Brockhaus F, Zhang J, Angyalosi G, He E, Geller DE.** 2011. Safety, efficacy and convenience of tobramycin inhalation powder in cystic fibrosis patients: the EAGER trial. J Cyst Fibros **10:**54–61. http://dx.doi.org/10.1016/j.jcf.2010.10.003.
88. **Gibson RL, Retsch-Bogart GZ, Oermann C, Milla C, Pilewski J, Daines C, Ahrens R, Leon K, Cohen M, McNamara S, Callahan TL, Markus R, Burns JL.** 2006. Microbiology, safety, and pharmacokinetics of aztreonam lysinate for inhalation in patients with cystic fibrosis. Pediatr Pulmonol **41:**656–665. http://dx.doi.org/10.1002/ppul.20429.
89. **Daddario MK, Hagerman JK, Klepser ME.** 2010. Clinical perspective on aztreonam lysine for inhalation in patients with cystic fibrosis. Infect Drug Resist **3:**123–132. http://dx.doi.org/10.2147/IDR.S7838.
90. **Godden DJ, Borland C, Lowry R, Higenbottam TW.** 1986. Chemical specificity of coughing in man. Clin Sci (Lond) **70:**301–306. http://dx.doi.org/10.1042/cs0700301.
91. **Lowry RH, Wood AM, Higenbottam TW.** 1988. Effects of pH and osmolarity on aerosol-induced cough in normal volunteers. Clin Sci (Lond) **74:**373–376. http://dx.doi.org/10.1042/cs0740373.
92. **Eschenbacher WL, Boushey HA, Sheppard D.** 1984. Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permeant anion causes cough alone. Am Rev Respir Dis **129:**211–215.
93. **Hess DR.** 2000. Nebulizers: principles and performance. Respir Care **45:**609–622.
94. **O'Doherty MJ, Thomas SH, Page CJ, Treacher DF, Nunan TO.** 1992. Delivery of a nebulized aerosol to a lung model during mechanical ventilation. Effect of ventilator settings and nebulizer type, position, and volume of fill. Am Rev Respir Dis **146:**383–388.
95. **O'Riordan TG, Greco MJ, Perry RJ, Smaldone GC.** 1992. Nebulizer function during mechanical ventilation. Am Rev Respir Dis **145:**1117–1122. http://dx.doi.org/10.1164/ajrccm/145.5.1117.
96. **O'Doherty MJ, Thomas SH.** 1997. Nebuliser therapy in the intensive care unit. Thorax **52**(Suppl 2)**:**S56–S59.
97. **Chait R, Craney A, Kishony R.** 2007. Antibiotic interactions that select against resistance. Nature **446:**668–671. http://dx.doi.org/10.1038/nature05685.
98. **Rahal JJ.** 2006. Novel antibiotic combinations against infections with almost completely resistant Pseudomonas aeruginosa and Acinetobacter species. Clin Infect Dis **43:**S95–S99. http://dx.doi.org/10.1086/504486.
99. **Klemmer A, Kramer I, Kamin W.** 2014. Physicochemical compatibility and stability of nebulizable drug admixtures containing dornase alfa and tobramycin. Pulm Pharmacol Ther **28:**53–59. http://dx.doi.org/10.1016/j.pupt.2013.08.003.
100. **Kramer I, Schwabe A, Lichtinghagen R, Kamin W.** 2009. Physicochemical compatibility of mixtures of dornase alfa and tobramycin containing nebulizer solutions. Pediatr Pulmonol **44:**134–141. http://dx.doi.org/10.1002/ppul.20955.
101. **Rubin BK.** 2015. Aerosol medications for treatment of mucus clearance disorders. Respir Care **60:**825–832. http://dx.doi.org/10.4187/respcare.04087.
102. **Adi H, Young PM, Chan HK, Stewart P, Agus H, Traini D.** 2008. Cospray dried antibiotics for dry powder lung delivery. J Pharm Sci **97:**3356–3366. http://dx.doi.org/10.1002/jps.21239.
103. **Tsifansky MD, Yeo Y, Evgenov OV, Bellas E, Benjamin J, Kohane DS.** 2008. Microparticles for inhalational delivery of antipseudomonal antibiotics. AAPS J **10:**254–260. http://dx.doi.org/10.1208/s12248-008-9033-8.
104. **Lee SH, Teo J, Heng D, Ng WK, Chan HK, Tan RB.** 2013. Synergistic combination dry powders for inhaled antimicrobial therapy: formulation, characterization and in vitro evaluation. Eur J Pharm Biopharm **83:**275–284. http://dx.doi.org/10.1016/j.ejpb.2012.09.002.
105. **Durante-Mangoni E, Signoriello G, Andini R, Mattei A, De Cristoforo M, Murino P, Bassetti M, Malacarne P, Petrosillo N, Galdieri N, Mocavero P, Corcione A, Viscoli C, Zarrilli R, Gallo C, Utili R.** 2013. Colistin and rifampicin compared with colistin alone for the treatment of serious infections due to extensively drug-resistant Acinetobacter baumannii: a multicenter, randomized clinical trial. Clin Infect Dis **57:**349–358. http://dx.doi.org/10.1093/cid/cit253.
106. **Zhou QT, Sun SP, Chan JG, Wang P, Barraud N, Rice SA, Wang J, Li J, Chan HK.** 2015. Novel inhaled combination powder containing amorphous colistin and crystalline rifapentine with enhanced antimicrobial activities against planktonic cells and biofilm of Pseudomonas aeruginosa for respiratory infections. Mol Pharm **12:**2594–2603. http://dx.doi.org/10.1021/mp500586p.
107. **Zhou QT, Gengenbach T, Denman JA, Yu HH, Li J, Chan HK.** 2014. Synergistic antibiotic combination powders of colistin and rifampicin provide high aerosolization efficiency and moisture protection. AAPS J **16:**37–47. http://dx.doi.org/10.1208/s12248-013-9537-8.
108. **Trapnell BC, McColley SA, Kissner DG, Rolfe MW, Rosen JM, McKevitt M, Moorehead L, Montgomery AB, Geller DE, Phase 2 FTI Study Group.** 2012. Fosfomycin/tobramycin for inhalation in patients with cystic fibrosis with Pseudomonas airway infection. Am J Respir Crit Care Med **185:**171–178. http://dx.doi.org/10.1164/rccm.201105-0924OC.
109. **Antoniu S.** 2015. Novel inhaled combined antibiotic formulations in the treatment of Pseudomonas aeruginosa airways infections in cystic fibrosis. Expert Rev Anti Infect Ther **13:**897–905. http://dx.doi.org/10.1586/14787210.2015.1041925.
110. **Pilcer G, De Bueger V, Traina K, Traore H, Sebti T, Vanderbist F, Amighi K.** 2013. Carrier-free combination for dry powder inhalation of antibiotics in the treatment of lung infections in cystic fibrosis. Int J Pharm **451:**112–120. http://dx.doi.org/10.1016/j.ijpharm.2013.04.069.
111. **Tamaoki J, Takeyama K, Tagaya E, Konno K.** 1995. Effect of clarithromycin on sputum production and its rheological properties in chronic respiratory tract infections. Antimicrob Agents Chemother **39:**1688–1690. http://dx.doi.org/10.1128/AAC.39.8.1688.
112. **Kawamura-Sato K, Iinuma Y, Hasegawa T, Horii T, Yamashino T, Ohta M.** 2000. Effect of subinhibitory concentrations of macrolides on

expression of flagellin in Pseudomonas aeruginosa and Proteus mirabilis. Antimicrob Agents Chemother **44:**2869–2872. http://dx.doi.org/10.1128/AAC.44.10.2869-2872.2000.

113. **Nalca Y, Jansch L, Bredenbruch F, Geffers R, Buer J, Haussler S.** 2006. Quorum-sensing antagonistic activities of azithromycin in Pseudomonas aeruginosa PAO1: a global approach. Antimicrob Agents Chemother **50:**1680–1688. http://dx.doi.org/10.1128/AAC.50.5.1680-1688.2006.
114. **Wallace SJ, Nation RL, Li J, Boyd BJ.** 2013. Physicochemical aspects of the coformulation of colistin and azithromycin using liposomes for combination antibiotic therapies. J Pharm Sci **102:**1578–1587. http://dx.doi.org/10.1002/jps.23508.
115. **Lee SH, Teo J, Heng D, Zhao Y, Ng WK, Chan HK, Tan RB.** 2014. Steroid-decorated antibiotic microparticles for inhaled anti-infective therapy. J Pharm Sci **103:**1115–1125. http://dx.doi.org/10.1002/jps.23874.
116. **Blum CA, Nigro N, Briel M, Schuetz P, Ullmer E, Suter-Widmer I, Winzeler B, Bingisser R, Elsaesser H, Drozdov D, Arici B, Urwyler SA, Refardt J, Tarr P, Wirz S, Thomann R, Baumgartner C, Duplain H, Burki D, Zimmerli W, Rodondi N, Mueller B, Christ-Crain M.** 2015. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet **385:**1511–1518. http://dx.doi.org/10.1016/S0140-6736(14)62447-8.
117. **Anonymous.** 2015. Global Initiative on Chronic Obstructive Lung Disease diagnosis and management guidelines. http://www.goldcopd.org/uploads/users/files/GOLD_Report_2015.pdf. Accessed 18 January 2016.
118. **Berlutti F, Morea C, Battistoni A, Sarli S, Cipriani P, Superti F, Ammendolia MG, Valenti P.** 2005. Iron availability influences aggregation, biofilm, adhesion and invasion of Pseudomonas aeruginosa and Burkholderia cenocepacia. Int J Immunopathol Pharmacol **18:**661–670.
119. **Moreau-Marquis S, Coutermarsh B, Stanton BA.** 2015. Combination of hypothiocyanite and lactoferrin (ALX-109) enhances the ability of tobramycin and aztreonam to eliminate Pseudomonas aeruginosa biofilms growing on cystic fibrosis airway epithelial cells. J Antimicrob Chemother **70:**160–166. http://dx.doi.org/10.1093/jac/dku357.
120. **Lee SH, Teo J, Heng D, Zhao Y, Ng WK, Chan HK, Tan LT, Tan RB.** 2015. A novel inhaled multi-pronged attack against respiratory bacteria. Eur J Pharm Sci **70:**37–44. http://dx.doi.org/10.1016/j.ejps.2015.01.005.
121. **Elmore RL, Contois ME, Kelly J, Noe A, Poirier A.** 1996. Stability and compatibility of admixtures of intravenous ciprofloxacin and selected drugs. Clin Ther **18:**246–255. http://dx.doi.org/10.1016/S0149-2918(96)80005-1.
122. **Weber B, Hochhaus G.** 2013. A pharmacokinetic simulation tool for inhaled corticosteroids. AAPS J **15:**159–171. http://dx.doi.org/10.1208/s12248-012-9420-z.
123. **De Backer JW, Vos WG, Vinchurkar SC, Claes R, Drollmann A, Wulfrank D, Parizel PM, Germonpre P, De Backer W.** 2010. Validation of computational fluid dynamics in CT-based airway models with SPECT/CT. Radiology **257:**854–862. http://dx.doi.org/10.1148/radiol.10100322.
124. **Delvadia RR, Longest PW, Byron PR.** 2012. In vitro tests for aerosol deposition. I. Scaling a physical model of the upper airways to predict drug deposition variation in normal humans. J Aerosol Med Pulm Drug Deliv **25:**32–40. http://dx.doi.org/10.1089/jamp.2011.0905.
125. **Miller DD, Amin MM, Palmer LB, Shah AR, Smaldone GC.** 2003. Aerosol delivery and modern mechanical ventilation: in vitro/in vivo evaluation. Am J Respir Crit Care Med **168:**1205–1209. http://dx.doi.org/10.1164/rccm.200210-1167OC.
126. **Diot P, Morra L, Smaldone GC.** 1995. Albuterol delivery in a model of mechanical ventilation. Comparison of metered-dose inhaler and nebulizer efficiency. Am J Respir Crit Care Med **152:**1391–1394.
127. **Williams L, Fletcher GC, Daniel M, Kinsella J.** 1999. A simple in vitro method for the evaluation of an ultrasonic nebulizer for drug delivery to intubated, ventilated patients and the effect of nebulizer and ventilator settings on the uptake of fluid from the nebulizer chamber. Eur J Anaesthesiol **16:**479–484. http://dx.doi.org/10.1097/00003643-199907000-00008.
128. **Thomas SH, O'Doherty MJ, Fidler HM, Page CJ, Treacher DF, Nunan TO.** 1993. Pulmonary deposition of a nebulised aerosol during mechanical ventilation. Thorax **48:**154–159. http://dx.doi.org/10.1136/thx.48.2.154.
129. **Mendelman PM, Smith AL, Levy J, Weber A, Ramsey B, Davis RL.** 1985. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am Rev Respir Dis **132:**761–765.
130. **Moore JE, Maeda Y, Goldsmith CE, Rendall JC, Elborn JS.** 2010. Direct comparison of in vitro susceptibility of wild-type clinical Pseudomonas aeruginosa isolated from adult patients with cystic fibrosis (CF) to TOBI and BRAMITOB (tobramycin inhalation solutions). J Cyst Fibros **9:**237–238. http://dx.doi.org/10.1016/j.jcf.2010.03.005.
131. **Montgomery AB, Rhomberg PR, Abuan T, Walters KA, Flamm RK.** 2014. Potentiation effects of amikacin and fosfomycin against selected amikacin-nonsusceptible Gram-negative respiratory tract pathogens. Antimicrob Agents Chemother **58:**3714–3719. http://dx.doi.org/10.1128/AAC.02780-13.
132. **Montgomery AB, Rhomberg PR, Abuan T, Walters KA, Flamm RK.** 2014. Amikacin-fosfomycin at a five-to-two ratio: characterization of mutation rates in microbial strains causing ventilator-associated pneumonia and interactions with commonly used antibiotics. Antimicrob Agents Chemother **58:**3708–3713. http://dx.doi.org/10.1128/AAC.02779-13.
133. **MacLeod DL, Barker LM, Sutherland JL, Moss SC, Gurgel JL, Kenney TF, Burns JL, Baker WR.** 2009. Antibacterial activities of a fosfomycin/tobramycin combination: a novel inhaled antibiotic for bronchiectasis. J Antimicrob Chemother **64:**829–836. http://dx.doi.org/10.1093/jac/dkp282.
134. **Anonymous.** 2005. Mesa Espanola de Normalizacion de la Sensibilidad y Resistencia a los Antimicrobianos. Recomendaciones del grupo MENSURA para la seleccion de antimicrobianos en el estudio de la sensibilidad y criterios para la interpretacion del antibiograma. MENSURA, Madrid, Spain.
135. **Morosini MI, Garcia-Castillo M, Loza E, Perez-Vazquez M, Baquero F, Canton R.** 2005. Breakpoints for predicting Pseudomonas aeruginosa susceptibility to inhaled tobramycin in cystic fibrosis patients: use of high-range Etest strips. J Clin Microbiol **43:**4480–4485. http://dx.doi.org/10.1128/JCM.43.9.4480-4485.2005.
136. **So W, Crandon JL, Hamada Y, Nicolau DP.** 10 November 2015. Antibacterial activity of achievable epithelial lining fluid exposures of Amikacin Inhale with or without meropenem. J Antimicrob Chemother http://dx.doi.org/10.1093/jac/dkv370.
137. **Gatmaitan BG, Carruthers MM, Lerner AM.** 1970. Gentamicin in treatment of primary Gram-negative pneumonias. Am J Med Sci **260:**90–94. http://dx.doi.org/10.1097/00000441-197008000-00003.
138. **Pines A, Raafat H, Plucinski K.** 1967. Gentamicin and colistin in chronic purulent bronchial infections. Br Med J **ii:**543–545. http://dx.doi.org/10.1136/bmj.2.5551.543.
139. **Panidis D, Markantonis SL, Boutzouka E, Karatzas S, Baltopoulos G.** 2005. Penetration of gentamicin into the alveolar lining fluid of critically ill patients with ventilator-associated pneumonia. Chest **128:**545–552. http://dx.doi.org/10.1378/chest.128.2.545.
140. **Carcas AJ, Garcia-Satue JL, Zapater P, Frias-Iniesta J.** 1999. Tobramycin penetration into epithelial lining fluid of patients with pneumonia. Clin Pharmacol Ther **65:**245–250. http://dx.doi.org/10.1016/S0009-9236(99)70103-7.
141. **Li M, Byron PR.** 2013. Tobramycin disposition in the rat lung following airway administration. J Pharmacol Exp Ther **347:**318–324. http://dx.doi.org/10.1124/jpet.113.207415.
142. **Marier JF, Brazier JL, Lavigne J, Ducharme MP.** 2003. Liposomal tobramycin against pulmonary infections of Pseudomonas aeruginosa: a pharmacokinetic and efficacy study following single and multiple intratracheal administrations in rats. J Antimicrob Chemother **52:**247–252. http://dx.doi.org/10.1093/jac/dkg317.
143. **Beaulac C, Sachetelli S, Lagace J.** 1999. Aerosolization of low phase transition temperature liposomal tobramycin as a dry powder in an animal model of chronic pulmonary infection caused by Pseudomonas aeruginosa. J Drug Target **7:**33–41. http://dx.doi.org/10.3109/10611869909085490.
144. **Beaulac C, Clement-Major S, Hawari J, Lagace J.** 1996. Eradication of mucoid Pseudomonas aeruginosa with fluid liposome-encapsulated tobramycin in an animal model of chronic pulmonary infection. Antimicrob Agents Chemother **40:**665–669.
145. **Beaulac C, Sachetelli S, Lagace J.** 1998. In-vitro bactericidal efficacy of sub-MIC concentrations of liposome-encapsulated antibiotic against gram-negative and gram-positive bacteria. J Antimicrob Chemother **41:**35–41. http://dx.doi.org/10.1093/jac/41.1.35.
146. **Stratton CW, Warner RR, Coudron PE, Lilly NA.** 1999. Bismuth-

mediated disruption of the glycocalyx-cell wall of Helicobacter pylori: ultrastructural evidence for a mechanism of action for bismuth salts. J Antimicrob Chemother **43:**659–666. http://dx.doi.org/10.1093/jac/43.5.659.

147. **Domenico P, Salo RJ, Novick SG, Schoch PE, Van Horn K, Cunha BA.** 1997. Enhancement of bismuth antibacterial activity with lipophilic thiol chelators. Antimicrob Agents Chemother **41:**1697–1703.
148. **Huang CT, Stewart PS.** 1999. Reduction of polysaccharide production in Pseudomonas aeruginosa biofilms by bismuth dimercaprol (BisBAL) treatment. J Antimicrob Chemother **44:**601–605. http://dx.doi.org/10.1093/jac/44.5.601.
149. **Domenico P, O'Leary R, Cunha BA.** 1992. Differential effects of bismuth and salicylate salts on the antibiotic susceptibility of Pseudomonas aeruginosa. Eur J Clin Microbiol Infect Dis **11:**170–175. http://dx.doi.org/10.1007/BF01967072.
150. **Veloira WG, Domenico P, LiPuma JJ, Davis JM, Gurzenda E, Kazzaz JA.** 2003. In vitro activity and synergy of bismuth thiols and tobramycin against Burkholderia cepacia complex. J Antimicrob Chemother **52:**915–919. http://dx.doi.org/10.1093/jac/dkg471.
151. **Wu CL, Domenico P, Hassett DJ, Beveridge TJ, Hauser AR, Kazzaz JA.** 2002. Subinhibitory bismuth-thiols reduce virulence of Pseudomonas aeruginosa. Am J Respir Cell Mol Biol **26:**731–738. http://dx.doi.org/10.1165/ajrcmb.26.6.2001-00020oc.
152. **Alhariri M, Omri A.** 2013. Efficacy of liposomal bismuth-ethanedithiol-loaded tobramycin after intratracheal administration in rats with pulmonary Pseudomonas aeruginosa infection. Antimicrob Agents Chemother **57:**569–578. http://dx.doi.org/10.1128/AAC.01634-12.
153. **Hirschl RB, Pranikoff T, Gauger P, Schreiner RJ, Dechert R, Bartlett RH.** 1995. Liquid ventilation in adults, children, and full-term neonates. Lancet **346:**1201–1202. http://dx.doi.org/10.1016/S0140-6736(95)92903-7.
154. **Hirschl RB, Overbeck MC, Parent A, Hernandez R, Schwartz S, Dosanjh A, Johnson K, Bartlett RH.** 1994. Liquid ventilation provides uniform distribution of perfluorocarbon in the setting of respiratory failure. Surgery **116:**159–167; discussion 167–168.
155. **Smith DJ, Gambone LM, Tarara T, Meays DR, Dellamary LA, Woods CM, Weers J.** 2001. Liquid dose pulmonary instillation of gentamicin PulmoSpheres formulations: tissue distribution and pharmacokinetics in rabbits. Pharm Res **18:**1556–1561. http://dx.doi.org/10.1023/A:1013078330485.
156. **Fox WW, Weis CM, Cox C, Farina C, Drott H, Wolfson MR, Shaffer TH.** 1997. Pulmonary administration of gentamicin during liquid ventilation in a newborn lamb lung injury model. Pediatrics **100:**E5.
157. **Zelinka MA, Wolfson MR, Calligaro I, Rubenstein SD, Greenspan JS, Shaffer TH.** 1997. A comparison of intratracheal and intravenous administration of gentamicin during liquid ventilation. Eur J Pediatr **156:**401–404. http://dx.doi.org/10.1007/s004310050625.
158. **Demaeyer P, Akodad EM, Gravet E, Schietecat P, Van Vooren JP, Drowart A, Yernault JC, Legros FJ.** 1993. Disposition of liposomal gentamicin following intrabronchial administration in rabbits. J Microencapsul **10:**77–88. http://dx.doi.org/10.3109/02652049309015314.
159. **Goldstein I, Wallet F, Nicolas-Robin A, Ferrari F, Marquette CH, Rouby JJ.** 2002. Lung deposition and efficiency of nebulized amikacin during Escherichia coli pneumonia in ventilated piglets. Am J Respir Crit Care Med **166:**1375–1381. http://dx.doi.org/10.1164/rccm.200204-363OC.
160. **Elman M, Goldstein I, Marquette CH, Wallet F, Lenaour G, Rouby JJ, Experimental ICU Study Group.** 2002. Influence of lung aeration on pulmonary concentrations of nebulized and intravenous amikacin in ventilated piglets with severe bronchopneumonia. Anesthesiology **97:**199–206. http://dx.doi.org/10.1097/00000542-200207000-00028.
161. **Hashimoto S, Wolfe E, Guglielmo B, Shanks R, Sundelof J, Pittet JF, Thomas E, Wiener-Kronish J.** 1996. Aerosolization of imipenem/cilastatin prevents Pseudomonas-induced acute lung injury. J Antimicrob Chemother **38:**809–818. http://dx.doi.org/10.1093/jac/38.5.809.
162. **Ferrari F, Liu ZH, Lu Q, Becquemin MH, Louchahi K, Aymard G, Marquette CH, Rouby JJ.** 2008. Comparison of lung tissue concentrations of nebulized ceftazidime in ventilated piglets: ultrasonic versus vibrating plate nebulizers. Intensive Care Med **34:**1718–1723. http://dx.doi.org/10.1007/s00134-008-1126-4.
163. **Tonnellier M, Ferrari F, Goldstein I, Sartorius A, Marquette CH, Rouby JJ.** 2005. Intravenous versus nebulized ceftazidime in ventilated piglets with and without experimental bronchopneumonia: comparative effects of helium and nitrogen. Anesthesiology **102:**995–1000. http://dx.doi.org/10.1097/00000542-200505000-00019.
164. **Marchand S, Lauda M, Le Moal G, Gobin P, Couet W, Roblot F.** 2013. Pharmacokinetics of daptomycin in a patient with severe renal failure not receiving dialysis. Antimicrob Agents Chemother **57:**2898–2899. http://dx.doi.org/10.1128/AAC.00230-13.
165. **Sabet M, Miller CE, Nolan TG, Senekeo-Effenberger K, Dudley MN, Griffith DC.** 2009. Efficacy of aerosol MP-376, a levofloxacin inhalation solution, in models of mouse lung infection due to Pseudomonas aeruginosa. Antimicrob Agents Chemother **53:**3923–3928. http://dx.doi.org/10.1128/AAC.00268-09.
166. **Kassamali Z, Rotschafer JC, Jones RN, Prince RA, Danziger LH.** 2013. Polymyxins: wisdom does not always come with age. Clin Infect Dis **57:**877–883. http://dx.doi.org/10.1093/cid/cit367.
167. **Nation RL, Li J, Turnidge JD.** 2013. The urgent need for clear and accurate information on the polymyxins. Clin Infect Dis **57:**1656–1657. http://dx.doi.org/10.1093/cid/cit522.
168. **Kassamali Z, Rotschafer JC, Jones RN, Prince RA, Danziger LH.** 2013. Reply to Nation et al. Clin Infect Dis **57:**1657–1658. (Reply.) http://dx.doi.org/10.1093/cid/cit525.
169. **Gales AC, Jones RN, Sader HS.** 2011. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006-09). J Antimicrob Chemother **66:**2070–2074. http://dx.doi.org/10.1093/jac/dkr239.
170. **Yapa SWS, Li J, Porter CJH, Nation RL, Patel K, McIntosh MP.** 2013. Population pharmacokinetics of colistin methanesulfonate in rats: achieving sustained lung concentrations of colistin for targeting respiratory infections. Antimicrob Agents Chemother **57:**5087–5095. http://dx.doi.org/10.1128/AAC.01127-13.
171. **Brain JD, Knudson DE, Sorokin SP, Davis MA.** 1976. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. Environ Res **11:**13–33. http://dx.doi.org/10.1016/0013-9351(76)90107-9.
172. **Gontijo AV, Gregoire N, Lamarche I, Gobin P, Couet W, Marchand S.** 2014. Biopharmaceutical characterization of nebulized antimicrobial agents in rats: 2. Colistin. Antimicrob Agents Chemother **58:**3950–3956. http://dx.doi.org/10.1128/AAC.02819-14.
173. **Lu Q, Girardi C, Zhang M, Bouhemad B, Louchahi K, Petitjean O, Wallet F, Becquemin MH, Le Naour G, Marquette CH, Rouby JJ.** 2010. Nebulized and intravenous colistin in experimental pneumonia caused by Pseudomonas aeruginosa. Intensive Care Med **36:**1147–1155. http://dx.doi.org/10.1007/s00134-010-1879-4.
174. **Barrowcliffe MP, Jones JG.** 1987. Solute permeability of the alveolar capillary barrier. Thorax **42:**1–10. http://dx.doi.org/10.1136/thx.42.1.1.
175. **Marchand S, Bouchene S, de Monte M, Guilleminault L, Montharu J, Cabrera M, Gregoire N, Gobin P, Diot P, Couet W, Vecellio L.** 4 June 2015. Pharmacokinetics of colistin methansulphonate (CMS) and colistin after CMS nebulisation in baboon monkeys. Pharm Res http://dx.doi.org/10.1007/s11095-015-1716-0.
176. **Newhouse MT, Hirst PH, Duddu SP, Walter YH, Tarara TE, Clark AR, Weers JG.** 2003. Inhalation of a dry powder tobramycin PulmoSphere formulation in healthy volunteers. Chest **124:**360–366. http://dx.doi.org/10.1378/chest.124.1.360.
177. **Le Conte P, Potel G, Peltier P, Horeau D, Caillon J, Juvin ME, Kergueris MF, Bugnon D, Baron D.** 1993. Lung distribution and pharmacokinetics of aerosolized tobramycin. Am Rev Respir Dis **147:**1279–1282. http://dx.doi.org/10.1164/ajrccm/147.5.1279.
178. **Baran D, de Vuyst P, Ooms HA.** 1990. Concentration of tobramycin given by aerosol in the fluid obtained by bronchoalveolar lavage. Respir Med **84:**203–204. http://dx.doi.org/10.1016/S0954-6111(08)80035-2.
179. **Burdette SD, Limkemann AJ, Slaughter JB, Beam WB, Markert RJ.** 2009. Serum concentrations of aerosolized tobramycin in medical, surgical, and trauma patients. Antimicrob Agents Chemother **53:**4568. http://dx.doi.org/10.1128/AAC.00490-09.
180. **Badia JR, Soy D, Adrover M, Ferrer M, Sarasa M, Alarcon A, Codina C, Torres A.** 2004. Disposition of instilled versus nebulized tobramycin and imipenem in ventilated intensive care unit (ICU) patients. J Antimicrob Chemother **54:**508–514. http://dx.doi.org/10.1093/jac/dkh326.
181. **Odio W, Van Laer E, Klastersky J.** 1975. Concentrations of gentamicin in bronchial secretions after intramuscular and endotracheal administration. J Clin Pharmacol **15:**518–524. http://dx.doi.org/10.1002/j.1552-4604.1975.tb01474.x.

182. **Al-Amoud AI, Clark BJ, Assi KA, Chrystyn H.** 2005. Determination of the bioavailability of gentamicin to the lungs following inhalation from two jet nebulizers. Br J Clin Pharmacol **59:**542–545. http://dx.doi.org/10.1111/j.1365-2125.2005.02360.x.
183. **Omri A, Beaulac C, Bouhajib M, Montplaisir S, Sharkawi M, Lagace J.** 1994. Pulmonary retention of free and liposome-encapsulated tobramycin after intratracheal administration in uninfected rats and rats infected with Pseudomonas aeruginosa. Antimicrob Agents Chemother **38:**1090–1095. http://dx.doi.org/10.1128/AAC.38.5.1090.
184. **Weers J, Metzheiser B, Taylor G, Warren S, Meers P, Perkins WR.** 2009. A gamma scintigraphy study to investigate lung deposition and clearance of inhaled amikacin-loaded liposomes in healthy male volunteers. J Aerosol Med Pulm Drug Deliv **22:**131–138. http://dx.doi.org/10.1089/jamp.2008.0693.
185. **Ehrmann S, Mercier E, Vecellio L, Ternant D, Paintaud G, Dequin PF.** 2008. Pharmacokinetics of high-dose nebulized amikacin in mechanically ventilated healthy subjects. Intensive Care Med **34:**755–762. http://dx.doi.org/10.1007/s00134-007-0935-1.
186. **Luyt CE, Clavel M, Guntupalli K, Johannigman J, Kennedy JI, Wood C, Corkery K, Gribben D, Chastre J.** 2009. Pharmacokinetics and lung delivery of PDDS-aerosolized amikacin (NKTR-061) in intubated and mechanically ventilated patients with nosocomial pneumonia. Crit Care **13:**R200. http://dx.doi.org/10.1186/cc8206.
187. **Montgomery AB, Vallance S, Abuan T, Tservistas M, Davies A.** 2014. A randomized double-blind placebo-controlled dose-escalation phase 1 study of aerosolized amikacin and fosfomycin delivered via the PARI investigational eFlow inline nebulizer system in mechanically ventilated patients. J Aerosol Med Pulm Drug Deliv **27:**441–448. http://dx.doi.org/10.1089/jamp.2013.1100.
188. **Stass H, Nagelschmitz J, Willmann S, Delesen H, Gupta A, Baumann S.** 2013. Inhalation of a dry powder ciprofloxacin formulation in healthy subjects: a phase I study. Clin Drug Invest **33:**419–427. http://dx.doi.org/10.1007/s40261-013-0082-0.
189. **Imberti R.** 2010. Intravenous colistimethate administration and colistin lung tissue concentrations. Intensive Care Med **36:**1795. http://dx.doi.org/10.1007/s00134-010-1960-z. (Reply, 36:1796-1797.)
190. **Imberti R, Cusato M, Villani P, Carnevale L, Iotti GA, Langer M, Regazzi M.** 2010. Steady-state pharmacokinetics and BAL concentration of colistin in critically ill patients after IV colistin methanesulfonate administration. Chest **138:**1333–1339. http://dx.doi.org/10.1378/chest.10-0463.
191. **Markou N, Fousteri M, Markantonis SL, Boutzouka E, Tsigou E, Baltopoulo G.** 2011. Colistin penetration in the alveolar lining fluid of critically ill patients treated with IV colistimethate sodium. Chest **139:**232–233. http://dx.doi.org/10.1378/chest.10-1860. (Reply, **139:**233-234.)
192. **Athanassa ZE, Markantonis SL, Fousteri MZ, Myrianthefs PM, Boutzouka EG, Tsakris A, Baltopoulos GJ.** 2012. Pharmacokinetics of inhaled colistimethate sodium (CMS) in mechanically ventilated critically ill patients. Intensive Care Med **38:**1779–1786. http://dx.doi.org/10.1007/s00134-012-2628-7.
193. **Dudhani RV, Turnidge JD, Nation RL, Li J.** 2010. fAUC/MIC is the most predictive pharmacokinetic/pharmacodynamic index of colistin against Acinetobacter baumannii in murine thigh and lung infection models. J Antimicrob Chemother **65:**1984–1990. http://dx.doi.org/10.1093/jac/dkq226.
194. **Westerman EM, de Boer AH, Le Brun PP, Touw DJ, Frijlink HW, Heijerman HG.** 2007. Dry powder inhalation of colistin sulphomethate in healthy volunteers: a pilot study. Int J Pharm **335:**41–45. http://dx.doi.org/10.1016/j.ijpharm.2006.11.021.
195. **Le Brun PP, de Boer AH, Mannes GP, de Fraiture DM, Brimicombe RW, Touw DJ, Vinks AA, Frijlink HW, Heijerman HG.** 2002. Dry powder inhalation of antibiotics in cystic fibrosis therapy: part 2. Inhalation of a novel colistin dry powder formulation: a feasibility study in healthy volunteers and patients. Eur J Pharm Biopharm **54:**25–32. http://dx.doi.org/10.1016/S0939-6411(02)00044-9.
196. **de Boer AH, Le Brun PP, van der Woude HG, Hagedoorn P, Heijerman HG, Frijlink HW.** 2002. Dry powder inhalation of antibiotics in cystic fibrosis therapy, part 1: development of a powder formulation with colistin sulfate for a special test inhaler with an air classifier as deagglomeration principle. Eur J Pharm Biopharm **54:**17–24. http://dx.doi.org/10.1016/S0939-6411(02)00043-7.
197. **Laennec RTH.** 1819. On mediate auscultation, or a treatise on the diagnosis of diseases of the lungs and heart. J-A Brosson et J-S Chaudé, Paris, France.
198. **McGuinness G, Naidich DP.** 2002. CT of airways disease and bronchiectasis. Radiol Clin North Am **40:**1–19. http://dx.doi.org/10.1016/S0033-8389(03)00105-2.
199. **Seitz AE, Olivier KN, Adjemian J, Holland SM, Prevots R.** 2012. Trends in bronchiectasis among Medicare beneficiaries in the United States, 2000 to 2007. Chest **142:**432–439. http://dx.doi.org/10.1378/chest.11-2209.
200. **Cole PJ.** 1986. Inflammation: a two-edged sword—the model of bronchiectasis. Eur J Respir Dis Suppl **147:**6–15.
201. **Pasteur MC, Helliwell SM, Houghton SJ, Webb SC, Foweraker JE, Coulden RA, Flower CD, Bilton D, Keogan MT.** 2000. An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med **162:**1277–1284. http://dx.doi.org/10.1164/ajrccm.162.4.9906120.
202. **Barker AF.** 2002. Bronchiectasis. N Engl J Med **346:**1383–1393. http://dx.doi.org/10.1056/NEJMra012519.
203. **Rubin BK.** 2003. Overview of cystic fibrosis and non-CF bronchiectasis. Semin Respir Crit Care Med **24:**619–628. http://dx.doi.org/10.1055/s-2004-815658.
204. **Tunney MM, Einarsson GG, Wei L, Drain M, Klem ER, Cardwell C, Ennis M, Boucher RC, Wolfgang MC, Elborn JS.** 2013. Lung microbiota and bacterial abundance in patients with bronchiectasis when clinically stable and during exacerbation. Am J Respir Crit Care Med **187:**1118–1126. http://dx.doi.org/10.1164/rccm.201210-1937OC.
205. **King PT, Holdsworth SR, Freezer NJ, Villanueva E, Holmes PW.** 2007. Microbiologic follow-up study in adult bronchiectasis. Respir Med **101:**1633–1638. http://dx.doi.org/10.1016/j.rmed.2007.03.009.
206. **Chan ED, Iseman MD.** 2013. Underlying host risk factors for nontuberculous mycobacterial lung disease. Semin Respir Crit Care Med **34:**110–123. http://dx.doi.org/10.1055/s-0033-1333573.
207. **Joish VN, Spilsbury-Cantalupo M, Operschall E, Luong B, Boklage S.** 2013. Economic burden of non-cystic fibrosis bronchiectasis in the first year after diagnosis from a US health plan perspective. Appl Health Econ Health Policy **11:**299–304. http://dx.doi.org/10.1007/s40258-013-0027-z.
208. **Bilton D, Henig N, Morrissey B, Gotfried M.** 2006. Addition of inhaled tobramycin to ciprofloxacin for acute exacerbations of Pseudomonas aeruginosa infection in adult bronchiectasis. Chest **130:**1503–1510. http://dx.doi.org/10.1378/chest.130.5.1503.
209. **Barker AF, Couch L, Fiel SB, Gotfried MH, Ilowite J, Meyer KC, O'Donnell A, Sahn SA, Smith LJ, Stewart JO, Abuan T, Tully H, Van Dalfsen J, Wells CD, Quan J.** 2000. Tobramycin solution for inhalation reduces sputum Pseudomonas aeruginosa density in bronchiectasis. Am J Respir Crit Care Med **162:**481–485. http://dx.doi.org/10.1164/ajrccm.162.2.9910086.
210. **Scheinberg P, Shore E.** 2005. A pilot study of the safety and efficacy of tobramycin solution for inhalation in patients with severe bronchiectasis. Chest **127:**1420–1426. http://dx.doi.org/10.1378/chest.127.4.1420.
211. **Ramsey BW, Pepe MS, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, Vasiljev KM, Borowitz D, Bowman CM, Marshall BC, Marshall S, Smith AL.** 1999. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. N Engl J Med **340:**23–30.
212. **Chalmers JD, Smith MP, McHugh BJ, Doherty C, Govan JR, Hill AT.** 2012. Short- and long-term antibiotic treatment reduces airway and systemic inflammation in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med **186:**657–665. http://dx.doi.org/10.1164/rccm.201203-0487OC.
213. **Martinez-Garcia MA, Soler-Cataluna JJ, Perpina-Tordera M, Roman-Sanchez P, Soriano J.** 2007. Factors associated with lung function decline in adult patients with stable non-cystic fibrosis bronchiectasis. Chest **132:**1565–1572. http://dx.doi.org/10.1378/chest.07-0490.
214. **Davies G, Wells AU, Doffman S, Watanabe S, Wilson R.** 2006. The effect of Pseudomonas aeruginosa on pulmonary function in patients with bronchiectasis. Eur Respir J **28:**974–979. http://dx.doi.org/10.1183/09031936.06.00074605.
215. **Olsen AM.** 1946. Streptomycin aerosol in the treatment of chronic bronchiectasis; preliminary report. Proc Staff Meet Mayo Clin **21:**53.
216. **Pasteur MC, Bilton D, Hill AT, British Thoracic Society Bronchiectasis Non-CF Guideline Group.** 2010. British Thoracic Society guideline

for non-CF bronchiectasis. Thorax **65**(Suppl 1)**:**i1–i58. http://dx.doi.org/10.1136/thx.2010.136119.
217. **Vendrell M, de Gracia J, Olveira C, Martinez MA, Giron R, Maiz L, Canton R, Coll R, Escribano A, Sole A.** 2008. Diagnosis and treatment of bronchiectasis Spanish Society of Pneumology and Thoracic Surgery. Arch Bronconeumol **44:**629–640. (In Spanish.) http://dx.doi.org/10.1016/S1579-2129(08)60117-2.
218. **Vendrell M, Munoz G, de Gracia J.** 2015. Evidence of inhaled tobramycin in non-cystic fibrosis bronchiectasis. Open Respir Med J **9:**30–36. http://dx.doi.org/10.2174/1874306401509010030.
219. **Orriols R, Roig J, Ferrer J, Sampol G, Rosell A, Ferrer A, Vallano A.** 1999. Inhaled antibiotic therapy in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection by Pseudomonas aeruginosa. Respir Med **93:**476–480. http://dx.doi.org/10.1016/S0954-6111(99)90090-2.
220. **Murray MP, Govan JR, Doherty CJ, Simpson AJ, Wilkinson TS, Chalmers JD, Greening AP, Haslett C, Hill AT.** 2011. A randomized controlled trial of nebulized gentamicin in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med **183:**491–499. http://dx.doi.org/10.1164/rccm.201005-0756OC.
221. **Lin HC, Cheng HF, Wang CH, Liu CY, Yu CT, Kuo HP.** 1997. Inhaled gentamicin reduces airway neutrophil activity and mucus secretion in bronchiectasis. Am J Respir Crit Care Med **155:**2024–2029. http://dx.doi.org/10.1164/ajrccm.155.6.9196111.
222. **Wilson R, Welte T, Polverino E, De Soyza A, Greville H, O'Donnell A, Alder J, Reimnitz P, Hampel B.** 2013. Ciprofloxacin dry powder for inhalation in non-cystic fibrosis bronchiectasis: a phase II randomised study. Eur Respir J **41:**1107–1115. http://dx.doi.org/10.1183/09031936.00071312.
223. **Serisier DJ, Bilton D, De Soyza A, Thompson PJ, Kolbe J, Greville HW, Cipolla D, Bruinenberg P, Gonda I, ORBIT-2 Investigators.** 2013. Inhaled, dual release liposomal ciprofloxacin in non-cystic fibrosis bronchiectasis (ORBIT-2): a randomised, double-blind, placebo-controlled trial. Thorax **68:**812–817. http://dx.doi.org/10.1136/thoraxjnl-2013-203207.
224. **Haworth CS, Foweraker JE, Wilkinson P, Kenyon RF, Bilton D.** 2014. Inhaled colistin in patients with bronchiectasis and chronic Pseudomonas aeruginosa infection. Am J Respir Crit Care Med **189:**975–982. http://dx.doi.org/10.1164/rccm.201312-2208OC.
225. **Patel IS, Vlahos I, Wilkinson TM, Lloyd-Owen SJ, Donaldson GC, Wilks M, Reznek RH, Wedzicha JA.** 2004. Bronchiectasis, exacerbation indices, and inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med **170:**400–407. http://dx.doi.org/10.1164/rccm.200305-648OC.
226. **Steinfort DP, Steinfort C.** 2007. Effect of long-term nebulized colistin on lung function and quality of life in patients with chronic bronchial sepsis. Intern Med J **37:**495–498. http://dx.doi.org/10.1111/j.1445-5994.2007.01404.x.
227. **Kelly MG, Murphy S, Elborn JS.** 2003. Bronchiectasis in secondary care: a comprehensive profile of a neglected disease. Eur J Intern Med **14:**488–492. http://dx.doi.org/10.1016/j.ejim.2003.10.002.
228. **Berlana D, Llop JM, Manresa F, Jodar R.** 2011. Outpatient treatment of Pseudomonas aeruginosa bronchial colonization with long-term inhaled colistin, tobramycin, or both in adults without cystic fibrosis. Pharmacotherapy **31:**146–157. http://dx.doi.org/10.1592/phco.31.2.146.
229. **Barker AF, O'Donnell AE, Flume P, Thompson PJ, Ruzi JD, de Gracia J, Boersma WG, De Soyza A, Shao L, Zhang J, Haas L, Lewis SA, Leitzinger S, Montgomery AB, McKevitt MT, Gossage D, Quittner AL, O'Riordan TG.** 2014. Aztreonam for inhalation solution in patients with non-cystic fibrosis bronchiectasis (AIR-BX1 and AIR-BX2): two randomised double-blind, placebo-controlled phase 3 trials. Lancet Respir Med **2:**738–749. http://dx.doi.org/10.1016/S2213-2600(14)70165-1.
230. **Brodt AM, Stovold E, Zhang L.** 2014. Inhaled antibiotics for stable non-cystic fibrosis bronchiectasis: a systematic review. Eur Respir J **44:**382–393. http://dx.doi.org/10.1183/09031936.00018414.
231. **Yang JW, Fan LC, Lu HW, Miao XY, Mao B, Xu JF.** 26 January 2015. Efficacy and safety of long-term inhaled antibiotic for patients with non-cystic fibrosis bronchiectasis: a meta-analysis. Clin Respir J http://dx.doi.org/10.1111/crj.12278.
232. **Evans DJ, Bara AI, Greenstone M.** 18 April 2007. Prolonged antibiotics for purulent bronchiectasis in children and adults. Cochrane Database Syst Rev http://dx.doi.org/10.1002/14651858.CD001392.pub2.
233. **Hnin K, Nguyen C, Carson KV, Evans DJ, Greenstone M, Smith BJ.** 2015. Prolonged antibiotics for non-cystic fibrosis bronchiectasis in children and adults. Cochrane Database Syst Rev **8:**CD001392. http://dx.doi.org/10.1002/14651858.CD001392.pub3.
234. **Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, Poppelwell L, Salih W, Pesci A, Dupont LJ, Fardon TC, De Soyza A, Hill AT.** 2014. The bronchiectasis severity index. An international derivation and validation study. Am J Respir Crit Care Med **189:**576–585. http://dx.doi.org/10.1164/rccm.201309-1575OC.
235. **Craven DE, Chroneou A, Zias N, Hjalmarson KI.** 2009. Ventilator-associated tracheobronchitis: the impact of targeted antibiotic therapy on patient outcomes. Chest **135:**521–528. http://dx.doi.org/10.1378/chest.08-1617.
236. **Craven DE.** 2008. Ventilator-associated tracheobronchitis (VAT): questions, answers, and a new paradigm? Crit Care **12:**157. http://dx.doi.org/10.1186/cc6912.
237. **Craven DE, Hjalmarson KI.** 2010. Ventilator-associated tracheobronchitis and pneumonia: thinking outside the box. Clin Infect Dis **51**(Suppl 1)**:**S59–S66. http://dx.doi.org/10.1086/653051.
238. **Torres A, Valencia M.** 2005. Does ventilator-associated tracheobronchitis need antibiotic treatment? Crit Care **9:**255–256. http://dx.doi.org/10.1186/cc3535.
239. **Fabregas N, Torres A, El-Ebiary M, Ramirez J, Hernandez C, Gonzalez J, de la Bellacasa JP, de Anta J, Rodriguez-Roisin R.** 1996. Histopathologic and microbiologic aspects of ventilator-associated pneumonia. Anesthesiology **84:**760–771. http://dx.doi.org/10.1097/00000542-199604000-00002.
240. **Rouby JJ, Martin De Lassale E, Poete P, Nicolas MH, Bodin L, Jarlier V, Le Charpentier Y, Grosset J, Viars P.** 1992. Nosocomial bronchopneumonia in the critically ill. Histologic and bacteriologic aspects. Am Rev Respir Dis **146:**1059–1066.
241. **Gerber J, Smirnov A, Wellmer A, Ragheb J, Prange J, Schutz E, Wettich K, Kalich S, Nau R.** 2001. Activity of LY333328 in experimental meningitis caused by a Streptococcus pneumoniae strain susceptible to penicillin. Antimicrob Agents Chemother **45:**2169–2172. http://dx.doi.org/10.1128/AAC.45.7.2169-2172.2001.
242. **A'Court CH, Garrard CS, Crook D, Bowler I, Conlon C, Peto T, Anderson E.** 1993. Microbiological lung surveillance in mechanically ventilated patients, using non-directed bronchial lavage and quantitative culture. Q J Med **86:**635–648. http://dx.doi.org/10.1093/qjmed/86.10.635.
243. **Soler N, Ewig S, Torres A, Filella X, Gonzalez J, Zaubet A.** 1999. Airway inflammation and bronchial microbial patterns in patients with stable chronic obstructive pulmonary disease. Eur Respir J **14:**1015–1022. http://dx.doi.org/10.1183/09031936.99.14510159.
244. **Nseir S, Di Pompeo C, Pronnier P, Beague S, Onimus T, Saulnier F, Grandbastien B, Mathieu D, Delvallez-Roussel M, Durocher A.** 2002. Nosocomial tracheobronchitis in mechanically ventilated patients: incidence, aetiology and outcome. Eur Respir J **20:**1483–1489. http://dx.doi.org/10.1183/09031936.02.00012902.
245. **Palmer LB, Smaldone GC, Chen JJ, Baram D, Duan T, Monteforte M, Varela M, Tempone AK, O'Riordan T, Daroowalla F, Richman P.** 2008. Aerosolized antibiotics and ventilator-associated tracheobronchitis in the intensive care unit. Crit Care Med **36:**2008–2013. http://dx.doi.org/10.1097/CCM.0b013e31817c0f9e.
246. **Athanassa ZE, Myrianthefs PM, Boutzouka EG, Tsakris A, Baltopoulos GJ.** 2011. Monotherapy with inhaled colistin for the treatment of patients with ventilator-associated tracheobronchitis due to polymyxin-only-susceptible Gram-negative bacteria. J Hosp Infect **78:**335–336. http://dx.doi.org/10.1016/j.jhin.2011.04.004.
247. **Maskin LP, Setten M, Rodriguez PO, Bonelli I, Attie S, Stryjewski ME, Valentini R.** 2015. Inhaled colistimethate sodium in ventilator-associated tracheobronchitis due to multidrug-resistant Gram-negative bacteria. Int J Antimicrob Agents **45:**199–200. http://dx.doi.org/10.1016/j.ijantimicag.2014.09.010.
248. **Hamer DH.** 2000. Treatment of nosocomial pneumonia and tracheobronchitis caused by multidrug-resistant Pseudomonas aeruginosa with aerosolized colistin. Am J Respir Crit Care Med **162:**328–330. http://dx.doi.org/10.1164/ajrccm.162.1.9910071.
249. **Falagas ME, Sideri G, Korbila IP, Vouloumanou EK, Papadatos JH, Kafetzis DA.** 2010. Inhaled colistin for the treatment of tracheobronchitis and pneumonia in critically ill children without cystic fibrosis. Pediatr Pulmonol **45:**1135–1140. http://dx.doi.org/10.1002/ppul.21302.
250. **Pereira GH, Muller PR, Levin AS.** 2007. Salvage treatment of pneumo-

nia and initial treatment of tracheobronchitis caused by multidrug-resistant Gram-negative bacilli with inhaled polymyxin B. Diagn Microbiol Infect Dis **58:**235–240. http://dx.doi.org/10.1016/j.diagmicrobio.2007.01.008.

251. **Agrafiotis M, Siempos II, Falagas ME.** 2010. Frequency, prevention, outcome and treatment of ventilator-associated tracheobronchitis: systematic review and meta-analysis. Respir Med **104:**325–336. http://dx.doi.org/10.1016/j.rmed.2009.09.001.
252. **Nseir S, Favory R, Jozefowicz E, Decamps F, Dewavrin F, Brunin G, Di Pompeo C, Mathieu D, Durocher A, VAT Study Group.** 2008. Antimicrobial treatment for ventilator-associated tracheobronchitis: a randomized, controlled, multicenter study. Crit Care **12:**R62. http://dx.doi.org/10.1186/cc6890.
253. **Bonten MJ, Bergmans DC, Ambergen AW, de Leeuw PW, van der Geest S, Stobberingh EE, Gaillard CA.** 1996. Risk factors for pneumonia, and colonization of respiratory tract and stomach in mechanically ventilated ICU patients. Am J Respir Crit Care Med **154:**1339–1346. http://dx.doi.org/10.1164/ajrccm.154.5.8912745.
254. **Fagon JY, Chastre J, Domart Y, Trouillet JL, Pierre J, Darne C, Gibert C.** 1989. Nosocomial pneumonia in patients receiving continuous mechanical ventilation. Prospective analysis of 52 episodes with use of a protected specimen brush and quantitative culture techniques. Am Rev Respir Dis **139:**877–884.
255. **Kaye KS, Pogue JM.** 24 October 2015. Infections caused by resistant Gram-negative bacteria: epidemiology and management. Pharmacotherapy http://dx.doi.org/10.1002/phar.1636.
256. **Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M.** 2006. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med **34:**1589–1596. http://dx.doi.org/10.1097/01.CCM.0000217961.75225.E9.
257. **Tacconelli E, Cataldo MA, Dancer SJ, De Angelis G, Falcone M, Frank U, Kahlmeter G, Pan A, Petrosillo N, Rodriguez-Bano J, Singh N, Venditti M, Yokoe DS, Cookson B, European Society of Clinical Microbiology.** 2014. ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. Clin Microbiol Infect **20**(Suppl 1):1–55. http://dx.doi.org/10.1111/1469-0691.12427.
258. **Ioannidou E, Siempos II, Falagas ME.** 2007. Administration of antimicrobials via the respiratory tract for the treatment of patients with nosocomial pneumonia: a meta-analysis. J Antimicrob Chemother **60:**1216–1226. http://dx.doi.org/10.1093/jac/dkm385.
259. **Falagas ME, Kasiakou SK.** 2007. Local administration of polymyxins into the respiratory tract for the prevention and treatment of pulmonary infections in patients without cystic fibrosis. Infection **35:**3–10. http://dx.doi.org/10.1007/s15010-007-6104-1.
260. **Melsen WG, Rovers MM, Groenwold RH, Bergmans DC, Camus C, Bauer TT, Hanisch EW, Klarin B, Koeman M, Krueger WA, Lacherade JC, Lorente L, Memish ZA, Morrow LE, Nardi G, van Nieuwenhoven CA, O'Keefe GE, Nakos G, Scannapieco FA, Seguin P, Staudinger T, Topeli A, Ferrer M, Bonten MJ.** 2013. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. Lancet Infect Dis **13:**665–671. http://dx.doi.org/10.1016/S1473-3099(13)70081-1.
261. **Bekaert M, Timsit JF, Vansteelandt S, Depuydt P, Vesin A, Garrouste-Orgeas M, Decruyenaere J, Clec'h C, Azoulay E, Benoit D, Outcomerea Study Group.** 2011. Attributable mortality of ventilator-associated pneumonia: a reappraisal using causal analysis. Am J Respir Crit Care Med **184:**1133–1139. http://dx.doi.org/10.1164/rccm.201105-0867OC.
262. **Zampieri FG, Nassar AP, Jr, Gusmao-Flores D, Taniguchi LU, Torres A, Ranzani OT.** 2015. Nebulized antibiotics for ventilator-associated pneumonia: a systematic review and meta-analysis. Crit Care **19:**150. http://dx.doi.org/10.1186/s13054-015-0868-y.
263. **Fagon JY, Chastre J, Hance AJ, Montravers P, Novara A, Gibert C.** 1993. Nosocomial pneumonia in ventilated patients: a cohort study evaluating attributable mortality and hospital stay. Am J Med **94:**281–288. http://dx.doi.org/10.1016/0002-9343(93)90060-3.
264. **Kollef MH.** 1993. Ventilator-associated pneumonia. A multivariate analysis. JAMA **270:**1965–1970.
265. **Rello J, Ollendorf DA, Oster G, Vera-Llonch M, Bellm L, Redman R, Kollef MH, VAP Outcomes Scientific Advisory Group.** 2002. Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. Chest **122:**2115–2121. http://dx.doi.org/10.1378/chest.122.6.2115.
266. **Restrepo MI, Anzueto A, Arroliga AC, Afessa B, Atkinson MJ, Ho NJ, Schinner R, Bracken RL, Kollef MH.** 2010. Economic burden of ventilator-associated pneumonia based on total resource utilization. Infect Control Hosp Epidemiol **31:**509–515. http://dx.doi.org/10.1086/651669.
267. **Kollef MH, Hamilton CW, Ernst FR.** 2012. Economic impact of ventilator-associated pneumonia in a large matched cohort. Infect Control Hosp Epidemiol **33:**250–256. http://dx.doi.org/10.1086/664049.
268. **Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC, Gibert C.** 1998. Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. Am J Respir Crit Care Med **157:**531–539. http://dx.doi.org/10.1164/ajrccm.157.2.9705064.
269. **Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP.** 2000. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature **407:**762–764. http://dx.doi.org/10.1038/35037627.
270. **Costerton JW, Stewart PS, Greenberg EP.** 1999. Bacterial biofilms: a common cause of persistent infections. Science **284:**1318–1322. http://dx.doi.org/10.1126/science.284.5418.1318.
271. **Hoffman LR, D'Argenio DA, MacCoss MJ, Zhang Z, Jones RA, Miller SI.** 2005. Aminoglycoside antibiotics induce bacterial biofilm formation. Nature **436:**1171–1175. http://dx.doi.org/10.1038/nature03912.
272. **Adair CG, Gorman SP, Byers LM, Jones DS, Feron B, Crowe M, Webb HC, McCarthy GJ, Milligan KR.** 2002. Eradication of endotracheal tube biofilm by nebulised gentamicin. Intensive Care Med **28:**426–431. http://dx.doi.org/10.1007/s00134-002-1223-8.
273. **Adair CG, Gorman SP, Feron BM, Byers LM, Jones DS, Goldsmith CE, Moore JE, Kerr JR, Curran MD, Hogg G, Webb CH, McCarthy GJ, Milligan KR.** 1999. Implications of endotracheal tube biofilm for ventilator-associated pneumonia. Intensive Care Med **25:**1072–1076. http://dx.doi.org/10.1007/s001340051014.
274. **Meers P, Neville M, Malinin V, Scotto AW, Sardaryan G, Kurumunda R, Mackinson C, James G, Fisher S, Perkins WR.** 2008. Biofilm penetration, triggered release and in vivo activity of inhaled liposomal amikacin in chronic Pseudomonas aeruginosa lung infections. J Antimicrob Chemother **61:**859–868. http://dx.doi.org/10.1093/jac/dkn059.
275. **Abazov VM, Abbott B, Abolins M, Acharya BS, Adams M, Adams T, Alexeev GD, Alkhazov G, Alton A, Alverson G, Alves GA, Ancu LS, Aoki M, Arnoud Y, Arov M, Askew A, Asman B, Atramentov O, Avila C, BackusMayes J, Badaud F, Bagby L, Baldin B, Bandurin DV, Banerjee S, Barberis E, Baringer P, Barreto J, Bartlett JF, Bassler U, Beale S, Bean A, Begalli M, Begel M, Belanger-Champagne C, Bellantoni L, Benitez JA, Beri SB, Bernardi G, Bernhard R, Bertram I, Besancon M, Beuselinck R, Bezzubov VA, Bhat PC, Bhatnagar V, Blazey G, Blessing S, Bloom K, Boehnlein A, et al.** 2010. Search for sneutrino production in eμ final states in 5.3 fb-1 of pp collisions at square root s =1.96 TeV. Phys Rev Lett **105:**191802. http://dx.doi.org/10.1103/PhysRevLett.105.191802.
276. **Falagas ME, Siempos II, Bliziotis IA, Michalopoulos A.** 2006. Administration of antibiotics via the respiratory tract for the prevention of ICU-acquired pneumonia: a meta-analysis of comparative trials. Crit Care **10:**R123. http://dx.doi.org/10.1186/cc5032.
277. **Dhand R, Guntur VP.** 2008. How best to deliver aerosol medications to mechanically ventilated patients. Clin Chest Med **29:**277–296. http://dx.doi.org/10.1016/j.ccm.2008.02.003.
278. **Luyt CE, Brechot N, Combes A, Trouillet JL, Chastre J.** 2013. Delivering antibiotics to the lungs of patients with ventilator-associated pneumonia: an update. Expert Rev Anti Infect Ther **11:**511–521. http://dx.doi.org/10.1586/eri.13.36.
279. **Nseir S.** 2008. Aerosolized antibiotics for ventilator-associated tracheobronchitis: let's go with the flow! Crit Care Med **36:**2191–2192. http://dx.doi.org/10.1097/CCM.0b013e31817c0a28.
280. **Kemming GI, Kreyling W, Habler O, Merkel M, Kleen M, Welte M, Messmer K, Zwissler B.** 1996. Aerosol production and aerosol droplet size distribution during mechanical ventilation (IPPV) with a new ultrasonic nebulizer. Eur J Med Res **1:**321–327.
281. **Harvey CJ, O'Doherty MJ, Page CJ, Thomas SH, Nunan TO, Treacher DF.** 1995. Effect of a spacer on pulmonary aerosol deposition from a jet nebuliser during mechanical ventilation. Thorax **50:**50–53. http://dx.doi.org/10.1136/thx.50.1.50.
282. **Davies JB, Bromilow J.** 2011. Bacterial filter obstruction with the use of

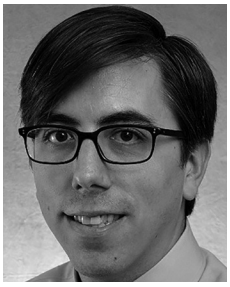
ultrasonic nebulisation. Anaesthesia **66:**394–395. http://dx.doi.org/10.1111/j.1365-2044.2011.06683.x.

283. **Ehrmann S, Roche-Campo F, Bodet-Contentin L, Razazi K, Dugernier J, Trenado-Alvarez J, Donzeau A, Vermeulen F, Thevoz D, Papanikolaou M, Edelson A, Leon Yoshido H, Piquilloud L, Lakhal K, Lopes C, Vicent C, Desachy A, Apiou-Sbirlea G, Isabey D, Brochard L, Reva Research Network, AT@ICU Study Group.** 24 November 2015. Aerosol therapy in intensive and intermediate care units: prospective observation of 2808 critically ill patients. Intensive Care Med http://dx.doi.org/10.1007/s00134-015-4114-5.
284. **Lu Q, Luo R, Bodin L, Yang J, Zahr N, Aubry A, Golmard JL, Rouby JJ, Nebulized Antibiotics Study Group.** 2012. Efficacy of high-dose nebulized colistin in ventilator-associated pneumonia caused by multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii. Anesthesiology **117:**1335–1347. http://dx.doi.org/10.1097/ALN.0b013e31827515de.
285. **Kwa AL, Loh C, Low JG, Kurup A, Tam VH.** 2005. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa. Clin Infect Dis **41:**754–757. http://dx.doi.org/10.1086/432583.
286. **Falagas ME, Siempos II, Rafailidis PI, Korbila IP, Ioannidou E, Michalopoulos A.** 2009. Inhaled colistin as monotherapy for multidrug-resistant gram (-) nosocomial pneumonia: a case series. Respir Med **103:**707–713. http://dx.doi.org/10.1016/j.rmed.2008.11.018.
287. **Falagas ME, Agrafiotis M, Athanassa Z, Siempos II.** 2008. Administration of antibiotics via the respiratory tract as monotherapy for pneumonia. Expert Rev Anti Infect Ther **6:**447–452. http://dx.doi.org/10.1586/14787210.6.4.447.
288. **Nick JA, Moskowitz SM, Chmiel JF, Forssen AV, Kim SH, Saavedra MT, Saiman L, Taylor-Cousar JL, Nichols DP.** 2014. Azithromycin may antagonize inhaled tobramycin when targeting Pseudomonas aeruginosa in cystic fibrosis. Ann Am Thorac Soc **11:**342–350. http://dx.doi.org/10.1513/AnnalsATS.201310-352OC.
289. **Mohr AM, Sifri ZC, Horng HS, Sadek R, Savetamal A, Hauser CJ, Livingston DH.** 2007. Use of aerosolized aminoglycosides in the treatment of Gram-negative ventilator-associated pneumonia. Surg Infect (Larchmt) **8:**349–357. http://dx.doi.org/10.1089/sur.2006.041.
290. **Arnold HM, Sawyer AM, Kollef MH.** 2012. Use of adjunctive aerosolized antimicrobial therapy in the treatment of Pseudomonas aeruginosa and Acinetobacter baumannii ventilator-associated pneumonia. Respir Care **57:**1226–1233. http://dx.doi.org/10.4187/respcare.01556.
291. **Hallal A, Cohn SM, Namias N, Habib F, Baracco G, Manning RJ, Crookes B, Schulman CI.** 2007. Aerosolized tobramycin in the treatment of ventilator-associated pneumonia: a pilot study. Surg Infect (Larchmt) **8:**73–82. http://dx.doi.org/10.1089/sur.2006.051.
292. **Niederman MS, Chastre J, Corkery K, Fink JB, Luyt CE, Garcia MS.** 2012. BAY41-6551 achieves bactericidal tracheal aspirate amikacin concentrations in mechanically ventilated patients with Gram-negative pneumonia. Intensive Care Med **38:**263–271. http://dx.doi.org/10.1007/s00134-011-2420-0.
293. **Czosnowski QA, Wood GC, Magnotti LJ, Croce MA, Swanson JM, Boucher BA, Fabian TC.** 2009. Adjunctive aerosolized antibiotics for treatment of ventilator-associated pneumonia. Pharmacotherapy **29:**1054–1060. http://dx.doi.org/10.1592/phco.29.9.1054.
294. **Ghannam DE, Rodriguez GH, Raad II, Safdar A.** 2009. Inhaled aminoglycosides in cancer patients with ventilator-associated Gram-negative bacterial pneumonia: safety and feasibility in the era of escalating drug resistance. Eur J Clin Microbiol Infect Dis **28:**253–259. http://dx.doi.org/10.1007/s10096-008-0620-5.
295. **Michalopoulos A, Fotakis D, Virtzili S, Vletsas C, Raftopoulou S, Mastora Z, Falagas ME.** 2008. Aerosolized colistin as adjunctive treatment of ventilator-associated pneumonia due to multidrug-resistant Gram-negative bacteria: a prospective study. Respir Med **102:**407–412. http://dx.doi.org/10.1016/j.rmed.2007.10.011.
296. **Rattanaumpawan P, Lorsutthitham J, Ungprasert P, Angkasekwinai N, Thamlikitkul V.** 2010. Randomized controlled trial of nebulized colistimethate sodium as adjunctive therapy of ventilator-associated pneumonia caused by Gram-negative bacteria. J Antimicrob Chemother **65:**2645–2649. http://dx.doi.org/10.1093/jac/dkq360.
297. **Tumbarello M, De Pascale G, Trecarichi EM, De Martino S, Bello G, Maviglia R, Spanu T, Antonelli M.** 2013. Effect of aerosolized colistin as adjunctive treatment on the outcomes of microbiologically documented ventilator-associated pneumonia caused by colistin-only susceptible gram-negative bacteria. Chest **144:**1768–1775. http://dx.doi.org/10.1378/chest.13-1018.
298. **Bergen PJ, Bulman ZP, Saju S, Bulitta JB, Landersdorfer C, Forrest A, Li J, Nation RL, Tsuji BT.** 2015. Polymyxin combinations: pharmacokinetics and pharmacodynamics for rationale use. Pharmacotherapy **35:**34–42. http://dx.doi.org/10.1002/phar.1537.
299. **Kalin G, Alp E, Coskun R, Demiraslan H, Gundogan K, Doganay M.** 2012. 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 **18:**872–877. http://dx.doi.org/10.1007/s10156-012-0430-7.
300. **Naesens R, Vlieghe E, Verbrugghe W, Jorens P, Ieven M.** 2011. A retrospective observational study on the efficacy of colistin by inhalation as compared to parenteral administration for the treatment of nosocomial pneumonia associated with multidrug-resistant Pseudomonas aeruginosa. BMC Infect Dis **11:**317. http://dx.doi.org/10.1186/1471-2334-11-317.
301. **Lin CC, Liu TC, Kuo CF, Liu CP, Lee CM.** 2010. 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:**323–331. http://dx.doi.org/10.1016/S1684-1182(10)60050-3.
302. **Doshi NM, Cook CH, Mount KL, Stawicki SP, Frazee EN, Personett HA, Schramm GE, Arnold HM, Murphy CV.** 2013. Adjunctive aerosolized colistin for multi-drug resistant gram-negative pneumonia in the critically ill: a retrospective study. BMC Anesthesiol **13:**45. http://dx.doi.org/10.1186/1471-2253-13-45.
303. **Mastoraki A, Douka E, Kriaras I, Stravopodis G, Manoli H, Geroulanos S.** 2008. Pseudomonas aeruginosa susceptible only to colistin in intensive care unit patients. Surg Infect (Larchmt) **9:**153–160. http://dx.doi.org/10.1089/sur.2007.004.
304. **Kofteridis DP, Alexopoulou C, Valachis A, Maraki S, Dimopoulou D, Georgopoulos D, Samonis G.** 2010. Aerosolized plus intravenous colistin versus intravenous colistin alone for the treatment of ventilator-associated pneumonia: a matched case-control study. Clin Infect Dis **51:**1238–1244. http://dx.doi.org/10.1086/657242.
305. **Kwa AL, Falagas ME, Michalopoulos A, Tam VH.** 2011. Benefits of aerosolized colistin for ventilator-associated pneumonia: absence of proof versus proof of absence? Clin Infect Dis **52:**1278–1279. http://dx.doi.org/10.1093/cid/cir134. (Reply, **52:**1279-1280. http://dx.doi.org/10.1093/cid/cir135.)
306. **van Leeuwen DHJ.** 2011. A closer look at aerosolized colistin. Clin Infect Dis **52:**1472–1473. http://dx.doi.org/10.1093/cid/cir248. (Reply, **52:**1473-1474.)
307. **Linden PK, Paterson DL.** 2006. Parenteral and inhaled colistin for treatment of ventilator-associated pneumonia. Clin Infect Dis **43**(Suppl 2)**:**S89–S94. http://dx.doi.org/10.1086/504485.
308. **Berlana D, Llop JM, Fort E, Badia MB, Jodar R.** 2005. Use of colistin in the treatment of multiple-drug-resistant gram-negative infections. Am J Health Syst Pharm **62:**39–47.
309. **Korbila IP, Michalopoulos A, Rafailidis PI, Nikita D, Samonis G, Falagas ME.** 2010. Inhaled colistin as adjunctive therapy to intravenous colistin for the treatment of microbiologically documented ventilator-associated pneumonia: a comparative cohort study. Clin Microbiol Infect **16:**1230–1236. http://dx.doi.org/10.1111/j.1469-0691.2009.03040.x.
310. **Michalopoulos A, Kasiakou SK, Mastora Z, Rellos K, Kapaskelis AM, Falagas ME.** 2005. Aerosolized colistin for the treatment of nosocomial pneumonia due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. Crit Care **9:**R53–R59. http://dx.doi.org/10.1186/cc3020.
311. **Zylberberg H, Vargaftig J, Barbieux C, Pertuiset N, Rothschild C, Viard JP.** 1996. Prolonged efficiency of secondary prophylaxis with colistin aerosols for respiratory infection due to Pseudomonas aeruginosa in patients infected with human immunodeficiency virus. Clin Infect Dis **23:**641–643. http://dx.doi.org/10.1093/clinids/23.3.641.
312. **Amin M, Rashad A, Fouad A, Abdel Azeem A.** 2013. Re-emerging of colistin for treatment of nosocomial pneumonia due to Gram negative multi-drug resistant pathogens in critically ill patients. Egypt J Chest Dis Tuberc **62:**447–451. http://dx.doi.org/10.1016/j.ejcdt.2013.05.012.
313. **Bogovic TZ, Baronica R, Tomasevic B, Miric M, Drvar Z, Pavlek M, Bratic V, Peric M, Budimir A, Bosnjak Z, Hrabac P.** 2014. Inhalation plus intravenous colistin versus intravenous colistin alone for treatment of ventilator associated pneumonia. Signa Vitae **9:**29–33.

314. **Liu D, Zhang J, Liu HX, Zhu YG, Qu JM.** 19 October 2015. Intravenous combined with aerosolized polymyxin versus intravenous polymyxin alone in the treatment of pneumonia caused by multidrug-resistant pathogens: a systematic review and meta-analysis. Int J Antimicrob Agents http://dx.doi.org/10.1016/j.ijantimicag.2015.09.011.
315. **Sobieszczyk ME, Furuya EY, Hay CM, Pancholi P, Della-Latta P, Hammer SM, Kubin CJ.** 2004. Combination therapy with polymyxin B for the treatment of multidrug-resistant Gram-negative respiratory tract infections. J Antimicrob Chemother **54:**566–569. http://dx.doi.org/10.1093/jac/dkh369.
316. **Stoutenbeek CP, van Saene HK, Miranda DR, Zandstra DF, Langrehr D.** 1986. Nosocomial gram-negative pneumonia in critically ill patients. A 3-year experience with a novel therapeutic regimen. Intensive Care Med **12:**419–423.
317. **Lu Q, Yang J, Liu Z, Gutierrez C, Aymard G, Rouby JJ.** 2011. Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by Pseudomonas aeruginosa. Am J Respir Crit Care Med **184:**106–115. http://dx.doi.org/10.1164/rccm.201011-1894OC.
318. **Radhakrishnan M, Jaganath A, Rao GS, Kumari HB.** 2008. Nebulized imipenem to control nosocomial pneumonia caused by Pseudomonas aeruginosa. J Crit Care **23:**148–150. http://dx.doi.org/10.1016/j.jcrc.2007.10.037.
319. **Horianopoulou M, Kanellopoulou M, Paraskevopoulos I, Kyriakidis A, Legakis NJ, Lambropoulos S.** 2004. Use of inhaled ampicillin-sulbactam against multiresistant Acinetobacter baumannii in bronchial secretions of intensive care unit patients. Clin Microbiol Infect **10:**85–86. http://dx.doi.org/10.1111/j.1469-0691.2004.00806.x.
320. **Karvouniaris M, Makris D, Zygoulis P, Triantaris A, Xitsas S, Mantzarlis K, Petinaki E, Zakynthinos E.** 24 September 2015. Nebulised colistin for ventilator-associated pneumonia prevention. Eur Respir J http://dx.doi.org/10.1183/13993003.02235-2014.
321. **Horianopoulou M, Lambropoulos S, Papafragas E, Falagas ME.** 2005. Effect of aerosolized colistin on multidrug-resistant Pseudomonas aeruginosa in bronchial secretions of patients without cystic fibrosis. J Chemother **17:**536–538. http://dx.doi.org/10.1179/joc.2005.17.5.536.
322. **Greenfield S, Teres D, Bushnell LS, Hedley-Whyte J, Feingold DS.** 1973. Prevention of gram-negative bacillary pneumonia using aerosol polymyxin as prophylaxis. I. Effect on the colonization pattern of the upper respiratory tract of seriously ill patients. J Clin Invest **52:**2935–2940.
323. **Rouby JJ, Poete P, Martin de Lassale E, Nicolas MH, Bodin L, Jarlier V, Korinek AM, Viars P.** 1994. Prevention of gram negative nosocomial bronchopneumonia by intratracheal colistin in critically ill patients. Histologic and bacteriologic study. Intensive Care Med **20:**187–192. http://dx.doi.org/10.1007/BF01704698.
324. **Claridge JA, Edwards NM, Swanson J, Fabian TC, Weinberg JA, Wood C, Croce MA.** 2007. Aerosolized ceftazidime prophylaxis against ventilator-associated pneumonia in high-risk trauma patients: results of a double-blind randomized study. Surg Infect (Larchmt) **8:**83–90. http://dx.doi.org/10.1089/sur.2006.042.
325. **Wolff RK, Dorato MA.** 1993. Toxicologic testing of inhaled pharmaceutical aerosols. Crit Rev Toxicol **23:**343–369. http://dx.doi.org/10.3109/10408449309104076.
326. **Rubin BK.** 2008. Aerosolized antibiotics for non-cystic fibrosis bronchiectasis. J Aerosol Med Pulm Drug Deliv **21:**71–76. http://dx.doi.org/10.1089/jamp.2007.0652.
327. **Canton R, Morosini MI.** 2011. Emergence and spread of antibiotic resistance following exposure to antibiotics. FEMS Microbiol Rev **35:**977–991. http://dx.doi.org/10.1111/j.1574-6976.2011.00295.x.
328. **Palmer LB, Smaldone GC.** 2014. Reduction of bacterial resistance with inhaled antibiotics in the intensive care unit. Am J Respir Crit Care Med **189:**1225–1233. http://dx.doi.org/10.1164/rccm.201312-2161OC.
329. **Burns JL, Van Dalfsen JM, Shawar RM, Otto KL, Garber RL, Quan JM, Montgomery AB, Albers GM, Ramsey BW, Smith AL.** 1999. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. J Infect Dis **179:**1190–1196. http://dx.doi.org/10.1086/314727.
330. **Sexauer WP, Fiel SB.** 2003. Aerosolized antibiotics in cystic fibrosis. Semin Respir Crit Care Med **24:**717–726. http://dx.doi.org/10.1055/s-2004-815667.
331. **Oermann CM, McCoy KS, Retsch-Bogart GZ, Gibson RL, McKevitt M, Montgomery AB.** 2011. Pseudomonas aeruginosa antibiotic susceptibility during long-term use of aztreonam for inhalation solution (AZLI). J Antimicrob Chemother **66:**2398–2404. http://dx.doi.org/10.1093/jac/dkr303.
332. **Brown RB, Phillips D, Barker MJ, Pieczarka R, Sands M, Teres D.** 1989. Outbreak of nosocomial Flavobacterium meningosepticum respiratory infections associated with use of aerosolized polymyxin B. Am J Infect Control **17:**121–125.
333. **Tsai RJ, Boiano JM, Steege AL, Sweeney MH.** 2015. Precautionary practices of respiratory therapists and other health-care practitioners who administer aerosolized medications. Respir Care **60:**1409–1417. http://dx.doi.org/10.4187/respcare.03817.
334. **Beaty CD, Ritz RH, Benson MS.** 1989. Continuous in-line nebulizers complicate pressure support ventilation. Chest **96:**1360–1363. http://dx.doi.org/10.1378/chest.96.6.1360.
335. **Craven DE, Lichtenberg DA, Goularte TA, Make BJ, McCabe WR.** 1984. Contaminated medication nebulizers in mechanical ventilator circuits. Source of bacterial aerosols. Am J Med **77:**834–838.
336. **McCoy KS.** 2007. Compounded colistimethate as possible cause of fatal acute respiratory distress syndrome. N Engl J Med **357:**2310–2311. http://dx.doi.org/10.1056/NEJMc071717.
337. **Hakeam HA, Almohaizeie AM.** 2006. Hypotension following treatment with aerosolized colistin in a patient with multidrug-resistant Pseudomonas aeruginosa. Ann Pharmacother **40:**1677–1680. http://dx.doi.org/10.1345/aph.1H019.
338. **Alothman GA, Alsaadi MM, Ho BL, Ho SL, Dupuis A, Corey M, Coates AL.** 2002. Evaluation of bronchial constriction in children with cystic fibrosis after inhaling two different preparations of tobramycin. Chest **122:**930–934. http://dx.doi.org/10.1378/chest.122.3.930.
339. **Cunningham S, Prasad A, Collyer L, Carr S, Lynn IB, Wallis C.** 2001. Bronchoconstriction following nebulised colistin in cystic fibrosis. Arch Dis Child **84:**432–433. http://dx.doi.org/10.1136/adc.84.5.432.
340. **Wilson FE.** 1981. Acute respiratory failure secondary to polymyxin-B inhalation. Chest **79:**237–239. http://dx.doi.org/10.1378/chest.79.2.237.
341. **Marschke G, Sarauw A.** 1971. Polymyxin inhalation therapeutic hazard. Ann Intern Med **74:**144–145. http://dx.doi.org/10.7326/0003-4819-74-1-144.
342. **Falagas ME, Kasiakou SK.** 2006. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. Crit Care **10:**R27. http://dx.doi.org/10.1186/cc3995.
343. **Kostyanev T, Bonten MJ, O'Brien S, Steel H, Ross S, Francois B, Tacconelli E, Winterhalter M, Stavenger RA, Karlen A, Harbarth S, Hackett J, Jafri HS, Vuong C, MacGowan A, Witschi A, Angyalosi G, Elborn JS, deWinter R, Goossens H.** 15 November 2015. The Innovative Medicines Initiative's New Drugs for Bad Bugs programme: European public-private partnerships for the development of new strategies to tackle antibiotic resistance. J Antimicrob Chemother http://dx.doi.org/10.1093/jac/dkv339.
344. **Kostyanev T, Bonten MJ, O'Brien S, Goossens H.** 2015. Innovative Medicines Initiative and antibiotic resistance. Lancet Infect Dis **15:**1373–1375. http://dx.doi.org/10.1016/S1473-3099(15)00407-7.
345. **Zarogoulidis P, Kioumis I, Ritzoulis C, Petridis D, Darwiche K, Porpodis K, Spyratos D, Parrish S, Browning R, Li Q, Turner JF, Freitag L, Zarogoulidis K.** 2013. New insights in the production of aerosol antibiotics. Evaluation of the optimal aerosol production system for ampicillin-sulbactam, meropenem, ceftazidime, cefepime and piperacillin-tazobactam. Int J Pharm **455:**182–188. http://dx.doi.org/10.1016/j.ijpharm.2013.07.040.

Continued next page

Eric Wenzler, Pharm.D., is an Infectious Diseases Pharmacotherapy Fellow at the University of Illinois at Chicago in Chicago, IL. He graduated from Ohio Northern University Raabe College of Pharmacy in 2012 and completed his Postgraduate Year 1 training in 2013 and Postgraduate Year 2 in Infectious Diseases in 2014 at The Ohio State University Wexner Medical Center in Columbus, OH. Dr. Wenzler's research interests include pharmacokinetics, pharmacodynamics, antimicrobial stewardship, and clinical microbiology.



Dustin R. Fraidenburg, M.D., is in his last year of a Pulmonary Critical Care fellowship at the University of Illinois at Chicago, where he previously completed his Internal Medicine residency training. He received his medical degree from Wayne State University in Detroit, MI. Dr. Fraidenburg's main research interests are in pulmonary vascular disease, studying the impact of lung inflammation on cellular and molecular mechanisms involved in pulmonary vasoreactivity.

Tonya Scardina, Pharm.D., is an Infectious Diseases Clinical Pharmacist at Loyola University Medical Center in Maywood, IL. She graduated from the University of Missouri—Kansas City School of Pharmacy in 2008. She completed her Postgraduate Year 1 training in 2009 and Infectious Diseases Pharmacotherapy Fellowship at the University of Illinois at Chicago in 2011. Dr. Scardina's main research interests include *Clostridium difficile* infection, antibiotic stewardship, and severe influenza.

Larry H. Danziger, Pharm.D., is a Professor of Pharmacy and Medicine at the University of Illinois at Chicago (UIC) and Codirector of the Section of Infectious Diseases in the Department of Pharmacy Practice. He is also the Executive Director of the Center for Advanced Design, Research and Exploration (CADRE), a center which reports to the UIC Office of the Vice Chancellor for Research. Dr. Danziger's research interests include basic and clinical research regarding both new and old antimicrobials. He played a critical role in the redevelopment of paromomycin, an antibiotic originally developed in the 1950s. In collaboration between the University of Illinois at Chicago and the World Health Organization, paromomycin was developed as a therapeutic agent for the treatment of visceral leishmaniasis.