CLINICAL INVESTIGATION

Emerging approaches to the treatment of ventilator-associated pneumonia

B Mourvillier[1,*], L Berra[1] & J Wiener-Kronish[1]
[1]Department of Anesthesia, Critical Care & Pain Management, Massachusetts General Hospital, 55, Fruit street, Boston, MA 02114, USA
*Author for correspondence:
Tel.: +1 617 726 3030
Fax: +1 617 726 3032
bmourvillier@gmail.com

Despite aggressive prevention efforts, ventilator-associated pneumonia is still the most common nosocomial infection in intensive care units. It increases mortality and morbidity. Since the most recent guidelines published in 2007, few new antibiotics have emerged and clinicians are facing more and more resistant pathogens. This review summarizes recent advances in optimizing treatment such as continuous infusion of antibiotics such as β-lactams, inhalation of antibiotics and high-dose aminoglycosides. Treatment of methicillin resistant *Staphylococcus aureus* and carbapenem resistant Gram-negative organisms are discussed. Eight-day duration of antimicrobial treatment for most of the cases is now well established and may even be shortened with a procalcitonin-guided algorithm. Immunomodulation and immunotherapy are also reviewed. Despite the lack of large randomized trials, most of these therapies appear to be useful for critically ill patients.

Keywords: aminoglycoside • colistin • continuous infusion • inhalated antibiotic • macrolides • methicillin-resistant *Staphylococcus aureus* (MRSA) • nosocomial pneumonia • ventilator-associated pneumonia

Ventilator-associated pneumonia (VAP) remains the most common hospital-acquired infection in the intensive care unit (ICU) [1–3]. It represents almost a third of hospital-acquired pneumonia, occurring in 9–40% of patients requiring mechanical ventilation [4]. VAP has been associated with prolonged ICU and hospital length of stay but also with increased mortality and morbidity ranging from 0 to 70% in the literature, depending on the reason of admission and critical care score range [4–6].

Although efforts should focus on prevention, optimal treatment must be provided when VAP occurs. The most recent guidelines on behalf of the American Thoracic Society (ATS) and Infectious Diseases Society of America were published in 2005 [7]. Since then many studies have showed promising results concerning treatment. The purpose of our review is to focus on emerging approaches for the treatment of VAP. Based on the review of recently published clinical studies, we discuss antibiotic administration modes (continuous infusion, inhalation and high-dose administration of aminoglycosides), the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant bacterial pneumonia, the duration of treatment, the role of macrolides and immunotherapy. Although most of these options are not validated by large, randomized, controlled trial, we will attempt to outline practical conclusions for each approach.

Antimicrobial therapy

Continuous antibiotic infusions

As far as pharmacodynamics and pharmacokinetics (PK) are concerned, antimicrobial concentration levels are difficult to predict in the context of critical care. Many parameters, such as high clearance or high distribution volume, may contribute to low concentration levels [8]. β-lactams are time-dependent antibiotics that must remain at specific concentration levels to be effective; at least 60% of the

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dosing interval above the minimal inhibitory concentration (MIC) is required for efficacy. Moreover, killing rates of Gram-negative bacteria increase when β-lactam plasma levels increase up to four-times the MIC, then reach a plateau [9]. In 2008, McKinnon also showed that patients with time above MIC of 100% had a significantly greater survival rate than patients who did not achieve this goal (82.1 vs 33.3%; p = 0.002) [10].

In addition, an important parameter for efficacy of treatment of pneumonia is the concentration of the antibiotic in the lung epithelial fluid. The previously described issues in plasmatic concentration are also reported in the lung, where effective concentrations are sometimes barely achievable [11]. Thus, delivery of antibiotics by a continuous infusion may be an effective method of administration with the ability to increase the concentration in the bloodstream and in the lung lining fluid with a lower risk of neurological or renal toxicity, since extremely high plasma levels at peak concentration are avoided [12,13].

The use of continuous antibiotic infusions for VAP treatment has been widely studied in the clinical setting. One of the most studied drugs is piperacillin-tazobactam (PTZ; Table 1). Using Monte Carlo simulations, from 16 septic ICU patient data sets with normal renal function, Roberts showed that only a loading dose of 4/0.5 g PTZ over 20 min followed by a continuous infusion of 12 or 16 g daily dose achieved the goal of 100% time above MIC up to MICs of 8 [14]. Recently, in a prospective study, Duszynska reported results of a continuous infusion of 10 g/1.25 g of PTZ, given after a loading dose of 2.0/0.25 g, adjusted to obtain a blood concentration four-times above the MIC [15]. While the maximum dose was 16 g of piperacillin, the median daily dose of PTZ was 11.25 g (range 6.75–13.5 g) and the initial PTZ regimen achieved adequate piperacillin concentrations on the first day of therapy in 11 patients (69%). The daily dose was reduced in seven patients and increased in six patients with resistant pathogens, but the increased dose resulted in target PTZ concentrations for only one additional pathogen. For the patients who had resistant pathogens (MIC ≥ 12 μg/ml), only three out of six patients had adequate concentrations of antibiotic in their plasma once in the first 4 days. No adverse effects were reported even in patients with high PTZ blood concentrations. Including one daily measurement, dose optimization allowed a cost reduction of €15 (US $20) per day compared with the recommended daily dose (16 g), representing €105 ($140) savings for a 7-day course.

Table 1. Characteristics of the studies concerning continuous infusion of piperacillin-tazobactam.

Study (year)	Study design	Patients (n)	Continuous or extended regimen	Main conclusion	Ref.
Lodise (2007)	Monocentric, retrospective	102 EI and 92 II	4-h infusion of 3.375 g every 8 h	Lower 14-day mortality rate in EI group for patients with APACHE II>17	[16]
Lorente (2009)	Monocentric, retrospective	37 CI and 46 II	Loading dose of PTZ 4.0/0.5 g over 30 min, followed by 4.0/0.5 g infused over 360 min every 6 h	Higher clinical cure rate in CI group	[14]
Roberts (2010)	Monte-Carlo simulation	8 CI and 8 II	Loading dose of 4.0/0.5 g PTZ followed by 12.0 g/24 h	CI associated with 50% T> MIC of 4	[12]
Yost (2011)	Multicenter, retrospective	186 EI and 173 II	3.375 g every 8 h as a 4-h infusion	No outcome difference between groups	[17]
Duszynska (2012)	Pilot study, prospective	16	Loading dose 2.0/0.25 g PTZ over 30 min followed by a daily CI of 10.0/1.25 g. Adjustment to obtain T>4 MIC 100% with a max dose of 16.0/2.0 g	11 patients (69%) with adequate concentration on day 1	[13]
Goncalves-Pereira (2012)	Multicenter, retrospective, propensity matched	173 each group	16.0/2.0 g PTZ per day (80.9% of the II group and 79.2% of the CI group)	No outcome difference between groups	[15]

CI: Continuous infusion; EI: Extended infusion; II: Intermittent infusion; MIC: Minimum inhibition concentration; PTZ: Piperacillin-tazobactam; T: time.

In a retrospective cohort study, Lorente *et al.* compared 37 episodes of VAP treated by continuous infusions of PTZ (loading dose of 4 g over 30 min and 16 g/day of piperacillin) to 46 episodes treated with intermittent dosing with 16 g piperacillin/day. PIP-TZ was associated with 7 mg/kg tobramycin extended interval for a total course of 14 days. The groups were comparable in terms of sex, age, APACHE II score at ICU admission, diagnosis, microorganism responsible for VAP, weight, creatinine clearance, SOFA score at the time of suspected VAP, vasopressor use, steroid use or the MIC of the responsible organism. Patients receiving continuous infusions had an overall significantly higher rate of clinical cure assessed by clinicians blinded to the therapeutic regimens (89.2 vs 56.5%; p = 0.001). Nevertheless this result was observed only for MICs of 8 (88.9 vs 40.0%; overall response [OR] = 10.79; 95% CI: 1.01–588.24; p = 0.049) or 16 μg/ml (87.5 vs 16.7%; OR = 22.89; 95% CI: 1.19–1880.78; p = 0.03) whereas cure rates were similar for lower MICs. No difference in terms of mortality rates or duration of mechanical ventilation were found [16].

Although not specifically evaluating VAP, a recently published, multicenter study used a propensity score analysis to compare outcome differences between 173 critically ill septic patients receiving continuous infusion of PTZ, matched to 173 patients receiving intermittent regimens [17]. 40% of the patients had nosocomial infections and 70% had pneumonia. Non-fermenting Gram-negative bacteria represented 45% of the causative pathogens. The total daily dose was 16 g of piperacillin plus 2 g of tazobactam in 80.9% of the intermittent dosing group and 79.2% of the continuous infusion group. The mean daily doses of piperacillin were 14.9 and 14.8 g, respectively (p = 0.84). A second antibiotic, effective against the isolated microorganism, was given to 29.5% of those receiving intermittent dosing and 32.9% continuous infusion (p = 0.77). The authors failed to find any differences in terms of ICU or hospital mortality rate or length of stay, even after stratifying patients according to SAPS II score.

In a retrospective study, Lodise compared 92 patients receiving intermittent infusions (II) to 102 patients receiving extended infusions for Pseudomonas infections. Extended infusions consisted of a 4-h infusion of 3.375 g of PTZ administered intravenously every 8 h. Pneumonia accounted for the most frequent infection with 53.9% of infections in the extended infusions group and 52.2% in the intermittent group. Median duration of stay prior to infection was 7 days. The authors reported a reduced 14-day mortality rate (12.2 vs 31.6%; p = 0.04) and decreased length of stay in the hospital (21 days [3–98] vs 38 [6–131]; p = 0.02) of patients with an APACHE 2 score above 17, in the extended infusions group [18].

This extended infusions of PTZ was also studied in a multicenter retrospective analysis on a larger cohort of ICU patients treated for Gram-negative bacterial infections [19]. The authors compared 186 patients treated with extended infusions (EI) of PTZ to 173 patients who received various regimens of intermittent antibiotics (cefepime, ceftazidime, imipenem-cilastatin, meropenem, doripenem or PTZ). They observed a significant reduction of mortality rate in the EI group (9.7 vs 17.9%; p = 0.02). No significant difference for ICU or hospital length of stay or antibiotic duration was found. Nevertheless, the two groups were not well balanced and significantly differed in terms of infection sources (30.7% of pneumonia in the EI group vs 43.4%; p = 0.01), aminoglycoside association (5.9% in the EI group vs 16.2%; p < 0.01) and pathogens (22.6% *Pseudomonas aeruginosa* in the EI group vs 39.9%; p < 0.01). These differences may have played a role in the primary result.

Ceftazidime or meropenem have also been used in continuous regimens. In a small sample of critically ill trauma patients with VAP, Hanes compared 2 g of ceftazidime every 8 h (15 patients) to 2 g over 30 min followed by 60 mg/kg/day as a continuous infusion (17 patients). The authors found that both regimens reached 100% time above MIC for all except one patient in the intermittent group. In this study the mean MIC was 0.55 μg/ml (0.047 to 6.0 μg/ml), and *Haemophilus influenzae* accounted for more than 50% of causative pathogens in both groups. In a prospective, randomized, controlled, open-labeled trial including 17 ICU patients treated for nosocomial pneumonia with a 3 g daily continuous infusion were compared with 18 with an infusion of 2 g every 8 h intermittent regimen [20]. The only significant outcome that favored continuous infusions was time to being afebrile. The authors also found a trend in quicker bacterial eradication (3.9 ± 3.8 vs 6.0 ± 4.0 days; p = 0.08). Lorente published a retrospective analysis of 56 patients receiving a continuous regimen of a loading dose of 1 g ceftazidime over 30 min, followed by 4 g a day, to 65 patients treated by II receiving ceftazidime 2 g over 30 min every 12 h [21]. In this study, the clinical cure rate was significantly higher in the patients receiving continuous infusions whatever the MIC (global cure rate: 89.3 vs 52.3%; p < 0.001) but it was significantly lower for MIC of 8 μg/ml compared with MIC ≤ 2 μg/ml. These results emphasize the importance of daily dose as suggested by the study recently published by a French group [22]. In this pharmacokinetic approach they reported that daily dose up to 10 or 12 g must be used to achieve a blood con-

centration of 40 mg/l (which is fivefold the European break point for *Pseudomonas*) 70% of the time when renal clearance evaluated by the Modification Diet in Renal Disease formula is above 120 ml/min [23].

Pharmacokinetic and pharmacodynamic parameters of cefepime have also been studied in a one-center, randomized study that included 50 critically ill patients, with 41 patients diagnosed with nosocomial pneumonia. Authors found that the continuous infusion of 2 g over 12 h twice daily was consistently associated with a serum concentration that was five-times the MIC and significantly higher (100 vs 82 ± 25%; $p < 0.01$) than in the patients receiving intermittent dosing (2 g over 30 min, twice daily) [24]. No other clinical study concerning cefepime has been published to date.

As far as carbapenems are concerned, instability of these antibiotics in concentrated solutions is a major issue limiting their use with continuous infusion, especially for imipenem [25]. Meropenem and doripenem appeared to remain stable at room temperature for at least 12 h for meropenem and 24 h for doripenem [26]. Continuous infusions of doripenem have not been studied and the recent stop of a clinical trial in nosocomial pneumonia (for which indication it is not approved) due to an increased mortality rate compared with imipenem, will probably limit further investigation[27,28]. Concerning meropenem, Lorente retrospectively reviewed 89 patients with VAP caused by Gram-negative bacilli, 42 of whom received continuous infusions (1 g over 6 h four times a day) and 47 intermittent infusions (1 g over 30 min every 6 h) [29]. Overall and *Pseudomonas* infection cure rates were statistically significantly in favor of the continuous infusions(90.47 vs 59.57% for overall VAP; $p < 0.001$ and 84.61% vs 40% for *P. aeruginosa;* $p = 0.02$). This difference was even more important when MIC was above 0.5 μg/ml (80.95 vs 29.41%; $p = 0.003$)

Recently, Chytra reported the results from one center, using a randomized controlled open-label trial comparing a loading dose of 2 g of meropenem followed by a continuous infusion of 4 g over 24 h to 2 g of meropenem over 30 min given every 8 h [30]. This study included 120 severely infected patients in the ICU in each group. Pneumonia accounted for more than 50% of all infections. Continuous infusion of meropenem was associated with a significantly higher success rate in eradicating organisms (90.6 vs 78.4%; $p = 0.02$) judged on the lack of a positive culture of a new sample at the end of the treatment. Clinical cure rate was similar in both groups (83.0% in the CI vs 75.0% patients in the II group; $p = 0.180$).

Finally, in a recent meta-analysis including 1229 patients in 14 studies, Falagas *et al.* found a lower mortality rate for patients treated with continuous infusions of PTZ or continuous infusions of carbapenems. They also found similar success rates for patients with pneumonia. Both community-acquired and nosocomial pneumonia were in one group (225 patients analyzed in this subgroup) [31]. In the same issue of the journal, Dulhunty and colleagues published the results of a multicenter, randomized, double-blind, controlled study comparing CI compared with II of PTZ, meropenem and ticarcillin-clavulanate [32]. In total, 60 patients were included with pneumonia as the first source of infection (36.8% in the CI group vs 43.2% in the II group) although not nosocomial (ICU length of stay prior to randomization was 1 day in each group). PTZ and meropenem were the most used antibiotics (PTZ 60% in CI group and 56.7% in II group; meropenem 33.3% in CI group and 40% in II group). The authors demonstrated a significantly higher clinical cure rate assessed by blinded clinicians in the CI group (70 vs 43%; $p = 0.037$) but failed to demonstrate higher ICU-free days or reduced mortality rate.

Vancomycin is another time dependent antibiotic that is widely used in the critical care setting. Nevertheless, unlike β-lactams, vancomycin has some postantibiotic effects and a longer serum half-life that make CI less accurate [33,34]. In 2001, Wysocki and colleagues reported the results of a multicenter, prospective, randomized controlled trial of ICU patients with suspected MRSA infections [35]. In total, 119 patients included in the final analysis received either vancomycin 15 mg/kg twice daily with a trough target concentration of 10–15 mg/l, or a 15-mg/kg loading dose followed by 30 mg/kg per 24-h infusion with a target plateau concentration of 20–25 mg/l. The initial dose of study medication was adjusted for baseline serum creatinine values based on the Moellering nomogram. Rates of treatment failure, infection-related death or overall death rates and safety were similar between the two groups, but in the CI group, target concentrations were achieved more quickly, with smaller blood dosage needed. *Post hoc* analysis failed to find subgroups of patients in whom CIV might be beneficial, including pneumonia, but the number of patients was small. Moreover, as this study did not reach statistical power to detect any difference between groups, results should be read with caution. However, one matched cohort study, designed to evaluate glycopeptide's efficacy to treat MRSA nosocomial pneumonia in critically ill patients, reported lower mortality rates with CI of vancomycin (25 vs 55%) [36]. Multiple regression analysis confirmed that CI was associated with improved survival. Since this study was not designed to compare CI to II, PK/pharmacodynamics data are lacking, limiting the final interpretation.

More recently continuous infusion of linezolid, the first oxazolidinone that inhibits protein synthesis, was evaluated in a pilot study in 16 critical care patients [37]. A CI of 1200 mg of linezolid after a loading dose of 300 mg over 30 min followed by 900 mg the first day, achieved a more stable serum concentration than intermittent regimens, which were characterized by wide variations and trough levels always below the susceptibility breakpoint (4 mg/l). AUC/MIC were the same in both groups, but CI allowed more patients to achieve a time with free serum concentrations higher than the MIC of 85% for 1 and 2 mg/l MICs. This value has been demonstrated to be related to outcome [38]. Infusion of 600 mg of linezolid as a loading dose followed by 1200 mg/day, was associated with epithelial lining fluid concentration above 4 mg/l with a median linezolid alveolar diffusion of 97% (80–108%) in 12 adult ICU patients [39].

Thus, CI appear to be an important administration route, since effective serum levels are more constantly achieved, especially when strains express high MCIs against β-lactams. This type of administration appears safe in the critical care patient population. PTZ and meropenem have been the most well evaluated drugs using continuous infusions. This strategy may even be cost effective. However, large randomized-controlled studies are missing, so it is impossible to definitely conclude that this type of infusion is superior. Continuous infusion should be considered when treating resistant pathogens. Wider use may also help control the emergence of resistance by avoiding suboptimal concentrations, but data are lacking to support this concept. As far as MRSA pneumonia is concerned, continuous infusion of vancomycin cannot be recommended because of the lack of definite data [40].

Nebulization

Aerosolization is another method of delivery for antibiotics. The potential advantage of aerosolization includes the achievement of high drug concentrations in the lungs, which cannot be achieved using intravenous administration. These high lung concentrations are achieved with relatively low systemic levels of antibiotics [41]. This characteristic is particularly interesting for highly resistant pathogens, or antibiotics with dose-dependant toxicity or poor lung diffusion such as aminoglycosides [42]. Animal studies have shown that many parameters can affect the effectiveness of aerosolized antibiotics. The first one is the type of aerosol. Jet nebulizers, using high-pressure gas to generate aerosols, are less efficient and the addition of gas into the ventilator circuit can alter tidal volumes and the pressures delivered to the ventilated patient [43]. Ultrasonic nebulizers are not commonly used with mechanical ventilation because they generate larger particles, are ineffective with viscous products and are expensive [44,45]. Vibrating-mesh nebulizers are the last available model and offer low residual volumes, a battery operated option and synchronization with inspiration. Other parameters influencing the effectiveness of aerosols include the patient's residual volume, the position of the nebulizer in the ventilator circuit, the size of aerosolized particles, the heat, humidity and the density of the carrying gas [46].

Although several antibiotics have been evaluated, inhaled colistin is the antibiotic that has been studied the most in VAP. In a controlled study [47], including 102 patients with Gram-negative bacterial VAP, Rattanaumpawan *et al.* randomly allocated patients to receive either 75 mg colistin every 12 h by aerosol or saline by aerosol, combined with systemic antibiotics which included imipenem or meropenem and colistin [48]. The most common causative agents in these patients were *Acinetobacter baumanii, P. aeruginosa* and *K.pneumoniae* and 45% of *A. baumanii* strains were multiresistant. The authors were unable to show any significant differences in terms of outcomes (death due to VAP 43.1% in the colistin group *vs* 36.7% in the saline group, p = 0,8), but they did find a large difference in eradicating bacteria using aerosolized colistin (60.9% in the colistin group *vs* 38.2% in the saline group, p = 0.03).

In another prospective observational study, Qin Lu reported results from 43 episodes of VAP treated with nebulized colistin for patients with multidrug resistant (MDR) *A. baumanii* (n = 11) and *P. aeruginosa* (n = 32) strains [49]. Patients received an aerosol of 167mg of basic colistin (higher dose than in the previous study) delivered every 8 h with a vibrating plate nebulizer for 14 days or until successful weaning from mechanical ventilation. Colistin was administered with a 3-day intravenous aminoglycoside in 15 patients. These patients were compared with 122 patients infected with sensitive strains who received 14 days of intravenous β-lactam (PTZ, ceftazidime or imipenem) associated with a 3-day course of either aminoglycoside (78%) or quinolone (22%). The authors found no differences in clinical cure rates of overall VAP (66.4 vs 67.4%) and mortality (23 vs 16%; p = 0.357) between the two groups. Similar results were found in a subgroup analysis for each pathogen.

The same authors also published the results from a randomized Phase II trial comparing aerosolized antibiotics to intravenously (iv.) administration of ceftazidime and amikacin in 20 patients in each group diagnosed with a VAP caused by *P. aeruginosa* [50].

Aerosolized regimens included eight daily administrations of 15 mg/kg ceftazidime over eight days and extended interval administration of 25 mg/kg amikacin for 3 days. The iv. course included a bolus of 30 mk/kg of ceftazidime followed by a continuous infusion of 90 mg/kg over 8 days. The authors found no differences between the two groups in terms of VAP cure rates, recurrences of VAP, length of hospital stay or duration of mechanical ventilation. At the end of the treatment, bronchoalveolar lavage culture was positive in 12 patients in the aerosol group compared with 11 patients in the iv. group. Resistant strains to at least one of the antibiotics were found only in the intravenous group. The authors did report that there was obstruction of the expiratory filter in three patients from the aerosolized drugs, leading to cardiac arrest in one patient with full recovery.

Arnold and Kollef recently published their experience with adjunctive aerosolized therapy for the treatment of *Pseduomonas* and *Acinetobacter* VAP [51]. They compared 19 patients who received iv. and aerosolized antibiotics (nine receiving colistin 150 mg twice daily, ten receiving tobramycin 300 mg twice daily) to 74 patients who received only iv. regimens. Patients in the aerosol group received iv. aminoglycosides more frequently (68.4 vs 27%; p = 0.001) and colistin (21.1 vs 2.7%; p = 0.02). Mean APACHE II score at the time of bronchoalveolar lavage were higher is the aerosol group (21.4 ± 5.7 vs 17.5 ± 5.3; p = 0.004). The authors found a lower 30-day mortality in the aerosol group (0.0 vs 17.6%) although the difference was not significant (p = 0.063). Kaplan–Meier curves depicted a significantly greater 30-day survival rate after VAP onset among patients receiving aerosolized therapy (p = 0.030 by the log rank test).

Aerosolized antibiotics have also been studied in the context of nosocomial tracheobronchitis. It is an entity defined by fever over 38°C with no other recognizable cause, purulent sputum production, endotracheal aspirate cultures of over 10^6 CFU/ml and no radiographic signs of pneumonia [52]. Palmer and colleagues randomized 43 patients at the time of tracheobronchitis diagnosis to receive either aerosolized antibiotics (gentamicin 80 mg/8 h in the case of Gram-negative pathogens or vancomycin 120 mg/8 h in case of Gram-positive organisms) or placebo [53]. Patients were treated for 14 days and followed for 14 days after treatment. The authors demonstrated a significant effect in the aerosol group, with reduction of pulmonary signs (35.7 vs 78.6%; p = 0.05, at day 14), reduction of resistance acquisition (0 vs 8 in the placebo group; p = 0.0056) and reduction of systemic antibiotic use (8/19 vs 17/24; p = 0.042). No statistically significant effect was found regarding mortality or ventilator-free days. It is important to note that at the time of randomization, 14 patients (73.6%) in the aerosol group and 18 patients (75.0%) in the placebo group met the actual criteria for VAP. Indeed, it is probably difficult to separate tracheobronchitis from actual pneumonia, questioning the evaluation of aerosolized antibiotics in this clinical setting [54].

The inhalation route has been used for other antibiotics, including aztreonam lysine, tobramycin, amikacin in a new formulation, levofloxacin, vancomycin and fosfomycin [55–57]. To date, aztreonam is the only approved drug for inhalation therapy, but this drug as an inhaled agent has only been studied in cystic fibrosis (CF) patients. Three doses (75, 150 and 225 mg) delivered through a dedicated device (a single patient, multiuse nebulizer that uses a vibrating and perforated membrane to generate the aerosol) were compared with placebo in a mixed population of adult and adolescents [58]. All doses were well tolerated and sputum aztreonam concentrations remained above MIC_{90} of most *P. aeruginosa* strains immediately after aerosol for all doses but only for 150 and 225 mg after 4 h. No data concerning lung concentrations are available. Levofloxacin and fosfomycin are currently also under evaluation in CF patients. Experience with inhaled vancomycin is limited to case reports [59,60].

The inhalation route has been utilized most for colistin and aminoglycosides. The use of this route appears to be safe and feasible, but requires appropriate devices and ventilator settings. Aerosolized antibiotics seem most relevant to treat highly resistant Gram-negative pathogens or when a patient's renal function is a major concern. Supremacy of the inhalation route to treat pneumonia has not been documented [61]. Currently published regimens are listed in Table 2.

High dose iv. aminoglycosides

According to the ATS guidelines on nosocomial pneumonia, aminoglycosides are part of the antimicrobial treatment that accompanies β-lactams [7]. Peak blood concentrations (C_{max}) over MIC is considered to be the best parameter to characterize *in vivo* exposure of bacteria to aminoglycoside concentrations [62,63]. This ratio should be between 8 and 10 to ensure maximal antibacterial activity and improve outcomes [64]. In a prospective study including 78 patients with Gram-negative pneumonia, Kashuba and colleagues showed that achievement of a C_{max}/MIC >10 within 48 h was strongly associated with positive clinical responses [65]. Recently, some authors pointed out that recommended doses of aminoglycosides are insufficient to achieve this goal in critically ill patients. Taccone studied 74 patients admitted for severe sepsis or septic shock, mainly related to pneu-

Table 2. Published regimens of aerosolized antibiotics for the treatment of ventilator-associated pneumonia.

Antibiotic	Regimen	Ref.
Amikacin	25 mg/kg once daily	[50]
Ceftazidime	15 mg/kg every 3 h	[50]
Colistin	75 mg every 12 h 150 mg every 12 h 167 mg every 8 h	[48–50]
Tobramycin	300 mg every 12 h	[51]

monia or abdominal infections, and showed that with a loading dose of 25 mg/kg of amikacin only 70% of the population reached a peak concentration eight-times the MIC breakpoints defined by EUCAST for *Enterobacteriaceae* and *P. aeruginosa* [66]. These results were emphasized by another group who studied 99 patients with severe sepsis or septic shock, randomly assigned to 15, 25 or 30 mg/kg of amikacin [67]. In this study, the maximal loading dose achieved a C_{max} above 60 µg/ml in 76% of the patients while only 39% reached the targeted value in the 25-mg/kg dose group and none in the 15-mg/kg group. No increase in renal complications were reported.

Higher doses of aminoglycosides appear to be mandatory in the critical care context to achieve targeted concentration. However, in the case of renal impairment, which happens in almost 30% of ICU patients [68], a high dose will result in prolonged intervals between doses. This optimal peak concentration may only be seldomly achieved, reducing the expected effect. Studies are still needed to better the characteristics of patients who do not achieve adequate peak levels.

MRSA nosocomial pneumonia treatment

Vancomycin has been the only major antimicrobial agent against MRSA for a long time. In 2001, Rubinstein and colleagues published the results of a randomized controlled trial comparing vancomycin with linezolid, a new class of antibiotics called oxazolidinone effective only against Gram-positive pathogens, for the treatment of nosocomial pneumonia [69]. The authors compared in an intention to treat analysis 203 patients receiving linezolid 600 mg every 12 h (with aztreonam) with 193 patients receiving vancomycin 1 g every 12 h (with aztreonam). Actual Gram-positive pneumonia represented 36 and 34.7% in the linezolid and vancomycin group, respectively. They found no difference in terms of clinical cure rates (53.4% in the linezolid group vs 52.1% in the vancomycin group; p = 0.79) in the overall population and in the clinical evaluable or microbiologically evaluable subgroups. The same author published the results of the extension of this study in a further 623 patients (321 in the linezolid group and 302 in the vancomycin group) with the same results [70]. In 2004, Kollef and colleagues published the pooled data of the two previous studies concerning 544 patients included with Gram-positive VAP [71]. In this retrospective analysis of two prospective randomized studies, they found a significantly higher rate of clinical cure (62.2 vs 21.2%; p = 0.001) and survival (84.1 vs 61.7%; p = 0.02) in the subgroup group of MRSA VAP treated with linezolid. These results led to a Phase IV, randomized, double-blind, multicenter study comparing linezolid 600 mg every 12 h with vancomycin 15 mg/kg every 12 h (in contrast to the flat 2-g/day dose in the previous studies) with dose adjustments in MRSA nosocomial pneumonia [72]. A total of 1184 patients with documented hospital-acquired or healthcare-associated pneumonia were included in the intention-to-treat cohort. There were 448 patients with confirmed MRSA pneumonia, who composed the modified intention-to-treat (mITT) cohort. The perprotocol (PP) group included patients who fulfilled all inclusion/exclusion criteria, received adequate study medication and had an observed outcome for that visit. Clinical cure was defined as resolution of signs and symptoms of pneumonia compared with baseline, improvement or lack of progression of chest x-ray results, and no additional antibiotic therapy required. The PP group included 348 patients (172 treated with linezolid and 176 with vancomycin). The primary end point was clinical outcome at the end of the study in the PP population. The authors found a significant difference in terms of clinical response in the PP and mITT population, which favored linezolid (57.6 vs 46.6%; p = 0.042 and 54.8 vs 44.9%, respectively). The incidence of adverse events was similar in the two groups except for renal dysfunction, which, in the mITT group, occurred in 18.2% of vancomycin-treated patients compared with 8.4% in the linezolid group.

Despite these results, controversy persists concerning the treatment of MRSA pneumonia. First, the baseline characteristics of the patients were imbalanced. The vancomycin group included more patients

who were mechanically ventilated (73.9 vs 66.9%; p = 0.15), kidney disease (36.9 vs 27.9%; p = 0.07) and MRSA bacteremia (10.8 vs 5.2%; p = 0.039), which is a risk factor for mortality [73]. Second, 52% of the vancomycin group patients whose data were available, had a day-3 trough level below 15–20 µg/ml, the value recommended by the IDSA to treat severe MRSA infections such as pneumonia [40]. Data were not available for 21% of the patients. Thus, vancomycin appears to remain a reasonable option for MRSA pneumonia.

As far as empirical treatment is concerned, linezolid use, especially for patients with prolonged ICU stays, may be a risk factor for the emergence of linezolid resistance [74]. Vancomycin should be utilized when MRSA infection is suspected. When definite MRSA is identified, one must take into account whether the patient condition, including renal function, will permit vancomycin and MRSA vancomycin MIC level. It is probably reasonable to reserve linezolid for documented MRSA pneumonia, especially when vancomycin MIC of the strain is above 2 µg/ml. If no MRSA is found, vancomycin or linezolid must be stopped.

Carbapenem-resistant Gram-negative pathogens

Because of the epidemic spread of Carbapenemase-producing *Enterobacteriaceae* (CPE) and the increasing frequency of other MDR bacteria [4,75], clinicians are now challenged by the treatment of nearly pan-resistant bacteria. This situation has led to the revival of colistin, used parentally or inhaled, as previously discussed. Few studies have evaluated iv. colistin in the context of VAP. In 2007, Rios retrospectively studied 61 episodes of MDR *Acinetobacter spp*. (unique pathogen in 36 cases) or *P. aeruginosa* (unique pathogen in 14 cases) VAP [76]. In total, 30 were carbapenem susceptible and 31 susceptible to colistin only. The same year, Kallel reported the results of a matched case–control study including 60 patients treated with iv. colistin for pan-drug-resistant *A. baumanii* or *P. aeruginosa* and 60 patients treated with imipenem for imipenem-susceptible strains [77]. Both studies found similar efficacy in each group: 51.6 vs 45.1% for mortality rates in the first study, 75 vs 71.7% for clinical cure in the second. Recently, these results were confirmed in a meta-analysis including 437 patients (14 studies) in the iv. colistin group and 359 patients (six studies) in the control group [78]. In the control group, the comparator was either imipenem for imipenem-susceptible strains, ampicillin-sulbactam or aerosolized colistin. Using a metaregression model to evaluate the effect of concomitant antibiotic treatment, the authors found no significant difference between the groups in terms of clinical response rates, in-hospital or in-ICU mortality or length of stay. They did find a trend towards better microbiological response with colistin (OR: 1.997; 95%CI: 0.97–4.12; p = 0.06). Thus, colistin appears to be effective. This study also raised issues of the optimal route to deliver colistin (choice of iv. or aerosolized) and the dose discrepancy, although the authors standardized the doses used to colistin base activity.

Optimal dosage of colistin is a matter of concern, especially in ICU patients. Different doses have been published but not compared with one another [79]. Recently, Plachouras and colleagues demonstrated that a 3 million unit (MU) dose (240 mg) of colistin methanesulfonate every 8 h resulted in a predicted maximum concentration of colistin in plasma of 0.6 and 2.3 mg/l for the first dose and at steady state, respectively [80]. As they also demonstrated that the half-life of colistin is 14.4 h, steady state is then obtained only after 3 days. Moreover, this steady state concentration corresponds to the MIC breakpoint suggested by EUCAST for *A. baumanii*, while for *P. aeruginosa* it has been suggested to be 4 mg/l [81]. These findings lead some authors to recommend a loading dose based on population PK studies in critically ill patients [82,83]. Garonzik and colleagues published suggested doses derived from equations based on the data of 105 critically ill patients. Recently, Dalfino reported the actual use of a loading dose of 9 MU followed by a maintenance dose adjusted on Cockcroft and Gault creatinine clearance estimates (4.5 MU every 12 h for normal clearance, every 24 h for clearance between 20–50 ml/min and every 48 h for clearance under 20 ml/min) [84]. Including 28 critically ill patients (18 with bloodstream infections and ten with VAP), they found an overall clinical cure rate of 82.1% (23/28), and 100% clinical cure (10/10) for VAP. Interestingly, bacteriological clearance rate was only 40% in VAP and all treatment failures were in the blood stream infections. No recurrent infection with the same pathogen was observed, but two episodes of super-infections appeared with intrinsically colistin-resistant organisms. Acute kidney injury developed in five patients with an onset of 7 days (interquartile range: 5.5–8.5 days), but all patients completed treatment by dose reduction without requiring renal replacement therapy. Renal failure is a matter of concern regarding iv. colistin. Recently published studies suggested that nephrotoxicity in ICU patients after colistin administration ranges from 0 to 36% [85].

In the context of MDR bacteria treatment, several studies reported combined treatment including

colistin (both iv. and aerosol), doripenem, or rifampicin [86–88]. Unfortunately, these studies failed to show convincing data on additional therapeutic benefit regarding clinical cure or mortality rates.

Regarding Carbapenem-resistant *P. aeruginosa*, some authors have reported synergistic effects and clinical success with combination therapy including carbapenem and fosfomycin [89,90]. To date, the largest population published included 25 patients treated with a doripenem–fosfomycin combination therapy retrospectively compared with 24 patients treated with colistin and fosfomycin, with 15 and 14 with VAP patients, respectively [91]. Patients received a 4-h infusion of 1 g doripenem with 2 g of fosfomycin every 8 h. The median *P. aeruginosa* MICs for imipenem and meropenem were > 32 mg/l and 4 mg/l (4–8 mg/l) for doripenem. Clinical cure, microbiological cure and all cause (28-day) mortality rates were similar in both groups suggesting that both treatments are effective.

Fosfomycin, a 40-year old antibiotic that inhibits bacterial cell wall, has gained renewed interest since it possesses *in vitro* activity against carbapenem-resistant *P. aeruginosa* and *Klebsiella pneumoniae* [92]. *In vitro* studies showed a synergistic effect with various antibiotics against clinically isolated MDR bacteria [93]. Nevertheless, its clinical use as a single agent is prohibited due to rapid emergence of resistance during therapy [94].

Tigecycline, a parenteral minocycline, whose spectrum includes MDR Gram-negative microorganisms, has also been studied in this context. A result of a recent meta-analysis has suggested that tigecycline may be less effective than comparators, especially in the clinical setting of VAP [95,96] with an excess risk of mortality. This issue is possibly a matter of dose, as suggested by Burkhardt [97]. Indeed, a Phase II trial evaluating two high doses of tigecycline (150 mg followed by 75 mg every 12 h and 200 mg followed by 100 mg every 12 h) in a small population with HAP (35.2% VAP) led to higher clinical response rates than in a previously reported Phase III study in the 100 mg group (85.0 vs 75.0%) [98]. Although neither approved nor recommended for the treatment of VAP, tigecycline may be considered in combination with another drug when no alternative treatment exists. Recently, data concerning 45 adults (35 ICU patients) treated with tigecycline (100 mg followed by 50 mg twice daily) for 21 episodes of VAP were reported. Pathogens were *A. baumanii* and *K. pneumonia* with tigecycline MIC from 1 to 8 and 0.5 to 3 mg/l respectively [99]. Successful clinical cure rate was 80% for VAP. More recently, Chan and colleagues published the results of a retrospective cohort of 55 patients with *A. baumanii* VAP. Patients received a 100 mg loading dose followed by 50 mg every 12 h. Only one patient received a monotherapy with tigecycline. Tigecycline-based therapy appeared to obtain the highest rate of clinical response.

The treatment of MDR pathogens, especially CPE, is not well established and mostly based on retrospective studies. In this context, carbapenem, ceftazidime, amikacin, fosfomycin, and amikacin MICS of the strain must be obtained. Colistin is also a corner stone of the treatment. It may be administered with other antibiotics even if the MICs are above the published breakpoints. The superiority of the iv. route is not established compared with inhalation. If the iv. route is preferred, one should consider the use of a loading dose of 9 MU and be mindful of potential nephrotoxicity (Box 1).

Duration reduction

The optimal duration of treatment for VAP is still a matter of debate. In 2003, Chastre *et al.* reported the results of a large multicenter, randomized, controlled trial comparing 8 versus 15 days of antibiotic therapy. In 402 randomized patients, they found no significant difference in terms of mortality (18.8 vs 17.2%; difference: 1.6%; 90% CI: -3.7–6.9%) or recurrent infections (28.9 vs 26.0%; difference: 2.9%; 90% CI: -3.2–9.1%) between the two arms [100]. They also found no differences in long-term outcomes at 60 days. Nevertheless they observed a higher rate of recurrence for *Pseudomonas* infections in the 8-day group, although other outcomes were similar. The number of MRSA pneumonia was too small to draw any conclusion (22 in the 8-day group vs 23 in the 15-day group). Moreover, no data concerning antibiotic resistance were published.

The evaluation of short antibiotic course was reassessed in a more recent trial dedicated to early onset VAP [101]. This prospective, multicenter, randomized study [102] included 225 patients (109 in the 15 days

Box 1. Published intravenous colistin regimens for patients with normal renal function.

- Intravenous only [80]
  - 240 mg every 8 h
- Intravenous with a loading dose [82]
  - Loading dose of 480 mg followed by 240 mg every 8 h
- Extended interval [84]
  - Loading dose of 720 mg followed by 360 mg every 12 h
- Combined aerosol and iv. [87]
  - Aerosol: 75 mg every 12 h
  - Intravenous: 240 mg every 8 h

group and 116 in the 8 days group). The primary outcome was clinical cure after 21 days. All patients were initially treated with amoxicillin, cefotaxime or ceftriaxone alone, and eventually given a non-amikacin aminoglycoside. Methicillin-sensitive *S. aureus* and streptococcus were the main causative pathogens, enterobacteriae were the third most common organisms found. In this study, Capellier and colleagues confirmed that an 8-day course was safe in the clinical setting where *Pseudomonas* and MRSA strains were absent (84.4% in the 15 day cohort vs 85.3% in the 8-day cohort; 95% CI -8.4–10.3%).

Procalcitonin (PCT), a 116 amino acid peptide precursor of calcitonin, has been extensively studied in the context of pneumonia, sepsis and critical care. Some authors have studied the ability of PCT to predict severity and outcomes, but others have suggested that it could be used to shorten the duration of antimicrobial therapy. Recently, Stolz and colleagues published a randomized controlled trial that compared two strategies that dictated the discontinuation of antibiotics in patients with VAP (ISRCTN61015974) [103]. In one arm, patients were treated according to the ATS recommendations and in the other, antibiotic duration was based on PCT levels measured on day-3, compared with day-0 (time of inclusion; the levels were blinded so that the attending physician in charge did not know the levels of PCT). When the PCT-levels were below 0.5µg/l or reduced by more than 80% on day 3 compared with day 0, antibiotic discontinuation was strongly encouraged. If the PCT level was above 0.5 or reduced by less than 80%, antibiotics were continued. This strategy was re-evaluated on a daily basis over 10 days. A group of 101 patients, representing 101 episodes of early onset VAP were included. *Pseudomonas*, *E. coli* and Methicillin-susceptible *S. aureus* were the main pathogens in both groups, although no resistance profile information was given. Authors reported a significant increase in antibiotic-free days in the group that received PCT levels (13 versus 9.5; p = 0.049), which represented a median time of 5 days. Outcomes were secondary end points, and no difference was noticed in terms of mechanical ventilation-free days or ICU-free days alive, length of stay, or death rates (either in-hospital or after 28 days).

Other PCT data are published in a multicenter French randomized controlled trial [104]. Although this study included all septic patients treated in the ICU, the authors had studied *a priori* the subgroup of VAP patients [105]. With a similar algorithm to the one published by Stolz, Bouadma *et al.* observed a significant reduction of 2.1 days in the antibiotic duration (7.3 vs 9.4; p = 0.021) in the PCT guided group. Again, no differences in terms of outcome were reported.

An 8-day antibiotic course appears safe in the context of early- or late-onset VAP. This duration can probably be shortened further when a PCT guided algorithm is used (Figure 1). Definite data are lacking when *Pseudomonas, Acinetobacter, Stenotrophomonas* or MRSA are concerned. If PCT is not used to guide antibiotic duration, a PCT level above 1.5 ng/ml after 3 days of treatment is strongly associated with a poor outcome [106], prompting clinicians to re-evaluate therapeutic regimens when PCT level remains high.

Macrolides

Although macrolides do not have any specific activity against the usual nosocomial pathogens, some studies suggest that macrolides may play a role in the treatment of nosocomial pneumonia. Recent studies showed that clarithromycin is able to decrease release of IL-1β, IL-6 and IL-8 in a viral infected model of human tracheal epithelial cells [107] and to inhibit mucin release through decrease of gene expression [108]. Macrolides may also reduce neutrophil counts and proinflammatory cytokines production in the alveolar space [109]. Azithromycin has shown its ability to increase monocyte influx and lung infiltration by alternative alveolar macrophages aiming at tissue rehabilitation, and decrease neutrophil influx [110,111]. As far as VAP is concerned, one multicenter, placebo-controlled, randomized study [112] evaluated the effect of 1 g of clarithromycin for 3 consecutive days in 200 VAP patients treated in the ICU [113]. The population included mostly late-onset VAP (*A. baumanii* and *P. aeruginosa* represented 80% of the identified pathogens) and 75% of the patients suffered from severe sepsis or septic shock. The patients who received clarithromycin improved earlier than the patients in the placebo group (7.0 vs 11.5 days; p = 0.006) leading to a reduction of mechanical ventilation duration (16.0 vs 22.5 days; p = 0.049). Despite this huge difference, no difference in mortality rates, even in the subgroup of sepsis-related cause of death was observed. The authors also reported a longer course for the development of multiple organ dysfunction syndrome after the diagnosis of VAP, which may be related to a restored balance between proinflammatory and anti-inflammatory cytokines [114].

Quorum sensing, a communication system for sessile forms of *P. aeruginosa,* is involved in the regulation of many *Pseudomonas* virulence factors. Among these virulence factors, rhamnolipids characterized by the production of glycolipidic surface-active molecules is quorum sensing-controlled. Macrolides, especially azithromycin, are potent inhibitors of one quorum sensing system of *P. aeruginosa* [115,116]. Although not concerning the treatment of VAP, one recent randomized controlled pilot study evaluated the effect of

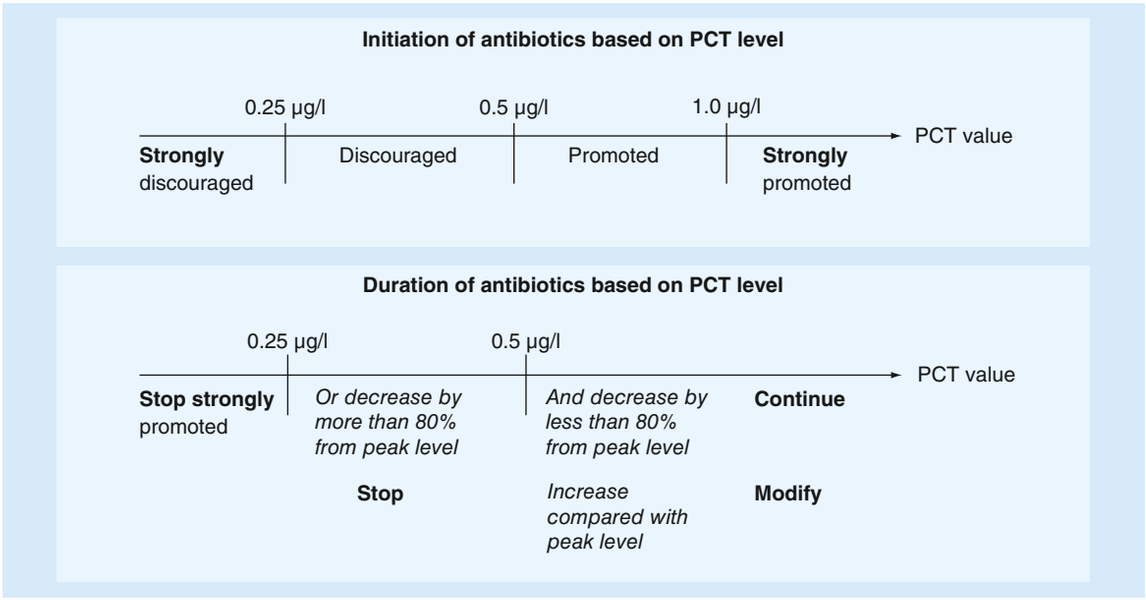

Figure 1. Procalcitonin algorithm for antibiotic duration.
PCT: Procalcitonin.
Data taken from from [103,105].

azithromycin in preventing *Pseudomonas* VAP [117]. These authors evaluated the effect of 300 mg intravenous azithromycin administered daily. They reported a reduced incidence of VAP in *Pseudomonas* colonized patients, where the *Pseudomonas* strain produced a lot of rhamnolipids. When evaluating the entire patient population, the authors found no statistically significant effect, although the reduction in rhamnolipids persisted. These results suggest that macrolides may benefit to patients with *Pseudomonas* colonization, especially if the bacteria produce rhamnolipids. To date, clinical evidences of benefit come from CF and chronic obstructive pulmonary disease patients, where azithromycin has been proven effective in randomized trials [118,119]. Moreover, the exact mechanism of effectiveness may be less obvious than suggested in former studies. Authors should also consider that *in vitro* macrolide susceptibility tests might not be accurate [109], or that macrolides have an effect on commensal bacteria [120].

Nevertheless, clinical studies that demonstrate that azithromycin or macrolides may effectively improve acute nosocomial pneumonia are lacking. Therefore, macrolide prescription cannot be recommended at this time.

Conclusion

Emerging approaches for the treatment of VAP are mostly concerned with optimizing antibimicrobial treatment. Continuous infusion of all β-lactams should be used more widely, particularly for patients with normal renal function, although its superiority to intermittent infusion is not definitely established by good randomized controlled trials. When highly resistant pathogens are involved, continuous infusions are often the only method to reach optimal blood concentrations.

Aerosolized antibiotics must be reserved for highly resistant pathogens at this time because of a lack of data concerning standard strains. High dose aminoglycosides should be used in order to reach optimal concentrations, but they should be utilized when other therapies are ineffective and they have renal toxicity. Vancomycin remains the first-line treatment when MRSA is suspected. Colistin seems essential in the treatment of carbapenemase strains, although no definite data exist to recommend iv. route over aerosol. VAP treatment should not extend more than 8 days, except for some pathogens (*P. aeruginosa, Acinetobacter, Stenotrophomonas* and MRSA). PCT may be an effective way to shorten or to guide duration of antibiotics in difficult situations. Macrolides need further study to better evaluate their benefit in VAP

Future perspective

Currently, no major new antibiotic is in the pipeline [121]. Immunotherapy may appear to be a major component of prevention and treatment in the next few years. Type III secretion system is one of the virulent mechanisms utilized by *Pseudomonas* and other Gram-negative bacteria; it is associated with higher mortality rates in VAP [122]. The PcrV protein is one of the three components of this system. Antibodies to PcrV protein have shown potential beneficial effects in animal

models of VAP. This data led to a clinical Phase II trial in mechanically ventilated patients [123]. In total, 39 *P. aeruginosa* colonized patients were randomized to receive a single iv. infusion of 3 or 10 mg/kg of the antibody to PcrV or to receive a placebo on a 1:1:1 basis. The safety and tolerability profile of this potential treatment was good, with no major differences in adverse event observed between the groups. The authors also reported a clear, clinically relevant reduction in *Pseudomonas* VAP, although the result was not statistically significant (33% in the 3 mg/kg group, 31% in the 10 mg/kg group and 60% in the placebo group; p = 0.092 and 0.085, respectively). The pharmacokinetic profile was also studied and showed that the antibody administration led to a prolonged high serum concentration as well as to high concentrations in the lung for the 10 mg/kg group.

PNAG is a surface antigen found on many bacteria including *S. aureus* and Gram-negative bacteria. Antibodies targeted to PNAG may constitute an interesting new treatment or prevention for severe infections [124]. In this perspective, a study evaluating the PK of a single iv. dose of MAb F598 administered to ICU patients on mechanical ventilation was begun, supported by Sanofi®. Unfortunately, this trial has been terminated due to difficulty in patient recruitment in the participating sites (last update 10 January 2013) [125].

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Executive Summary

- Continuous infusion has potential benefits but definite data from good randomized controlled trials are lacking.
- Aerosolized antibiotics are relevant to treat highly resistant pathogens.
- Aminoglycoside regimens should include high, extended interval dose for the treatment of Gram-negative pathogens.
- Vancomycin is preferred to linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* ventilator associated pneumonia, through levels must be strictly watched.
- Colistin is frequently utilized for the treatment of multidrug resistant strains.
- The use of procalcitonin to reduce antibiotic duration in ventilator associated pneumonia is safe and easy to use at bedside.

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