Pseudomonas aeruginosa: Evolution of Antimicrobial Resistance and Implications for Therapy

Kate McCarthy, MBBS, FRACP, FRCPA[1]

[1] University of Queensland, UQ Centre for Clinical Research, Brisbane, Australia

Semin Respir Crit Care Med 2015;36:44–55.

Address for correspondence Kate McCarthy, MBBS, FRACP, FRCPA, University of Queensland Centre for Clinical Research, Building 71/918, Royal Brisbane and Womens Hospital Campus, Herston, QLD 4029, Australia (e-mail: kate.mccarthy1@uqconnect.edu.au).

Abstract

Pseudomonas aeruginosa is a formidable pathogen in the infection arena. It is able to easily adapt to the environment which it inhabits and can also colonize and invade the human host to cause serious infections. In 2011, it was responsible for 7.1% of all health care–associated infection in the United States. The morbidity and mortality of both blood stream infections and ventilator-associated pneumonia are significant. On a global scale, we have seen the development of not only multidrug resistance but also extensive and pan drug resistance in this organism. This is often associated with limited clonal types of which we now have epidemiological evidence of spread. With this has come reduced antibiotic treatment options. Consideration of antibiotic infusions, combination therapy, and inhalational therapy has occurred in an attempt to gain the upper ground. Gram-negative resistance has appropriately been described as a global emergency.

Keywords

- *Pseudomonas aeruginosa*
- bacteremia
- ventilator-associated pneumonia
- epidemiology
- resistance
- antibiotic treatment

Pseudomonas aeruginosa was the second most common cause of pneumonia and the third most common gram-negative cause of blood stream infection (BSI) in the Centers for Disease Control and Prevention Multistate Point-Prevalence Survey of health care–associated infections (HCAI).[1] The morbidity and mortality of both BSIs and more specifically ventilator-associated pneumonia (VAP) is significant. This review will focus on BSI and VAP, particularly the epidemiology, mechanisms of carbapenem resistance, and the latest evidence regarding treatment for this pathogen. Although there have been several changes in the epidemiology of *P. aeruginosa* infections in patients with cystic fibrosis (CF), this is beyond the scope of this article.

Clinical Epidemiology

Rates of Infection

The Centers for Disease Control and Prevention found that *P. aeruginosa* accounted for 7.1% of HCAI in the United States in 2011.[1] It was the second most common cause of pneumonia in the hospital setting and the third most common gram-negative cause of BSIs. The European Centre for Disease Prevention and Control 2011 Point-Prevalence Survey for HCAIs found a similar figure, with 8.9% of all infections caused by *P. aeruginosa*.[2] Kollef et al performed a recent global prospective epidemiological study and found that the prevalence of *P. aeruginosa* causing VAP was 4.1% globally and this did not differ significantly between the United States, Europe, Latin America, or in the Asia Pacific regions.[3]

Blood Stream Infection

Traditionally, *P. aeruginosa* BSI has been regarded as only a hospital acquired infection (HAI); however, infections acquired outside the hospital setting have been reported.[4,5] Al-Hasan et al in their unique population-based study in the United States found that 78% of the monomicrobial *P. aeruginosa* BSI were either hospital or health care associated and 12% were community acquired.[4] When Marra et al looked at the location of acquisition for HAIs in an American institution with nine intensive care units (ICUs) they found

Issue Theme Antimicrobial Resistance: Management of Superbugs; Guest Editor, David L. Paterson, MBBS, PhD, FRACP, FRCPA

DOI http://dx.doi.org/10.1055/s-0034-1396907.
ISSN 1069-3424.

that the BSI originated predominantly in the ICU setting.[6] The rates of *P. aeruginosa* BSI have been shown to increase exponentially across age and a male predominance has been found.[4,7–9] Studies looking at case matched analysis of risk factors for *P. aeruginosa* BSI are few. Joo et al performed a case–control study to look at nonneutropenic patients with a solid tumor. They found that the independent risk factors for a *P. aeruginosa* BSI were the presence of lung cancer and previous antimicrobial therapy.[10] The strictness of definition for source of infection varies between studies. Marra et al required the identification of the same isolate from the BSI at a secondary site. They found that the most frequent sources of BSI was the respiratory tract and central venous catheters.[6] Kang et al also found other primary sites of infection to be important including the urinary tract, and soft tissue.[7] Chen et al were one of the first groups to identify the pancreatobiliary tract as a potential source of BSI.[11] The mortality from a *P. aeruginosa* BSI is significant and has been found to be up to 42%, depending on the population studied.[9,12–15] Risk factors for in-hospital mortality in *P. aeruginosa* BSI have been focused upon by Joo et al. They found in their population the risk factors of corticosteroid use, nosocomial acquisition, polymicrobial infection, an increasing Charleston weighted comorbidity index, and ICU care to be associated with death on multivariate analysis.[9]

Ventilator-Associated Pneumonia

The published epidemiology of most studies on VAP reflects the fact that VAP can be caused by a large range of bacterial pathogens. If we focus on the small number of studies looking at *P. aeruginosa* alone, the patients tend to be predominantly male with a mean age greater than 50 years.[16–19] Peña et al found that 58% of patients with non-multidrug resistant (MDR) *P. aeruginosa* pneumonia had underlying comorbidities and this increased to 85% in the MDR group.[16] The rate of septic shock at presentation varies between studies from 10.2% to 49.2%, reflecting the differing patient populations studied.[18,19] Planquette et al described a large cohort of patients with VAP caused by *P. aeruginosa*. The median time to VAP onset was 12 days (interquartile range [IQR], 8–20 days). The majority of patients experienced one episode of pneumonia and 62 of the 314 patients experienced a recurrence. Approximately one-third of patients experienced a treatment failure at day 14 either defined as a recurrence or death. On multivariate analysis, the factors significantly associated with treatment failure as opposed to discharge from ICU at day 14 were limitation of life support in the first 48 hours of ICU stay, vasopressor therapy, delay to first VAP onset of less than 12 days, and a MDR or an extensively drug-resistant (XDR) pathogen. The crude ICU mortality was 38.5%.[18] Similar figures of 44.5 and 42.1% have been found by other cohorts studying *P. aeruginosa* VAP.[17,19]

Epidemiology of Carbapenem Resistance

Laboratory Definitions

Two important changes have been made to the definitions surrounding carbapenem resistance and *P. aeruginosa* that are important to mention in this discussion. First in 2011, new standardized definitions were proposed for MDR, XDR, and pan drug-resistant (PDR) bacteria in response to the variable definitions of these terms in the medical literature. Epidemiologically significant antimicrobial categories were constructed for each bacterium. MDR was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. XDR was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories and PDR was defined as nonsusceptibility to all agents in all antimicrobial categories.[20] It is important to note that MDR is not synonymous with carbapenem resistance. Secondly in 2012, the Clinical Laboratory Standards Institute lowered the breakpoint for sensitivity for carbapenems (excluding ertapenem) in *P. aeruginosa* isolates in the routine laboratory setting. This was to capture isolates expressing resistance mechanisms in the previously defined susceptible range. A breakpoint of less than or equal to 2 mg/L was the new definition of susceptibility to meropenem.[21] The current European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines also define an identical susceptibility breakpoint for meropenem and *P. aeruginosa*.[22]

Rates of Carbapenem Resistance

Croughs et al published 13 years of susceptibility data on *P. aeruginosa* isolates from ICUs in the Netherlands. In 2008, 8.3% of *P. aeruginosa* isolates were resistant to carbapenems and this had increased to 17% by 2010. This change was statistically significant.[23] Slekovec et al looked at *P. aeruginosa* susceptibility trends in six French hospitals over 2001 to 2011. The majority of isolates were from BSIs. The proportion of carbapenem resistant isolates significantly increased over this time to over 25% of the isolates. The proportion of MDR isolates was 20% in 2001 and had a nonsignificant decrease over the time period studied.[24] Turning to the United States, Eurofin's surveillance network database collects routine clinical microbiology data, from a nationally representative sample of microbiology laboratories in 217 hospitals.[25] Laboratories are included based on their geography and the demographics of the population they serve. Zilberberg and Shorr examined the data on *P. aeruginosa* isolates originating from pneumonia or BSI infections from 2000 to 2009. They found a net small rise in MDR *P. aeruginosa* from 10.7% in 2000 to 13.5% in 2009 among BSI isolates and from 19.2% in 2000 to 21.7% in 2009 among pneumonia specimens. For BSI isolates, the likelihood of a MDR isolate originating from the ICU was double that from a non-ICU location.[25] The USA Healthcare Safety Network found that in 2009 to 2010, 26.1% of catheter-associated BSIs and 30.2% of isolates from VAP were MDR.[26] In the data discussed there is evidence of geographical, hospital-specific, and intrahospital variation.

Risk Factors for Infection with a Carbapenem-Resistant or Multidrug-Resistant Organism

Studies utilizing a case–control methodology to look at independent risk factors for carbapenem resistance have found prior antibiotic use, both carbapenem and noncarbapenem antibiotics, to be significant.[27–29] The lack of

consistent identification of particular antibiotics between studies is most likely due to the heterogeneity of the patients being studied, varying antibiotic usage patterns, the type of study being performed, and the varying sample sizes in the studies. In the *P. aeruginosa* BSI literature, other identified independent risk factors for carbapenem resistance have included transfer from another facility, longer duration of hospital stay, ICU admission, arterial catheter administration, and higher number of total white blood cells on the day of diagnosis.[27,28,30–32] Johnson et al published a large retrospective cohort study on *P. aeruginosa* BSI and focused on MDR. They found previous transplantation, hospital acquired BSI, and prior ICU admission, all independent risk factors for MDR isolates.[13] A large prospective observational cohort study by Morata et al found that the independent risk factors for a MDR *P. aeruginosa* BSI were bladder catheterization and prior steroid or antibiotic therapy use.[15] The American Thoracic Society lists several risk factors for MDR organisms (MDRO) in their guidelines for the management of ventilator, hospital, or health care acquired pneumonia.[33] As expected, some are mirrored in the risk factors already mentioned for BSI. Xie et al found that in their setting, utilization of these risk factors only had a positive predictive value of 38.2% and a negative predictive value of 63.5%.[34] This makes the point that it is important to have epidemiological knowledge relevant to the patient population in which you practice.

Outcome in the Setting of Antimicrobial Resistance

The complexity and the severity of illness in the patient who develops either a BSI or VAP make teasing out the factors affecting mortality difficult. In addition, the diagnosis of VAP is difficult to establish with complete certainty as there is no pathognomonic finding. The effect of *P. aeruginosa* antibiotic resistance on outcome in the setting of BSI has been variable results. A higher 30-day mortality rate in the setting of MDR *P. aeruginosa* bacteremia was seen in the observational studies by Morata et al (32.3%), Lautenbach et al (17.4%), and Tam et al (40%).[15,28,35] However, no significant difference in mortality was seen in *P. aeruginosa* BSI caused by either carbapenem resistant or MDR *P. aeruginosa* in four further studies.[13,31,36,37] Specifically in the MDR *P. aeruginosa* BSI, both lower Charleston morbidity index and a high-risk source of the bacteremia (respiratory tract, intra-abdominal, soft tissue) have been found to be independent risk factors for mortality.[30,37] Esterly et al stratified carbapenem minimum inhibitory concentration (MIC) for *P. aeruginosa* BSI isolates and looked at mortality. They showed increasing MIC was an independent prediction of mortality.[38] Peña et al in a prospective multicenter observational study also found that the impact of resistance on mortality was statistically significant. This was from the 5th day after the onset of bacteremia and reached peak values at day 30.[30] Suárez et al also showed a time-dependent effect on mortality with a higher mortality in the setting of carbapenem resistance evident from 4 days after the onset of bacteremia.[14] The observed effect of antimicrobial resistance, in the setting of a BSI, on the length of hospital stay is also mixed.[13,15,16,28,36] If we turn to VAP, contrasting findings regarding the effect of antibiotic resistance on mortality in *P. aeruginosa* VAP have been observed.[16–18,39,40] This is also seen when recurrence of VAP has been grouped with mortality as an outcome measure.[16,18,40] Both Tumbarello et al and Parker et al found that MDR was associated with longer median duration of mechanical ventilation.[17,39]

Mechanisms of Carbapenem Resistance

Pseudomonas aeruginosa can develop carbapenem resistance by several mechanisms both mutational and acquired. They include alteration in porins, enzyme production, and efflux systems. It is also possible that modification of penicillin-binding profiles may also play a role.[41] There is differing selectivity for the carbapenem affected by these mechanisms and more than one mechanism may be required for carbapenem resistance to manifest. Although porin changes are the most common cause of carbapenem resistance in *P. aeruginosa*, the recent predominance of successful clones carrying enzymatic resistance, particularly Verona integron-encoded metallo-beta-lactamase (VIM)-2, is of global concern.

Porin Changes

The main route of carbapenem entry into gram-negative bacteria is via the OprD porin which functions like a channel. Mutational loss of the *OprD* gene and hence loss of this porin is described as the most common cause of imipenem resistance in *P. aeruginosa*.[42] Combination of this porin change with overexpression of AmpC, an Ambler class C β-lactamase, or efflux pump overexpression is required for meropenem or doripenem resistance to manifest.[43] The exact role of the OprD porin and carbapenem resistance is still unfolding.[44]

Efflux Pumps

The terminology of the commonly observed efflux pump is a compound of designations for the pump, the linker lipoprotein, and the exit portal. The pumps can reduce drug accumulation in the bacteria. There are five superfamilies of pumps with the resistance-nodulation-division (RND) family being the most common. Several of the pumps share common substrates. For example, when the MexAB-OprM system is upregulated, this may cause reduction in meropenem susceptibility in addition to fluoroquinolone, penicillin, and cephalosporin susceptibility. Upregulation of other efflux pump systems, for example, MexCD-OprJ and MexEF-OprN confers resistance to fluoroquinolones and some β-lactams, whereas upregulation of MexXY-OprM reduces susceptibility to aminoglycosides and fluoroquinolones.[44,45] Efflux pump upregulation can therefore be a potential cause of MDR in a *P. aeruginosa* isolate.

Metallo-Beta-Lactamases

Metallo-beta-lactamases (MBL) are the predominant group of carbapenemases found in *P. aeruginosa*. They confer resistance to all of the β lactams except aztreonam and require zinc ions for their activity. These genes are normally encoded in class 1 integrons along with other resistance determinants.

Association of the integron with a plasmid or a transposon allows transfer between bacteria. IMP and VIM types are the most common MBLs found in *P. aeruginosa*. IMP-1 was the first mobile *P. aeruginosa* MBL discovered in Japan in 1991.[46] Most of the IMP types have a defined geographical distribution apart from IMP-1 and IMP-7.[47] As variants are found, they are numbered sequentially. Recently, IMP-43 and IMP-44 MBLs have been described in two MDR *P. aeruginosa* isolates in Japan.[48] VIM was first described in 1997.[49] Of the 41 VIM types now described, many have been found in *P. aeruginosa* isolates (www.lahey.org/Studies). Again there is generally a defined geographical distribution of the allotypes but VIM-1 and VIM-2 have seen globally with a predominance of VIM-2.[47] Other types of MBLs in *P. aeruginosa* have been described, SPM-1, GIM-1, AIM-1, and FIM-1 but are not as common as the IMP and VIM types.[50–52] NDM-1 was first reported in 2009 by Yong et al in a *Klebsiella pneumoniae* isolate from India.[53] Subsequently, global dissemination in Enterobacteriaceae has been documented which is of major concern.[54] Emergence in *P. aeruginosa* was subsequently documented by Jovcic et al in Serbia in 2011.[55] A new zinc-dependent intrinsic imipenemase, *P. aeruginosa* 5542, dubed PIB-1 France, has just been identified by Fajardo et al.[56]

Other Beta-Lactamases

The β-lactamase enzymes are divided into four functional groups A to D. Much less common enzymatic causes of resistance in *P. aeruginosa* are type A or D β-lactamases. The class A serine carbapenemases described in this bacterium are either GES or KPC types. They are plasmid encoded enzymes and thus potentially transferrable. Not all GES types manifest carbapenemase activity in contrast to KPC types. A second mechanism of carbapenem resistance, such as membrane impermeability, must be present for imipenem resistance to be manifested in the setting of the GES 2 enzyme. GES enzymes have been described worldwide, as with KPC enzymes, but are less prolific. KPC enzymes are more typically described in Enterobacteriaceae but have recently also been described in *P. aeruginosa*.[57] Martínez et al recently published a case report of a *P. aeruginosa* isolate coharboring KPC-2 and an IMP-18 carbapenemase.[58] Oxacillinases or class D enzymes are common in *P. aeruginosa* but their spectrum is such that carbapenems are not normally a substrate. Only two OXA enzymes, OXA 40 and 198, have been described in isolated reports to express carbapenemase activity.[59,60]

Molecular Epidemiology

Pseudomonas aeruginosa has been shown in general to have a nonclonal population structure. Recombination plays a large part in the diversity of the bacterial isolates. When a range of clinical and wider environmental isolates were studied, it was shown that clinical *P. aeruginosa* isolates are genotypically indistinguishable from environmental isolates. Isolates from the same clone can be found from different habitats separated by large geographical distances.[61–64] In this context, a multiclonal outbreak of *P. aeruginosa* BSI has been described in the literature.[65] There is, however, the punctuation by highly successful epidemic clones or clonal complexes. This has been traditionally described in the CF patient population where it is not associated with MBLs.[66] The successful clones seem to be geographically isolated and have been attributed to random recombination and subsequent local transmission.[61,64] In the non-CF setting, early studies regarding carbapenem resistance showed no evidence of clonality.[67] Since then the published literature on antibiotic resistant isolates has shown that the majority of isolates belong to a few successful clones such as ST111, ST175, or ST235. This has been seen worldwide.[68–72] It is felt to reflect the ability of these particular clones to acquire genetic resistance mechanisms. However, the study by Edelstein et al now provides longitudinal data showing evidence of cross-transmission of these clones, thus contributing to the spread of resistance.[72]

Treatment: Bloodstream Infection

Empirical Therapy

Since 1958, when colistin first became available, the range of antibiotics against *P. aeruginosa* has increased. We have also developed a better understanding of how to use such drugs. Gentamicin monotherapy for *P. aeruginosa* BSI, for example, is no longer considered adequate.[73] There are many treatment studies published regarding empirical therapy in the setting of *P. aeruginosa* BSI. Comparison of these observational studies is inherently difficult due to variance in factors such as patient populations, study methodology used, critical definitions, antibiotic resistance rates, and time to appropriate therapy. Morata et al on analysis of their prospective single-center cohort of *P. aeruginosa* BSI were able to show that inappropriate empirical therapy itself was an independent risk factor for 30-day mortality, regardless of the susceptibility pattern.[15] Appropriate therapy being defined as utilization of at least one effective antimicrobial based on in vitro susceptibilities. However, conflicting results have been obtained regarding appropriate empirical therapy and mortality benefit.[7,15,74–77] A recent study by Schechner et al focused on mortality in *P. aeruginosa* BSI. They looked at patients who were found to have a BSI within 72 hours of hospital admission. The majority of infections were healthcare associated. They did not find that inadequate empirical antimicrobial therapy was a risk factor for mortality on multivariate analysis. On subgroup analysis with sepsis severity at presentation stratified, in the severe sepsis group, inadequate empirical therapy had a relative risk of mortality of 1.8 (confidence interval 1.1–2.8, $p = 0.051$). This was not statistically significant.[12] Recent studies have focused on empirical treatment in the setting of antimicrobial resistance. MDR has been shown to be associated with a patient being more likely to receive inappropriate empirical antibiotic therapy. Identification of the patient groups who would benefit most from appropriate empirical therapy is still to occur.[14,15,35,78]

Definitive Therapy

Moving on to the comparisons of definitive therapy in the literature, despite the limitations described earlier, a

mortality benefit of definitive treatment with an effective antimicrobial has been shown more consistently.[36,74,79,80] Vidal et al found that inappropriate definitive therapy became an independent risk factor for death, only when the subset of patients with intravenous catheter-associated bacteremia, were excluded from the analysis. They hypothesized that this was due to a removable focus making the benefit of antibiotic therapy less easy to show.[81] The concept of using the minimum inhibitory concentration of a β-lactam antibiotic for a bacterial isolate to help guide dosing of that antibiotic has been increasingly explored. This is the minimum concentration of antibiotic that is required to inhibit the growth of the organism in vitro. We would expect appropriate antibiotic dosing to achieve antibiotic concentrations above the MIC for the sensitive bacterium. The role of achieving antibiotic concentrations above the MIC for longer time periods by dosed bolus administration or an infusion is a subject of interest. Lodise et al published one of the earliest studies utilizing piperacillin–tazobactam infusions for *P. aeruginosa* infection. The main source of infection in the patients studied was the respiratory tract. They aimed to obtain a free drug concentration exceeding the MIC for 50% of the dosing interval for the range of MICs in a susceptible *P. aeruginosa* isolate. By Monte Carlo simulation, this was found to be best achieved by a 4-hour infusion of 3.375 g every 8 hours. A total of 194 patients were included in the study and they found that in patients with an Acute Physiological and Chronic Health Evaluation Score of greater than 17, 14-day mortality was significantly lower. They also found that there were significant benefits on length of hospital stay defined from the time of sample collection.[82] Dulhunty et al focused on the ICU setting and conducted a prospective multicenter double-blind concealed randomized controlled trial in patients with severe sepsis. Sixty patients were enrolled and the study directly compared the effects of continuous and intermittent administration of β-lactam antibiotics. They found that continuous administration achieved significant pharmacokinetic (PK) separation in time above the MIC and a higher rate of clinical cure. The study was not powered to look at survival.[83] Further studies are planned. Taccone et al presented a case report of an XDR *P. aeruginosa* BSI causing septic shock. In the setting of drug resistance, they administered meropenem to achieve levels four times above the MIC of the isolate for 40% of the dosing interval. The patient recovered.[84] Further studies in this area are required.

Definitive Therapy in the Setting of Antibiotic Resistance

In the setting of MDR, there are reduced antibiotic options which have led to review of older drugs such as the polymyxins and fosfomycin. These drugs would be generally used in this setting as part of an antibiotic combination. The polymyxins, colistin, and polymyxin B were initially discarded due to neuro- and nephrotoxicity.[85] Falagas et al looked at a single-center cohort of MDR gram-negative infections treated with colistin. Twelve percent of the infections were BSIs and 26.4% of the infections were due to *P. aeruginosa*. Of the 12 *P. aeruginosa* infections that received monotherapy, 9 were cured.[86] Comment cannot be made on the combination regimens containing colistin as the definition of MDR meant that prescribed therapy may have consisted of two active agents. Paul et al subsequently conducted a large single-center prospective study of all patients with a MDR gram-negative bacterial infection. This study in comparison had a higher rate of bacteremias and only utilised monotherapy with a comparator arm. Only infections caused by isolates susceptible to colistin, carbapenems, or ampicillin–sulbactam were included. The treating physician chose the antibiotic regimen and in analysis the β-lactam therapies were used as a control group. The 30-day mortality rate was 39% in the colistin group versus 28.8% in the comparator groups. This was statistically significant. On subgroup analysis of BSIs, colistin therapy was independently associated with the 30-day mortality, but not in the analysis of the overall cohort. It is important to note that the patients treated with colistin had several poorer prognostic features as compared with those treated with alternative antibiotics. Colistin was an independent risk factor for renal failure.[87] Park et al conducted a retrospective cohort study, at a single center, and focused on the treatment of monomicrobial *P. aeruginosa* or *Acinetobacter baumannii* bacteremia. Eighty-one of the 149 episodes studied were due to *P. aeruginosa*. Very few patients in the study received combination therapy. Seventy-three patients received appropriate empirical therapy and nine patients in total received colistin. Seven of these nine patients were dead at day 14. On multivariate analysis, adequate colistin therapy was a risk factor for mortality.[88] Unfortunately, the nonrandomized nature of the studies described does not allow for adequate control of confounding. Randomized controlled trials are required to look at colistin's efficacy and safety to guide further useage.

Fosfomycin was first available for use in 1973 and now has been revisited particularly in the setting of XDR or PDR gram-negative bacteria. Geographical variation in fosfomycin resistance has been described. Falagas et al on review of studies looking at resistance rates found a range from 50 to greater than 90% susceptibility.[89] Limited literature regarding the drug in the treatment of BSIs is published. Dinh et al prospectively followed a cohort of patients treated with intravenous fosfomycin. Of these, nine patients with a bacteremia were reported. Of these, two were caused by *P. aeruginosa*. The majority had a concomitant antibiotic. Four patients had a favorable outcome, three unfavorable, and two insufficient follow-up.[90] Pontikis et al in a multicenter ICU study prospectively followed all fosfomycin treated patients who were XDR or PDR and fosfomycin susceptible. Combination therapy with colistin was used. Of the 18 primary bacteremias, 11 were successfully treated. In the entire cohort, fosfomycin resistance developed in three cases. The main adverse event was reversible hypokalemia.[91] Colistin has attractive PK characteristics, with good distribution into many tissue and body fluids including cerebrospinal fluid and lung tissue.[92] The development of resistance to fosfomycin during therapy is one of the main concerns regarding utilization of this drug in the setting of MDR.

Combination Therapy

Combination therapy was first looked at in the setting of gram-negative bacterial studies where better overall outcomes were shown.[93,94] It is difficult to extrapolate these studies to practice today as there were small numbers of *P. aeruginosa* infections in the cohorts. The antibiotics used also had less intrinsic activity against *P. aeruginosa* than the agents we use today. In latter studies focusing on *P. aeruginosa* BSI, antibiotic combinations normally consisted of a β-lactam with an aminoglycoside or fluoroquinolone. Observational studies looking at definitive therapy have failed to find a benefit of combination therapy over monotherapy.[74,79–81,95,96] The heterogeneity of these studies has previously been discussed. Contrasting results regarding the benefit of such therapy in the empirical setting have been seen.[13,80] Three studies in 2013 were published on this topic. Bowers et al looked at empiric combination therapy versus monotherapy for *P. aeruginosa* bacteremia. They performed an international multicenter retrospective cohort study of 384 patients. They did not find on multivariate analysis that combination therapy was significantly different to monotherapy in regard to the end points studied.[97] Peña et al looked at both empirical and definitive therapy and performed a post hoc analysis of prospectively collected data. A total of 593 patients were analyzed. They found no significant difference with outcome with the utilization of single or combination drug therapy.[98] The lack of benefit of combination therapy was again reflected in Hu et al's meta-analysis of studies on this topic.[99]

Why has research continued in this area? There are many postulated benefits of combination therapy. Micek et al found that appropriate initial antimicrobial therapy was administered significantly more often among patients receiving combination therapy than monotherapy. This was in the setting of limited antibiotic resistance.[75] MDR has also been shown to be associated with a patient being more likely to receive inappropriate empirical antibiotic therapy.[14,15,35,78] It is postulated that combination therapy would reduce this occurrence. Drug synergy is another postulated benefit. Studies utilizing checkerboard testing, time-kill assays, or E tests among various antibiotics have shown synergy.[100,101] As surmised by Traugott et al, clinical correlation of this testing is not clear.[102] Siqueira et al looked at morphological changes on a MDR *P. aeruginosa* clinical isolate and found summation of the effects in the setting of meropenem and ciprofloxacin combination therapy.[103] Combination therapy has also been found to be synergistic in pharmacodynamics (PD) models in regard to *P. aeruginosa* kill rate.[104] Prevention of the development of resistance is another possible benefit. In the mouse model this has been shown with the combination of tobramycin and cefepime, and meropenem and levofloxacin.[104,105] The utilization of such therapy must be weighed up against increased expense in comparison to monotherapy, the potential for additional or additive drug toxicity and the risk of acquiring another MDRO. The clinical question remains if there is a target population that we have not yet defined in clinical studies that would benefit from combination therapy. There were two studies published in 2010 by Kumar et al which suggest this might be the case. The first study was a retrospective cohort study and then the second a meta-analysis that showed an independent survival benefit in the setting of combination antibiotic therapy for bacterial infection causing septic shock.[106,107] Further studies are required. The literature behind the utilization of colistin or fosfomycin in an infection that has limited antibiotic treatment options has already been discussed. Other agents that are often considered are carbapenems, aminoglycosides, and rifampicin. The evidence for utilization of additional agents is mainly based on preclinical studies, case reports, or limited numbers of cases in a wider cohort. Paul et al performed a comprehensive review of colistin monotherapy versus combination therapy in the setting of carbapenem-resistant gram-negative bacteria. They did not find any benefit on all-cause mortality of the utilization of combination therapy.[108] Further research to guide management in this setting is urgently required.

Treatment: Ventilator-Associated Pneumonia

Empirical Therapy

Tumbarello et al and Garnacho-Montero et al have published two of the more recent studies in the literature focusing on empirical therapy in *P. aeruginosa* VAP. Tumbarello et al conducted a retrospective analysis of prospectively collected data on 110 patients in the general ICU of a single Italian hospital over a 2-year period. Seventy-one of the cases were VAP. Thirty percent of the strains were meropenem resistant and 38% MDR. In logistic regression analysis, infection caused by MDR *P. aeruginosa* isolates was independently associated with inadequate empirical therapy. The use of combination therapy in the empiric phase was independently associated with a lower risk of inadequate empirical therapy. Inappropriate empirical therapy was independently associated with mortality. Survivors who received inappropriate empirical therapy also had significantly longer median periods of post–pneumonia onset mechanical ventilation.[17] Garnacho-Montero et al performed a retrospective multicenter observational cohort study in five ICUs in Spanish hospitals. One hundred and eighty-three episodes of monomicrobial *P. aeruginosa* pneumonia were analyzed. Inappropriate empirical therapy was independently associated with mortality. They were unable to assess whether one antibiotic regimen was superior to another. They found that the rate of appropriate initial antimicrobial treatment was significantly higher in patients with combination therapy than in monotherapy. Resistance rates of the isolates were not provided. They also found a trend toward greater in-hospital mortality in those patients receiving monotherapy in the prescribed empirical therapy regimen compared with those patients receiving combination therapy.[19] It would have been interesting to see this comparison within the group of patients with appropriate therapy and the outcome of mono and combination therapy as opposed to all patients in the study. Peña et al focused on the impact of MDR on *P. aeruginosa* VAP. In their retrospective cohort from a tertiary care hospital, 60 of the 91 episodes

were caused by MDR or XDR strains. On logistic regression, they identified inadequate empirical antibiotic therapy as a risk factor for mortality.[16] These studies in combination suggest the need for the utilization of antibiograms in the individual ICU and the use of combination therapy where there are higher rates of resistance to the mono-therapy planned. Interestingly, not all studies even agree with the importance of empirical therapy including a recent study by Planquette et al and a meta-analysis by Aarts et al looking at all causes of VAP.[18,109] This reflects the heterogeneity of the population we are studying, varying antibiotic resistance rates, varying clinical and differing study characteristics.

Definitive Therapy

Kollef et al particularly looked at the role of de-escalation of broad spectrum antibiotic therapy once the antibiogram had been received in VAP. They studied 20 ICUs throughout the United States in a prospective fashion. They found that de-escalation of therapy was associated with a reduction in mortality on univariate analysis.[110] It is important to note that the differences in patients who did or did not have their antibiotic therapy de-escalated were not looked at. Hence, it may not be the de-escalation of therapy itself that was the cause of the better outcome in this group. Garnacho-Montero et al in their previously mentioned study on *P. aeruginosa* pneumonia did not find a significant difference in mortality rates between utilization of single agent appropriate definitive therapy and appropriate definitive combination therapy. Median duration of antimicrobial treatment was 14 days (IQR 10–19.5 days). In this study, survivor's length of ICU days and hospital stays were again similar between the two treatment groups as were rates of recurrent VAP. The development of resistance was documented in only three cases and in two of these cases in the setting of combination therapies.[19] Thus the routine use of combination definitive therapy is not currently supported by the literature.

However, there may be more to this story. Louie et al in a neutropenic murine model of *P. aeruginosa* pneumonia looked at a range of dosing combinations for meropenem and tobramycin. They found that the interaction of the two drugs was additive and suppressed all resistance amplification.[111]

Optimization of antimicrobial therapy according to PK and PD principles has been demonstrated to significantly improve clinical and microbiological outcomes in ICU patients. However, achieving such optimization in the individual severely ill patient can be difficult.[112] There are few studies that have directly compared antibiotic regimens in *P. aeruginosa* VAP. Luyt et al compared doripenem, meropenem, and imipenem in a prospective fashion to treat *P. aeruginosa* VAP. The doripenem MICs of the isolates were lower than the other carbapenems studied. However, resistance rates to the antibiotics were similar. There was no significant difference in mortality rates or VAP recurrence rates between the drugs. No carbapenem was superior to another for preventing carbapenem resistance emergence.[40] Kollef et al looked at the length of antibiotic therapy in all cause VAP, in a double-blinded randomized controlled trial. They compared 7 days of doripenem therapy with 10 days of imipenem–cilastatin therapy. In the modified intention to treat analysis for *P. aeruginosa* VAP, the 28-day mortality was significantly higher in the shorter length doripenem arm.[113] Comparison of 8 versus 15 days of clinician chosen antibiotic therapy of all-cause VAP was compared by Chastre et al. In the subgroup of patients with nonfermenting gram-negative bacteria, including *P. aeruginosa*, a shorter course of antibiotic therapy resulted in a higher VAP recurrence rates although other outcome measures did not differ.[114] This suggests that shorter courses are not appropriate for *P. aeruginosa* VAP. Can the antibiotic therapy be tailored to the patient? In the Cochrane review regarding length of therapy in HAP, Pugh et al concluded that of the small number of studies available that looked at discontinuation strategies, the combination of clinical features and a procalcitonin level could possibly be used to guide length of therapy at the bedside.[115]

Treatment in the Setting of Antimicrobial Resistance

Intravenous colistin has so far been discussed in the context of BSI treatment. Florescu et al recently performed a systematic review and metaregression analysis regarding the utilization of colistin for VAP. The study looked at both nebulized and intravenous administration and the organisms treated included but were not isolated to *P. aeruginosa*. Six dual arm studies were identified, in four the route of administration was intravenous and in two inhaled. The clinical response, hospital mortality, and length of ICU stay did not differ significantly between colistin and control groups. Nephrotoxicity and neurotoxicity did not differ significantly between the arms. In the meta-analysis of the single arm studies, favorable results were shown and a low rate of nephrotoxicity.[116] Rigatto et al performed a prospective cohort study on the comparison of polymyxin B with other antimicrobials in the treatment of VAP and tracheobronchitis caused by *P. aeruginosa* or *A. baumannii*. On multivariate analysis, the use of polymyxin B and the development of renal failure during therapy were independently associated with the 30-day mortality.[117] It is clear that we need a better understanding of how to use the polymyxins and in whom. The role of combination therapy in this setting has not been addressed in the literature.

Inhalational Therapy

Local delivery of antibiotics to the respiratory tract is a subject of ongoing investigation. Theoretical benefits of local delivery include increased antibiotic concentration at the site of infection and low systemic absorption leading to decreased adverse effects and superinfection. However, few antibiotics are specially formulated for nebulized administration. Lu et al performed a randomized controlled trial on 40 patients with VAP caused by *P. aeruginosa*. The comparison was nebulized ceftazidime and amikacin as opposed to the intravenous combination of these drugs. After 8 days of antibiotic administration, there were similar outcomes between the groups. There was rapid and early reduction in bacterial growth in the nebulized antibiotic arm. Three adverse events related to

obstruction of the respiratory filter were reported. It is important to note 13 patients had to be excluded from the study due to positive blood cultures for *P. aeruginosa*.[118] Larger trials are required to study this further. BAY41–6551 is a new drug–device combination that has been trialed in the setting of VAP. It consists of amikacin specially formulated for inhaled administration via a proprietary gasless vibrating mesh nebulizer which integrates with standard mechanical ventilation equipment. The aerosol contains a high proportion of fine particles, 3 to 5 μm in diameter, which is optimal for delivery to the distal airways. A dose finding study showed that it was well tolerated.[110] Arikace is a novel formulation of inhaled liposomal amikacin that has the ability to penetrate biofilms. The formulation also permits sustained release of the drug.[119] This has been studied in patients with CF but not in VAP so far.

Aerosolized colistin has been looked at in the setting of MDR *P. aeruginosa*. When aerosolized, the drug achieves high concentrations in the respiratory tract avoiding systemic effects.[120] Observational studies looking at the utilization of inhaled colistin, usually in the context of MDR gram-negative bacteria, have had small numbers of *P. aeruginosa* in the cohort and have found conflicting results.[121–124] Lu et al performed a prospective study in which they compared 122 patients with *P. aeruginosa* or *A. baumannii* VAP. The MDR group was treated with nebulized colistimethate (prodrug of colistin) as monotherapy or with 3 days of intravenous aminoglycosides. The control group who all had susceptible isolates received an intravenous β-lactam antibiotic and this was combined with either an aminoglycoside or fluoroquinolone for the first 3 days. There was no difference in cure rates between the groups, mortality or recurrence of infection. However, the duration of ventilation after inclusion was longer in patients of the MDR group. Renal impairment was observed in 12% of the nebulization group.[125] Rattanaumpawan et al performed a randomized controlled trial of nebulized colistimethate as adjunctive therapy for VAP caused by gram-negative bacteria. *Pseudomonas aeruginosa* made up 39% of the isolates. Forty-five percent of the *A. baumannii* and 5% of the *P. aeruginosa* isolates were resistant to carbapenems, aminoglycosides, and fluoroquinolones. All patients received systemic antibiotics according to clinician decision and then were randomized to receive nebulized colistimethate or normal saline. There was no statistically significant difference in outcome between the two groups. The group that received inhaled colistimethate had significantly higher rates of bacterial eradication. There was no significant difference in renal impairment or bronchospasm between the two groups.[126] Thus, currently evidence is lacking to support the routine use of nebulized antibiotics for VAP.

Conclusion

Pseudomonas aeruginosa BSI and VAP occurs in the sick host and cause high rates of mortality. Drug-resistant isolates are an everyday reality for many hospitals and we now have evidence of the ability of certain resistant clones to spread by cross-transmission. We are attempting to treat resistant infections from a small evidence base and with the threat of limited new drugs on the horizon. Further studies looking at how we can better use our existing antibiotic armamentarium are required and the quest for new antibiotics needs to be fully supported.

Disclaimer

The views expressed in this article are the author's and not an official position of the institutions listed.

References

1 Magill SS, Edwards JR, Bamberg W, et al; Emerging Infections Program Healthcare-Associated Infections and Antimicrobial Use Prevalence Survey Team. Multistate point-prevalence survey of health care-associated infections. N Engl J Med 2014;370(13): 1198–1208

2 Zarb P, Coignard B, Griskeviciene J, et al; National Contact Points for the ECDC pilot point prevalence survey; Hospital Contact Points for the ECDC pilot point prevalence survey. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro Surveill 2012;17(46):20316

3 Kollef MH, Chastre J, Fagon JY, et al. Global prospective epidemiologic and surveillance study of ventilator-associated pneumonia due to Pseudomonas aeruginosa*. Crit Care Med 2014;42(10): 2178–2187

4 Al-Hasan MN, Wilson JW, Lahr BD, Eckel-Passow JE, Baddour LM. Incidence of Pseudomonas aeruginosa bacteremia: a population-based study. Am J Med 2008;121(8):702–708

5 Cheong HS, Kang CI, Wi YM, et al. Clinical significance and predictors of community-onset Pseudomonas aeruginosa bacteremia. Am J Med 2008;121(8):709–714

6 Marra AR, Bar K, Bearman GM, Wenzel RP, Edmond MB. Systemic inflammatory response syndrome in adult patients with nosocomial bloodstream infection due to Pseudomonas aeruginosa. J Infect 2006;53(1):30–35

7 Kang C-I, Kim S-H, Kim H-B, et al. Pseudomonas aeruginosa bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. Clin Infect Dis 2003;37(6):745–751

8 Scheetz MH, Hoffman M, Bolon MK, et al. Morbidity associated with Pseudomonas aeruginosa bloodstream infections. Diagn Microbiol Infect Dis 2009;64(3):311–319

9 Joo EJ, Kang CI, Ha YE, et al. Risk factors for mortality in patients with Pseudomonas aeruginosa bacteremia: clinical impact of antimicrobial resistance on outcome. Microb Drug Resist 2011; 17(2):305–312

10 Joo EJ, Kang CI, Ha YE, et al. Clinical predictors of *Pseudomonas aeruginosa* bacteremia among Gram-negative bacterial infections in non-neutropenic patients with solid tumor. J Infect 2011;63(3):207–214

11 Chen SCLR, Lawrence RH, Byth K, Sorrell TC. *Pseudomonas aeruginosa* bacteraemia. Is pancreatobiliary disease a risk factor? Med J Aust 1993;159(9):592–597

12 Schechner V, Gottesman T, Schwartz O, et al. Pseudomonas aeruginosa bacteremia upon hospital admission: risk factors for mortality and influence of inadequate empirical antimicrobial therapy. Diagn Microbiol Infect Dis 2011;71(1):38–45

13 Johnson LE, D'Agata EM, Paterson DL, et al. Pseudomonas aeruginosa bacteremia over a 10-year period: multidrug resistance and outcomes in transplant recipients. Transpl Infect Dis 2009; 11(3):227–234

14 Suárez C, Peña C, Gavaldà L, et al. Influence of carbapenem resistance on mortality and the dynamics of mortality in Pseudomonas aeruginosa bloodstream infection. Int J Infect Dis 2010; 14(Suppl 3):e73–e78

15 Morata L, Cobos-Trigueros N, Martínez JA, et al. Influence of multidrug resistance and appropriate empirical therapy on the 30-day mortality rate of Pseudomonas aeruginosa bacteremia. Antimicrob Agents Chemother 2012;56(9):4833–4837

16 Peña C, Gómez-Zorrilla S, Oriol I, et al. Impact of multidrug resistance on Pseudomonas aeruginosa ventilator-associated pneumonia outcome: predictors of early and crude mortality. Eur J Clin Microbiol Infect Dis 2013;32(3):413–420

17 Tumbarello M, De Pascale G, Trecarichi EM, et al. Clinical outcomes of Pseudomonas aeruginosa pneumonia in intensive care unit patients. Intensive Care Med 2013;39(4):682–692

18 Planquette B, Timsit JF, Misset BY, et al; OUTCOMEREA Study Group. Pseudomonas aeruginosa ventilator-associated pneumonia. predictive factors of treatment failure. Am J Respir Crit Care Med 2013;188(1):69–76

19 Garnacho-Montero J, Sa-Borges M, Sole-Violan J, et al. Optimal management therapy for Pseudomonas aeruginosa ventilator-associated pneumonia: an observational, multicenter study comparing monotherapy with combination antibiotic therapy. Crit Care Med 2007;35(8):1888–1895

20 Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18(3): 268–281

21 Institute CLS. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement. In: Document M100–S22. Wayne, PA; 2012

22 EUCAST.. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 3.1; 2013

23 Croughs PD, Li B, Hoogkamp-Korstanje JA, Stobberingh E. Antibiotic Resistance Surveillance Group. Thirteen years of antibiotic susceptibility surveillance of Pseudomonas aeruginosa from intensive care units and urology services in the Netherlands. Eur J Clin Microbiol Infect Dis 2013;32(2):283–288

24 Slekovec C, Robert J, Trystram D, et al; ONERBA. Pseudomonas aeruginosa in French hospitals between 2001 and 2011: back to susceptibility. Eur J Clin Microbiol Infect Dis 2014;33(10): 1713–1717

25 Zilberberg MD, Shorr AF. Prevalence of multidrug-resistant Pseudomonas aeruginosa and carbapenem-resistant Enterobacteriaceae among specimens from hospitalized patients with pneumonia and bloodstream infections in the United States from 2000 to 2009. J Hosp Med 2013;8(10):559–563

26 Sievert DM, Ricks P, Edwards JR, et al; National Healthcare Safety Network (NHSN) Team and Participating NHSN Facilities. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infect Control Hosp Epidemiol 2013; 34(1):1–14

27 Onguru P, Erbay A, Bodur H, et al. Imipenem-resistant Pseudomonas aeruginosa: risk factors for nosocomial infections. J Korean Med Sci 2008;23(6):982–987

28 Lautenbach E, Synnestvedt M, Weiner MG, et al. Imipenem resistance in Pseudomonas aeruginosa: emergence, epidemiology, and impact on clinical and economic outcomes. Infect Control Hosp Epidemiol 2010;31(1):47–53

29 Nakamura A, Miyake K, Misawa S, et al. Meropenem as predictive risk factor for isolation of multidrug-resistant Pseudomonas aeruginosa. J Hosp Infect 2013;83(2):153–155

30 Peña C, Suarez C, Gozalo M, et al; Spanish Network for Research in Infectious Diseases REIPI. Prospective multicenter study of the impact of carbapenem resistance on mortality in Pseudomonas aeruginosa bloodstream infections. Antimicrob Agents Chemother 2012;56(3):1265–1272

31 Tuon FF, Gortz LW, Rocha JL. Risk factors for pan-resistant Pseudomonas aeruginosa bacteremia and the adequacy of antibiotic therapy. Braz J Infect Dis 2012;16(4):351–356

32 Harris AD, Smith D, Johnson JA, Bradham DD, Roghmann MC. Risk factors for imipenem-resistant Pseudomonas aeruginosa among hospitalized patients. Clin Infect Dis 2002;34(3): 340–345

33 American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171(4):388–416

34 Xie J, Ma X, Huang Y, et al. Value of American Thoracic Society guidelines in predicting infection or colonization with multidrug-resistant organisms in critically ill patients. PLoS ONE 2014;9(3): e89687

35 Tam VH, Rogers CA, Chang KT, Weston JS, Caeiro JP, Garey KW. Impact of multidrug-resistant Pseudomonas aeruginosa bacteremia on patient outcomes. Antimicrob Agents Chemother 2010; 54(9):3717–3722

36 Willmann M, Kuebart I, Marschal M, et al. Effect of metallo-β-lactamase production and multidrug resistance on clinical outcomes in patients with Pseudomonas aeruginosa bloodstream infection: a retrospective cohort study. BMC Infect Dis 2013; 13:515

37 Suárez C, Peña C, Tubau F, et al. Clinical impact of imipenem-resistant Pseudomonas aeruginosa bloodstream infections. J Infect 2009;58(4):285–290

38 Esterly JS, Wagner J, McLaughlin MM, Postelnick MJ, Qi C, Scheetz MH. Evaluation of clinical outcomes in patients with bloodstream infections due to Gram-negative bacteria according to carbapenem MIC stratification. Antimicrob Agents Chemother 2012; 56(9):4885–4890

39 Parker CM, Kutsogiannis J, Muscedere J, et al; Canadian Critical Care Trials Group. Ventilator-associated pneumonia caused by multidrug-resistant organisms or Pseudomonas aeruginosa: prevalence, incidence, risk factors, and outcomes. J Crit Care 2008;23(1):18–26

40 Luyt CE, Aubry A, Lu Q, et al. Imipenem, meropenem, or doripenem to treat patients with Pseudomonas aeruginosa ventilator-associated pneumonia. Antimicrob Agents Chemother 2014; 58(3):1372–1380

41 Moyá B, Beceiro A, Cabot G, et al. Pan-β-lactam resistance development in Pseudomonas aeruginosa clinical strains: molecular mechanisms, penicillin-binding protein profiles, and binding affinities. Antimicrob Agents Chemother 2012;56(9):4771–4778

42 Fournier D, Richardot C, Müller E, et al. Complexity of resistance mechanisms to imipenem in intensive care unit strains of Pseudomonas aeruginosa. J Antimicrob Chemother 2013;68(8): 1772–1780

43 Riera E, Cabot G, Mulet X, et al. Pseudomonas aeruginosa carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem and doripenem. J Antimicrob Chemother 2011;66(9):2022–2027

44 Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22(4):582–610

45 Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin Infect Dis 2002;34(5):634–640

46 Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 1991;35(1):147–151

47 Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? Lancet Infect Dis 2011;11(5): 381–393

48 Tada T, Miyoshi-Akiyama T, Shimada K, Shimojima M, Kirikae T. IMP-43 and IMP-44 metallo-β-lactamases with increased carbapenemase activities in multidrug-resistant Pseudomonas aeruginosa. Antimicrob Agents Chemother 2013;57(9): 4427–4432

49 Lauretti L, Riccio ML, Mazzariol A, et al. Cloning and characterization of blaVIM, a new integron-borne metallo-beta-lactamase gene from a Pseudomonas aeruginosa clinical isolate. Antimicrob Agents Chemother 1999;43(7):1584–1590

50 Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR. Molecular characterization of a beta-lactamase gene, blaGIM-1, encoding a new subclass of metallo-beta-lactamase. Antimicrob Agents Chemother 2004;48(12):4654–4661

51 Yong D, Toleman MA, Bell J, et al. Genetic and biochemical characterization of an acquired subgroup B3 metallo-β-lactamase gene, blaAIM-1, and its unique genetic context in Pseudomonas aeruginosa from Australia. Antimicrob Agents Chemother 2012;56(12):6154–6159

52 Pollini S, Maradei S, Pecile P, et al. FIM-1, a new acquired metallo-β-lactamase from a Pseudomonas aeruginosa clinical isolate from Italy. Antimicrob Agents Chemother 2013;57(1): 410–416

53 Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 2009;53(12):5046–5054

54 Rolain JM, Parola P, Cornaglia G. New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect 2010;16(12):1699–1701

55 Jovcic B, Lepsanovic Z, Suljagic V, et al. Emergence of NDM-1 metallo-β-lactamase in Pseudomonas aeruginosa clinical isolates from Serbia. Antimicrob Agents Chemother 2011;55(8): 3929–3931

56 Fajardo A, Hernando-Amado S, Oliver A, Ball G, Filloux A, Martinez JL. Characterization of a novel Zn2+-dependent intrinsic imipenemase from Pseudomonas aeruginosa. J Antimicrob Chemother 2014;69(11):2972–2978

57 Villegas MV, Lolans K, Correa A, Kattan JN, Lopez JA, Quinn JP. Colombian Nosocomial Resistance Study Group. First identification of Pseudomonas aeruginosa isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. Antimicrob Agents Chemother 2007;51(4):1553–1555

58 Martínez T, Vázquez GJ, Aquino EE, Ramírez-Ronda R, Robledo IE. First report of a Pseudomonas aeruginosa clinical isolate coharbouring KPC-2 and IMP-18 carbapenemases. Int J Antimicrob Agents 2012;39(6):542–543

59 Sevillano E, Gallego L, García-Lobo JM. First detection of the OXA-40 carbapenemase in P. aeruginosa isolates, located on a plasmid also found in A. baumannii. Pathol Biol (Paris) 2009;57(6): 493–495

60 El Garch F, Bogaerts P, Bebrone C, Galleni M, Glupczynski Y. OXA-198, an acquired carbapenem-hydrolyzing class D beta-lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother 2011;55(10):4828–4833

61 Pirnay JP, Bilocq F, Pot B, et al. Pseudomonas aeruginosa population structure revisited. PLoS ONE 2009;4(11):e7740

62 Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG. Development of a multilocus sequence typing scheme for the opportunistic pathogen Pseudomonas aeruginosa. J Clin Microbiol 2004; 42(12):5644–5649

63 Wiehlmann L, Wagner G, Cramer N, et al. Population structure of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 2007;104(19): 8101–8106

64 Kidd TJ, Ritchie SR, Ramsay KA, Grimwood K, Bell SC, Rainey PB. Pseudomonas aeruginosa exhibits frequent recombination, but only a limited association between genotype and ecological setting. PLoS ONE 2012;7(9):e44199

65 Micol JB, de Botton S, Guieze R, et al. An 18-case outbreak of drug-resistant Pseudomonas aeruginosa bacteriemia in hematology patients. Haematologica 2006;91(8):1134–1138

66 Cardoso O, Alves AF, Leitão R. Metallo-beta-lactamase VIM-2 in Pseudomonas aeruginosa isolates from a cystic fibrosis patient. Int J Antimicrob Agents 2008;31(4):375–379

67 Harris A, Torres-Viera C, Venkataraman L, DeGirolami P, Samore M, Carmeli Y. Epidemiology and clinical outcomes of patients with multiresistant Pseudomonas aeruginosa. Clin Infect Dis 1999;28(5):1128–1133

68 Viedma E, Juan C, Villa J, et al. VIM-2-producing multidrug-resistant Pseudomonas aeruginosa ST175 clone, Spain. Emerg Infect Dis 2012;18(8):1235–1241

69 García-Castillo M, Del Campo R, Morosini MI, et al. Wide dispersion of ST175 clone despite high genetic diversity of carbapenem-nonsusceptible Pseudomonas aeruginosa clinical strains in 16 Spanish hospitals. J Clin Microbiol 2011;49(8):2905–2910

70 Cholley P, Thouverez M, Hocquet D, van der Mee-Marquet N, Talon D, Bertrand X. Most multidrug-resistant Pseudomonas aeruginosa isolates from hospitals in eastern France belong to a few clonal types. J Clin Microbiol 2011;49(7):2578–2583

71 Yoo JS, Yang JW, Kim HM, et al. Dissemination of genetically related IMP-6-producing multidrug-resistant Pseudomonas aeruginosa ST235 in South Korea. Int J Antimicrob Agents 2012; 39(4):300–304

72 Edelstein MV, Skleenova EN, Shevchenko OV, et al. Spread of extensively resistant VIM-2-positive ST235 Pseudomonas aeruginosa in Belarus, Kazakhstan, and Russia: a longitudinal epidemiological and clinical study. Lancet Infect Dis 2013;13(10): 867–876

73 Jackson GG, Riff LJ. Pseudomonas bacteremia: pharmacologic and other bases for failure of treatment with gentamicin. J Infect Dis 1971;124(Suppl):S185–S191

74 Siegman-Igra Y, Ravona R, Primerman H, Giladi M. Pseudomonas aeruginosa bacteremia: an analysis of 123 episodes, with particular emphasis on the effect of antibiotic therapy. Int J Infect Dis 1998;2(4):211–215

75 Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. Pseudomonas aeruginosa bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother 2005;49(4):1306–1311

76 Lodise TP Jr, Patel N, Kwa A, et al. Predictors of 30-day mortality among patients with Pseudomonas aeruginosa bloodstream infections: impact of delayed appropriate antibiotic selection. Antimicrob Agents Chemother 2007;51(10):3510–3515

77 Osih RB, McGregor JC, Rich SE, et al. Impact of empiric antibiotic therapy on outcomes in patients with Pseudomonas aeruginosa bacteremia. Antimicrob Agents Chemother 2007;51(3): 839–844

78 Tumbarello M, Repetto E, Trecarichi EM, et al. Multidrug-resistant Pseudomonas aeruginosa bloodstream infections: risk factors and mortality. Epidemiol Infect 2011;139(11):1740–1749

79 Chatzinikolaou I, Abi-Said D, Bodey GP, Rolston KV, Tarrand JJ, Samonis G. Recent experience with Pseudomonas aeruginosa bacteremia in patients with cancer: Retrospective analysis of 245 episodes. Arch Intern Med 2000;160(4):501–509

80 Chamot E, Boffi El Amari E, Rohner P, Van Delden C. Effectiveness of combination antimicrobial therapy for Pseudomonas aeruginosa bacteremia. Antimicrob Agents Chemother 2003;47(9): 2756–2764

81 Vidal F, Mensa J, Almela M, et al. Epidemiology and outcome of Pseudomonas aeruginosa bacteremia, with special emphasis on the influence of antibiotic treatment. Analysis of 189 episodes. Arch Intern Med 1996;156(18):2121–2126

82 Lodise TP Jr, Lomaestro B, Drusano GL. Piperacillin-tazobactam for Pseudomonas aeruginosa infection: clinical implications of an extended-infusion dosing strategy. Clin Infect Dis 2007;44(3): 357–363

83 Dulhunty JM, Roberts JA, Davis JS, et al. Continuous infusion of beta-lactam antibiotics in severe sepsis: a multicenter double-blind, randomized controlled trial. Clin Infect Dis 2013;56(2): 236–244

84 Taccone FS, Cotton F, Roisin S, Vincent JL, Jacobs F. Optimal meropenem concentrations to treat multidrug-resistant Pseudomonas aeruginosa septic shock. Antimicrob Agents Chemother 2012;56(4):2129–2131

85 Koch-Weser J, Sidel VW, Federman EB, Kanarek P, Finer DC, Eaton AE. Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy. Ann Intern Med 1970;72(6):857–868

86 Falagas ME, Rafailidis PI, Ioannidou E, et al. Colistin therapy for microbiologically documented multidrug-resistant Gram-negative bacterial infections: a retrospective cohort study of 258 patients. Int J Antimicrob Agents 2010;35(2):194–199

87 Paul M, Bishara J, Levcovich A, et al. Effectiveness and safety of colistin: prospective comparative cohort study. J Antimicrob Chemother 2010;65(5):1019–1027

88 Park JH, Choi SH, Chung JW. The impact of early adequate antimicrobial therapy on 14-day mortality in patients with monomicrobial Pseudomonas aeruginosa and Acinetobacter baumannii bacteremia. J Infect Chemother 2013;19(5):843–849

89 Falagas ME, Kastoris AC, Karageorgopoulos DE, Rafailidis PI. Fosfomycin for the treatment of infections caused by multidrug-resistant non-fermenting Gram-negative bacilli: a systematic review of microbiological, animal and clinical studies. Int J Antimicrob Agents 2009;34(2):111–120

90 Dinh A, Salomon J, Bru JP, Bernard L. Fosfomycin: efficacy against infections caused by multidrug-resistant bacteria. Scand J Infect Dis 2012;44(3):182–189

91 Pontikis K, Karaiskos I, Bastani S, et al. Outcomes of critically ill intensive care unit patients treated with fosfomycin for infections due to pandrug-resistant and extensively drug-resistant carbapenemase-producing Gram-negative bacteria. Int J Antimicrob Agents 2014;43(1):52–59

92 Roussos N, Karageorgopoulos DE, Samonis G, Falagas ME. Clinical significance of the pharmacokinetic and pharmacodynamic characteristics of fosfomycin for the treatment of patients with systemic infections. Int J Antimicrob Agents 2009;34(6):506–515

93 Klastersky J, Cappel R, Daneau D. Therapy with carbenicillin and gentamicin for patients with cancer and severe infections caused by gram-negative rods. Cancer 1973;31(2):331–336

94 Schimpff SC, Gaya H, Klastersky J, Tattersall MH, Zinner SH. Three antibiotic regimens in the treatment of infection in febrile granulocytopenic patients with cancer. The EORTC international antimicrobial therapy project group. J Infect Dis 1978;137(1): 14–29

95 Hilf M, Yu VL, Sharp J, Zuravleff JJ, Korvick JA, Muder RR. Antibiotic therapy for Pseudomonas aeruginosa bacteremia: outcome correlations in a prospective study of 200 patients. Am J Med 1989; 87(5):540–546

96 Bliziotis IA, Petrosillo N, Michalopoulos A, Samonis G, Falagas ME. Impact of definitive therapy with beta-lactam monotherapy or combination with an aminoglycoside or a quinolone for Pseudomonas aeruginosa bacteremia. PLoS ONE 2011;6(10):e26470

97 Bowers DR, Liew YX, Lye DC, Kwa AL, Hsu LY, Tam VH. Outcomes of appropriate empiric combination versus monotherapy for Pseudomonas aeruginosa bacteremia. Antimicrob Agents Chemother 2013;57(3):1270–1274

98 Peña C, Suarez C, Ocampo-Sosa A, et al; Spanish Network for Research in Infectious Diseases (REIPI). Effect of adequate single-drug vs combination antimicrobial therapy on mortality in Pseudomonas aeruginosa bloodstream infections: a post Hoc analysis of a prospective cohort. Clin Infect Dis 2013;57(2): 208–216

99 Hu Y, Li L, Li W, et al. Combination antibiotic therapy versus monotherapy for Pseudomonas aeruginosa bacteraemia: a meta-analysis of retrospective and prospective studies. Int J Antimicrob Agents 2013;42(6):492–496

100 Lingscheid T, Tobudic S, Poeppl W, Mitteregger D, Burgmann H. In vitro activity of doripenem plus fosfomycin against drug-resistant clinical blood isolates. Pharmacology 2013;91(3-4):214–218

101 He W, Kaniga K, Lynch AS, Flamm RK, Davies TA. In vitro Etest synergy of doripenem with amikacin, colistin, and levofloxacin against Pseudomonas aeruginosa with defined carbapenem resistance mechanisms as determined by the Etest method. Diagn Microbiol Infect Dis 2012;74(4):417–419

102 Traugott KA, Echevarria K, Maxwell P, Green K, Lewis JS II. Monotherapy or combination therapy? The Pseudomonas aeruginosa conundrum. Pharmacotherapy 2011;31(6):598–608

103 Siqueira VL, Cardoso RF, Caleffi-Ferracioli KR, et al. Structural changes and differentially expressed genes in Pseudomonas aeruginosa exposed to meropenem-ciprofloxacin combination. Antimicrob Agents Chemother 2014;58(7):3957–3967

104 Louie A, Grasso C, Bahniuk N, et al. The combination of meropenem and levofloxacin is synergistic with respect to both Pseudomonas aeruginosa kill rate and resistance suppression. Antimicrob Agents Chemother 2010;54(6):2646–2654

105 Drusano GL, Bonomo RA, Bahniuk N, et al. Resistance emergence mechanism and mechanism of resistance suppression by tobramycin for cefepime for Pseudomonas aeruginosa. Antimicrob Agents Chemother 2012;56(1):231–242

106 Kumar A, Zarychanski R, Light B, et al; Cooperative Antimicrobial Therapy of Septic Shock (CATSS) Database Research Group. Early combination antibiotic therapy yields improved survival compared with monotherapy in septic shock: a propensity-matched analysis. Crit Care Med 2010;38(9):1773–1785

107 Kumar A, Safdar N, Kethireddy S, Chateau D. A survival benefit of combination antibiotic therapy for serious infections associated with sepsis and septic shock is contingent only on the risk of death: a meta-analytic/meta-regression study. Crit Care Med 2010;38(8):1651–1664

108 Paul M, Carmeli Y, Durante-Mangoni E, et al. Combination therapy for carbapenem-resistant Gram-negative bacteria. J Antimicrob Chemother 2014;69(9):2305–2309

109 Aarts MA, Hancock JN, Heyland D, McLeod RS, Marshall JC. Empiric antibiotic therapy for suspected ventilator-associated pneumonia: a systematic review and meta-analysis of randomized trials. Crit Care Med 2008;36(1):108–117

110 Kollef MH, Morrow LE, Niederman MS, et al. Clinical characteristics and treatment patterns among patients with ventilator-associated pneumonia. Chest 2006;129(5):1210–1218

111 Louie A, Liu W, Fikes S, Brown D, Drusano GL. Impact of meropenem in combination with tobramycin in a murine model of Pseudomonas aeruginosa pneumonia. Antimicrob Agents Chemother 2013;57(6):2788–2792

112 Fish DN, Kiser TH. Correlation of pharmacokinetic/pharmacodynamic-derived predictions of antibiotic efficacy with clinical outcomes in severely ill patients with Pseudomonas aeruginosa pneumonia. Pharmacotherapy 2013;33(10):1022–1034

113 Kollef MH, Chastre J, Clavel M, et al. A randomized trial of 7-day doripenem versus 10-day imipenem-cilastatin for ventilator-associated pneumonia. Crit Care 2012;16(6):R218

114 Chastre J, Wolff M, Fagon JY, et al; PneumA Trial Group. Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. JAMA 2003;290(19): 2588–2598

115 Pugh R, Grant C, Cooke RP, Dempsey G. Short-course versus prolonged-course antibiotic therapy for hospital-acquired pneumonia in critically ill adults. Cochrane Database Syst Rev 2011; (10):CD007577

116 Florescu DF, Qiu F, McCartan MA, Mindru C, Fey PD, Kalil AC. What is the efficacy and safety of colistin for the treatment of ventilator-associated pneumonia? A systematic review and meta-regression. Clin Infect Dis 2012;54(5):670–680

117 Rigatto MH, Ribeiro VB, Konzen D, Zavascki AP. Comparison of polymyxin B with other antimicrobials in the treatment of ventilator-associated pneumonia and tracheobronchitis caused by Pseudomonas aeruginosa or Acinetobacter baumannii. Infection 2013;41(2):321–328
118 Lu Q, Yang J, Liu Z, Gutierrez C, Aymard G, Rouby JJ. Nebulized Antibiotics Study Group. Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by Pseudomonas aeruginosa. Am J Respir Crit Care Med 2011;184(1):106–115
119 Li Z, Zhang Y, Wurtz W, et al. Characterization of nebulized liposomal amikacin (Arikace) as a function of droplet size. J Aerosol Med Pulm Drug Deliv 2008;21(3):245–254
120 Ratjen F, Rietschel E, Kasel D, et al. Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. J Antimicrob Chemother 2006;57(2):306–311
121 Korbila IP, Michalopoulos A, Rafailidis PI, Nikita D, Samonis G, Falagas ME. Inhaled colistin as adjunctive therapy to intravenous colistin for the treatment of microbiologically documented ventilator-associated pneumonia: a comparative cohort study. Clin Microbiol Infect 2010;16(8):1230–1236
122 Naesens R, Vlieghe E, Verbrugghe W, Jorens P, Ieven M. 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 2011; 11:317
123 Michalopoulos A, Fotakis D, Virtzili S, et al. Aerosolized colistin as adjunctive treatment of ventilator-associated pneumonia due to multidrug-resistant Gram-negative bacteria: a prospective study. Respir Med 2008;102(3):407–412
124 Kofteridis DP, Alexopoulou C, Valachis A, et al. Aerosolized plus intravenous colistin versus intravenous colistin alone for the treatment of ventilator-associated pneumonia: a matched case-control study. Clin Infect Dis 2010;51(11):1238–1244
125 Lu Q, Luo R, Bodin L, et al; Nebulized Antibiotics Study Group. Efficacy of high-dose nebulized colistin in ventilator-associated pneumonia caused by multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii. Anesthesiology 2012;117(6): 1335–1347
126 Rattanaumpawan P, Lorsutthitham J, Ungprasert P, Angkasekwinai N, Thamlikitkul V. Randomized controlled trial of nebulized colistimethate sodium as adjunctive therapy of ventilator-associated pneumonia caused by Gram-negative bacteria. J Antimicrob Chemother 2010;65(12):2645–2649