Framework for optimisation of the clinical use of colistin and polymyxin B: the Prato polymyxin consensus

Roger L Nation, Jian Li, Otto Cars, William Couet, Michael N Dudley, Keith S Kaye, Johan W Mouton, David L Paterson, Vincent H Tam, Ursula Theuretzbacher, Brian T Tsuji, John D Turnidge

In the face of diminishing therapeutic options for the treatment of infections caused by multidrug-resistant, Gram-negative bacteria, clinicians are increasingly using colistin and polymyxin B. These antibiotics became available clinically in the 1950s, when understanding of antimicrobial pharmacology and regulatory requirements for approval of drugs was substantially less than today. At the 1st International Conference on Polymyxins in Prato, Italy, 2013, participants discussed a set of key objectives that were developed to explore the factors affecting the safe and effective use of polymyxins, identify the gaps in knowledge, and set priorities for future research. Participants identified several factors that affect the optimum use of polymyxins, including: confusion caused by several different conventions used to describe doses of colistin; an absence of appropriate pharmacopoeial standards for polymyxins; outdated and diverse product information; and uncertainties about susceptibility testing and breakpoints. High-priority areas for research included: better definition of the effectiveness of polymyxin-based combination therapy compared with monotherapy via well designed, randomised controlled trials; examination of the relative merits of colistin versus polymyxin B for various types of infection; investigation of pharmacokinetics in special patient populations; and definition of the role of nebulised polymyxins alone or in combination with intravenous polymyxins for the treatment of pneumonia. The key areas identified provide a roadmap for action regarding the continued use of polymyxins, and are intended to help with the effective and safe use of these important, last-line antibiotics.

Lancet Infect Dis 2015; 15: 225–34

Published Online October 21, 2014 http://dx.doi.org/10.1016/S1473-3099(14)70850-3

Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia (Prof R L Nation PhD, Prof J Li PhD); **Department of Medical Sciences, Section of Infectious Diseases, Uppsala University, Uppsala, Sweden** (Prof O Cars MD); **Faculté de Médecine et de Pharmacie, Université de Poitiers, CHU Poitiers and Inserm U1070, Poitiers, France** (Prof W Couet PhD); **The Medicines Company, San Diego, CA, USA** (M N Dudley Pharm D); **Division of Infectious Diseases, Wayne State University, Detroit Medical Center, Detroit, MI, USA** (Prof K S Kaye MD); **Erasmus University Medical Centre, Rotterdam, Netherlands** (Prof J W Mouton MD); **The University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia** (Prof D L Paterson FRACP); **Department of Clinical Sciences and Administration, University of Houston College of Pharmacy, Houston, TX, USA** (Prof V H Tam Pharm D); **Center for Anti-Infective Agents, Vienna, Austria** (U Theuretzbacher PhD); **School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA** (B T Tsuji Pharm D); **and Departments of Pathology and Paediatrics and School of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, SA, Australia** (Prof J D Turnidge FRACP)

Introduction

In an era of increasing rates of multidrug resistance and a dry drug-development pipeline for new antimicrobial agents,[1,2] the polymyxin antibiotics—colistin and polymyxin B—have had a substantial resurgence in their use for treatment of infections caused by Gram-negative bacteria; in particular, multidrug-resistant (MDR) *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*.[3–7] Colistin (also known as polymyxin E) and polymyxin B became clinically available in the 1950s but soon fell out of favour, mainly because of concerns about their potential to cause toxic effects in the kidneys.[3–7] However, in recent years, polymyxins have been increasingly used as a last-line treatment for infections that are resistant to other available antibiotics. Although polymyxin resistance rates are relatively low, there is concern that this situation is changing.[8,9] This problem underscores the importance of ensuring the best possible use of these old, unfamiliar, polymyxin antibiotics.

Being products of bacterial fermentation, colistin and polymyxin B are multicomponent antibiotics; they have very similar chemical structures, differing by one aminoacid in the peptide ring (figure)[10–12] The polymyxins are relatively large lipopeptide molecules and are poorly absorbed after oral administration.[10] For the treatment of life-threatening systemic infections including those in the urinary tract, they are given by the intravenous route, or by nebulisation for the treatment of respiratory tract infections.[3–7] The parenteral and nebulisation products for colistin are in the form of the sodium salt of colistin methanesulphonate, also known as colistimethate (figure). Colistimethate is an inactive prodrug and conversion in vivo to the active antibacterial colistin is needed.[13] However, pharmaceutical products of polymyxin B contain its sulphate salt, and therefore polymyxin B is given in its active form.

Having received marketing approval in the 1950s, the polymyxins were not subjected to the drug development procedures and regulatory scrutiny needed for modern drugs. Thus, information to guide their clinical use has been scarce.[3,6,14–17] Substantial progress has been made in the contemporary study of the preclinical and clinical pharmacology of polymyxins,[15–19] which has increased understanding to guide their clinical use. In view of the growing importance of polymyxins in the antibacterial armamentarium against serious Gram-negative infections and the recent advances that have taken place, the 1st International Conference on Polymyxins was held in Prato, Italy on May 2–4, 2013. Details of the programme and presenters can be found on the conference website.[20] The meeting objectives were framed by two key questions: (1) what factors affect the ability of clinicians to make optimum use of the polymyxins? and (2) where are the gaps in knowledge and what research is needed? This report describes the outcomes of this conference.

Approach of the conference

The session and lecture topics at the conference[20] were generated by the organisers and designed to address the two key questions with regard to polymyxins. All lectures were plenary in nature and were delivered by international research and clinical leaders who were identified through a review of published work and the uptake of that work. Other invitees who contributed to the programme were from the European Medicines Agency (EMA), the European Commission, and the United States National Institutes of Health.

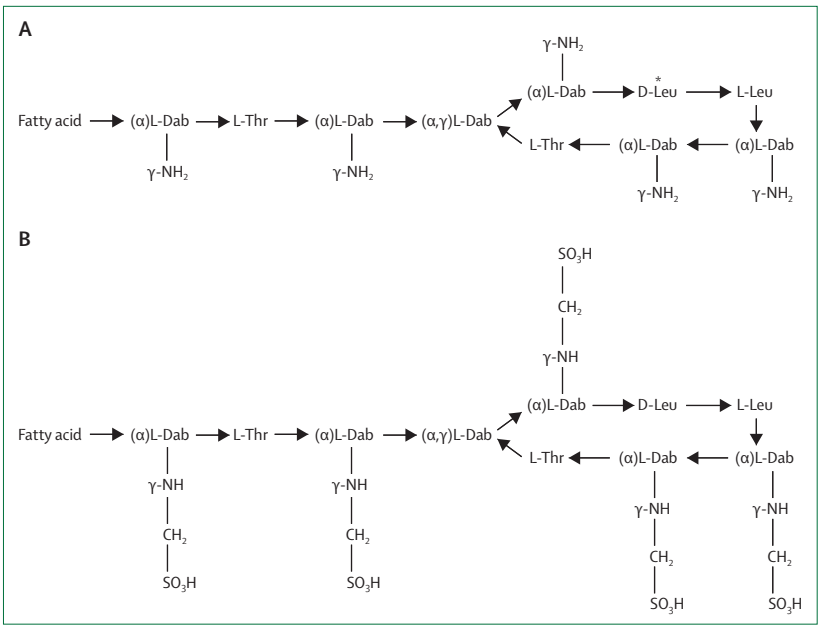

Figure: **Structures of polymyxins**
(A) Structures of colistin A and B and polymyxin B1 and B2. For polymyxin B, D-Phe (phenylalanine) replaces the D-Leu (leucine) marked with the asterisk. (B) Structures of colistin methanesulphonate A and B. Fatty acid: 6-methyloctanoic acid for colistin A and polymyxin B1; and 6-methylheptanoic acid for colistin B and polymyxin B2. α and γ show the respective $-NH_2$ involved in the peptide linkage.
Thr=threonine. Dab=α,γ-diaminobutyric acid.

Correspondence to:
Prof Roger L Nation, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC 3052, Australia
roger.nation@monash.edu

For more on the **2013 Polymyxin conference** see http://monash.edu/pharm/about/events/polymyxins

The conference delegates, from 27 countries, were infectious diseases specialists, clinical microbiologists, pharmacologists, clinical pharmacists, pharmaceutical scientists, and pharmaceutical company drug-regulatory specialists. The 146 attendees heard about the substantial advances that have happened in recent years in understanding of the complex chemistry, microbiology, pharmacology, and clinical use of the polymyxins,[4–6,16–18,21,22] and work that is in progress. A key aspect of the programme was the identification of topics that arose from the two key conference questions. The identified topics were carried forward to the final session in which they were presented and discussed, with active input from conference attendees who had the opportunity to agree or disagree with the proposed high-priority areas. The recommendations in this report were informed by the discussion among attendees at the conference, particularly within the consensus session. The consensus panel members—the authors of this report—were members of the conference-organising committee, or invited speakers at the conference.

What factors affect the ability of clinicians to make the best use of polymyxins?

Different conventions are used to describe doses of colistin

Unfortunately, a number of different dosing terminologies are used around the world to express colistin doses. This causes much confusion and affects the ability of clinicians to ensure the optimum and safe use of the drug. As noted, colistin is not given directly by the parenteral or inhalational routes; the product given contains the prodrug, colistimethate (figure). Colistimethate is microbiologically inactive and needs cleavage of the methanesulphonate chemical moieties in vivo to generate the active antibacterial colistin.[3,6,13,16–18] Two main conventions are used worldwide, both of which rely on in-vitro microbiological assays, to describe the contents of parenteral or inhalational vials and corresponding doses for colistimethate products. The first is based on the number of international units (IU). This convention is used in Europe, India, and a few other regions. Recently, product information documents for some brands in these areas have introduced a description of the vial contents or dose in terms of the number of mg of colistimethate, in addition to the number of IU. The second convention is based on the number of mg of colistin base activity (CBA). This convention is used in the remaining regions of the world where parenteral colistin is available, including North and South America, southeast Asia, and Australia.

It is important to understand the equivalence across these conventions. One million IU is equivalent to about 30 mg of CBA, which corresponds to about 80 mg of the prodrug colistimethate.[3] Therefore a specific number of mg of CBA is equivalent to about 2·7 times (ie, 80/30) that number of mg of colistimethate. The two possible ways of expressing a colistin dose in mg (ie, as mg of CBA or as mg of colistimethate) can lead to medication errors that threaten patient safety. Such a situation has already been documented; a dose of colistimethate that was about 2·7 times higher than intended was given to a patient, which lead to acute renal failure and death.[23]

The amount of drug contained in a parenteral vial should be expressed as mg of CBA or number of IU, consistent with the convention in the region where the product is used. Additionally, dose information in the respective prescribing information documents should clearly state the dose as mg of CBA or number of IU. The expression of dose as mg of colistimethate in the dose section of product information should cease. Regulators and pharmaceutical manufacturers should immediately assess vial labelling and product prescribing information for colistimethate parenteral products, and revise content where necessary. With regard to the reporting of clinical studies on colistin, journals of infectious diseases, antimicrobial chemotherapy, and clinical pharmacology, have been requested to implement at the editorial level procedures to avoid future confusion arising from different dosing terminologies.[14]

Pharmacopoeial standards for polymyxins

Polymyxins are supplied as products containing mixtures of many components produced by fermentation. With colistin, subsequent chemical modification is undertaken to produce the prodrug colistimethate in which sulphomethylation might occur at between one and all five of the primary amine groups on colistin (figure). In

addition to colistin itself and the fully (ie, penta) sulphomethylated form, there are 30 possible partly sulphomethylated derivatives for each of the two major components colistin A and colistin B (figure) and the other colistin components entering the sulphomethylation procedure. Thus, the fermentation and subsequent chemical modification processes produce very complex mixtures of components.[24,25] Variations in the nature of the heterogeneity among different colistimethate batches or drug products could affect the rate of conversion to colistin. Such effects could contribute to variability across colistimethate batches or products in pharmacokinetic profiles for colistin formed in vivo; such pharmacokinetic variability has been recently reported.[24] Potentially, this situation could lead to different pharmacodynamic and toxicodynamic responses. Quality control limits for the components of colistin and polymyxin B do not exist at all in the US Pharmacopeia.[26,27] Limits for components of these two polymyxins are present in the corresponding European standard, although they are rather wide for colistin.[28,29] Neither of these regions have limits for the components of colistimethate.[26,28] Therefore, there is potential for substantial brand-to-brand and even batch-to-batch variation in the composition of polymyxin B, colistin, and, in particular, colistimethate. Because polymyxins have a narrow therapeutic window,[16,21] their pharmacopoeial standards should be re-assessed urgently.

Outdated and diverse product information

Several important sections within product information documents for polymyxins need correction and updating to incorporate new knowledge. Essentially all of the prescribing information supplied with colistimethate parenteral products contains decades-old information about the pharmacokinetics of colistin that was obtained with microbiological assays. Such assays do not measure the colistin concentration that is actually present in the plasma at the time of collection of a blood sample from a patient. This discrepancy arises because during the incubation period of microbiological assays, colistin continues to be formed from colistimethate that is also present in the sample.[3] Thus, the colistin concentrations reported in the product information are artifactually raised and do not show the actual concentration of colistin, the antibacterial entity, that was present in vivo. This is especially important because during the last few years, therapeutic drug monitoring (TDM) services that use specific chromatographic methods have been established in several institutions.[30–32] Clinicians should not interpret the individual plasma colistin concentration measurements generated with these specific assays in patients against the artifactually raised concentrations of colistin reported in product information literature. The pharmacokinetic section of product information documents should be updated with newly generated data that are based on specific chromatographic methods, and appropriate handling and processing of samples that minimise any ex-vivo conversion of colistimethate to colistin.[25,33] The susceptibility testing section of many product information documents also needs modification because the general implication is that such testing is undertaken with colistimethate, but, susceptibility testing should actually be undertaken with the microbiologically active colistin.[13,16] Furthermore, the range of recommended daily maintenance doses varies widely across countries (even within Europe),[34] and usually does not contain the recommendation for a loading dose, although there is good evidence that therapy should be initiated in this manner.[35–37]

The foregoing examples show that there is an urgent need for the product information of parenteral polymyxin drug products to be updated and harmonised according to current evidence. The harmonisation will need input and cooperation from the pharmaceutical companies marketing the different brands and the regulators from different regions.

Availability of colistin and polymyxin B around the world

Some countries (eg, South Africa and Japan) do not have parenteral products of either colistin or polymyxin B that are registered for use in human beings. In some of these countries it might be possible for institutions to apply for importation of a product that is registered in another jurisdiction, but this could be an impediment to timely initiation of the antibiotic. Such a situation is not desirable in view of the last-line therapeutic status of polymyxins in an era of global spread of MDR Gram-negative pathogens, particularly carbapenemase-producing Enterobacteriaceae.[38,39] Additionally, a delay in initiation of appropriate antibiotic therapy has a negative effect on the outlook for critically ill patients.[40–42] Thus, jurisdictions where the products are not currently registered should think about maintaining a supply of at least one of the polymyxins for urgent patient use.

Is the availability of polymyxin parenteral products ideal in other countries? Parenteral products of colistin (ie, the inactive prodrug colistimethate) and polymyxin B are available in some countries (eg, USA, Brazil, and Singapore), but in others only the colistimethate formulation is available (eg, countries throughout Europe). Although colistin and polymyxin B have very similar antibacterial activity in vitro and both can cause nephrotoxic effects,[4,8,17] there are important differences in their pharmacology in man. These differences arise because polymyxin B is given intravenously in its active form, whereas colistin is given in the form of its prodrug colistimethate that is converted to the active colistin via a slow and incomplete (about 20% conversion with good renal function) process that is subject to substantial interpatient variability.[35–37,43] This variability in the rate and extent of conversion to colistin probably arises, at least partly, because of batch-to-batch variability in the multicomponent composition of colistimethate products.[24]

Recent studies have suggested that upon initiation of therapy with colistimethate, even with a loading dose, plasma colistin concentrations increase slowly, resulting in a delay in achievement of concentrations that might be associated with antibacterial activity.[35,37] Such a delay is probably detrimental, in view of the known link between timely initiation of antibiotic therapy and patient outcome.[40–42] Another consideration is the ability of currently approved maintenance doses to achieve plasma concentrations of formed colistin that are likely to be effective. Daily maintenance doses of colistimethate within the currently approved range are able to generate average steady-state plasma concentrations of colistin of about 4–9 mg/L in patients with diminished renal function. However, in patients with creatinine clearance more than 80 mL/min, it is not possible to reliably achieve an average steady-state plasma colistin concentration of 2 mg/L.[35] These limitations do not apply to polymyxin B because it is not given as a prodrug. Moreover, colistimethate maintenance doses might need adjustment according to renal function but even at a specific creatinine clearance there is very large interpatient variability in the plasma colistin concentration achieved at a specific daily dose of colistimethate,[35] which are characteristics that make dose selection difficult. By contrast, polymyxin B dose requirements are not affected by kidney function, and the interpatient variability is substantially smaller even across a very wide range of creatinine clearance measurements.[44]

The two polymyxins also differ substantially from each other with regard to the concentrations achieved in urine. This difference arises because urinary excretion is a minor clearance pathway for polymyxin B,[44] whereas it is a major clearance route for colistimethate which is partly converted within the urinary tract to colistin.[43] Although colistin (administered as colistimethate) and polymyxin B are potentially nephrotoxic, the relative risk of this adverse effect in patients is unclear.[21,45] However, comparative studies of almost 400 patients suggest that the incidence of nephrotoxic effects is higher with colistimethate than with polymyxin B.[46,47] Thus, across a range of clinical pharmacological properties, polymyxin B seems to be a better choice than colistimethate for many infections, particularly those outside the urinary tract. In the interest of individual patients and the preservation of activity of the polymyxins as a class, parenteral products of both colistimethate and polymyxin B should be available to clinicians in all parts of the world.

Inappropriate use of polymyxins

An area of concern with polymyxins is their clinical use in some parts of the world for selective decontamination of the digestive tract (SDD) of patients.[48,49] This exposes gut flora to polymyxin and has been reported to lead to rapid emergence of resistance to these last-line antibiotics.[48] If SDD is undertaken, alternatives to polymyxins should be used.

Until recently, parenteral polymyxins had only been prescribed rarely in human medicine, and thus use of this antibiotic class for veterinary or agricultural purposes has been judged by regulatory agencies to have little or no actual or potential effect on human medicine. Colistin has been used as a growth promoter, and for disease prevention and treatment of animals, especially in swine and poultry farming worldwide. In Europe, veterinary formulations of colistin are approved and they are fifth on the list of antibacterial drugs used in food-producing animals.[50] Although there is currently no evidence of spread of polymyxin-resistant bacteria from food-producing animals to human beings, the European Medicines Agency recommended restricting polymyxin use to the treatment of infected animals and those in contact with them, and to remove all indications for preventive use in animals.[50] In several other parts of the world, there are few quantitative data on the use of polymyxins in animals. The relatively recent necessity to resurrect polymyxins in human medicine, and the need to maintain their activity, needs urgent action internationally with policies controlling polymyxin use in animals.

Susceptibility testing and breakpoints

The internationally recognised reference method for susceptibility testing is a broth microdilution assay described in the ISO 20776-1 standard.[51] At the time that this standard was promulgated (2006) there had been no particular issues identified with the susceptibility testing of the polymyxin class, apart from the difficulty of achieving zone diameter correlates for minimum inhibitory concentrations (MICs) with the disk-diffusion method.[52] Subsequently, many investigators identified an issue with the adherence of colistin and polymyxin B to plastic and other materials,[53,54] with the extent of binding being affected by factors such as the nature of the plastic and whether it was surface treated.[53]

As has been suggested for other highly adherent agents such as oritavancin[55] and dalbavancin,[56] MIC testing for polymyxins could be undertaken using a surfactant such as polysorbate 80 in the stock solutions, dilutions, and growth medium.[57,58] In a study done on behalf of the Clinical and Laboratory Standards Institute (CLSI),[59] the MICs of two quality-control strains were measured for both colistin and polymyxin B in the presence and absence of polysorbate 80. The presence of polysorbate 80 had a consistent effect on lowering MICs of the two strains, but disappointingly did not reduce the overall assay variance (unpublished data, JD Turnidge, Adelaide University, Adelaide, SA, Australia). Results of studies using tissue-culture treated microtitre trays have shown substantially raised MICs compared with untreated plates, an effect that was not abolished by addition of polysorbate 80.[53] Thus, present data do not provide compelling evidence supporting the routine addition of polysorbate 80 during MIC testing.

Additionally, the reference assay should be suitably standardised before any further work on susceptibility testing and pharmacodynamics can be undertaken. The features of the reference test method and the breakpoints for polymyxins are under review jointly by CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST); the working group charged with this task is co-chaired by authors of this report. Until the work on reference MIC testing is agreed to internationally, and breakpoints are established using the full suite of microbiological, pharmacodynamic, and clinical data,[60] the epidemiological cutoff values as published on the EUCAST website should be used.[61]

Therapeutic drug monitoring in routine clinical practice

Colistin and polymyxin B show concentration-dependent bacterial killing. Additionally, they have narrow therapeutic windows and nephrotoxic effects are the major dose-limiting adverse effect.[16,17] These characteristics provide a strong argument for measurement of plasma drug concentrations to assist in optimisation of dose regimens in individual patients, to maximise antibacterial effect, and minimise the potential for emergence of resistance and development of polymyxin-induced nephrotoxic effects.

For colistimethate, the very substantial interpatient variability in the steady-state plasma colistin concentration achieved from a specific daily dose of colistimethate at a specific degree of kidney function[35] provides an additional compelling case for TDM. On the basis of pharmacokinetic and pharmacodynamic studies[35–37,62] and analyses suggesting that the likelihood of a decline in kidney function increases with plasma colistin concentrations of more than about 2·5 mg/L (especially in patients with good baseline kidney function), an average steady-state plasma colistin concentration of 2 mg/L seems to be a reasonable target value (unpublished data, A Forrest, University at Buffalo, Buffalo, NY, USA). If it is not possible to achieve a plasma colistin concentration of about 2 mg/L (this is probable in patients with good kidney function) or if the infecting pathogen has an MIC greater than 1 mg/L, combination therapy is advised. In some countries, TDM services have already been established to assist in optimisation of dose regimens.[30–32] The analytical methods used must be specific for the active entity; microbiological assays are not appropriate because of continued conversion of colistimethate to colistin during the assay incubation.[3,10,13] Samples of plasma should be transported to the TDM laboratory in such a way as to halt this ex-vivo conversion. If these precautions are not followed, the measured concentration of colistin might be substantially higher than that present in the patient at the time of collection of the sample, which will render the result misleading.[3,10,13]

TDM-assisted dose individualisation for critically ill patients is widely used for aminoglycosides (antibiotics with a low therapeutic index) and several other antibacterial classes,[63,64] even though the clinical benefits have not been formally assessed in large studies. In view of the factors that argue for TDM to be applied to polymyxins as outlined, TDM services should be used wherever possible. However, prospective studies should be undertaken to assess its benefit.

Where are the gaps in knowledge and what research is needed?

Prospective studies with TDM and adaptive feedback control

As discussed, TDM has been introduced in some parts of the world to optimise polymyxin dose regimens for patients.[30–32] In view of the narrow therapeutic window of the polymyxins there is a strong rationale for TDM; however, the benefit of this approach should be formally investigated in appropriate prospective research studies. Integration of TDM with adaptive feedback control[65] to further refine individualisation of therapy should also be investigated and assessed.

Mechanisms of action, resistance, and toxic effects

Despite their growing importance within the antibiotic armamentarium, the mechanism(s) by which polymyxins ultimately kill bacterial cells is still not known. Although the interaction of the polymyxins with the lipopolysaccharide of the outer membrane of Gram-negative bacteria is recognised as the initial step in the antibacterial effect, this does not account for their ultimate killing action.[11,12] Additionally, there is a growing but incomplete understanding of resistance mechanisms.[11,12] Elucidation of mechanisms of action and resistance is likely to be beneficial for management of patients—eg, in guiding the rational selection of other antibiotics to use in combination with a polymyxin to increase activity and minimise potential for emergence of resistance.

Polymyxin-induced nephrotoxicity is associated with changes in mitochondrial morphology and membrane potential, and apoptosis of tubular cells.[66] However many questions remain unanswered including precise mechanisms of polymyxin uptake into and trafficking within renal tubular cells (ie, cellular pharmacokinetics). Such knowledge would assist in identification and assessment in preclinical studies of potential methods to ameliorate polymyxin-induced nephrotoxic effects, for ultimate clinical translation and testing in patients. Importantly, a more complete understanding of the mechanisms of action, resistance, and nephrotoxic effects will greatly assist in the discovery of next-generation polymyxin antibiotics. These new agents could be designed to have increased activity against bacterial strains, including those that are resistant to current polymyxins, or have lower propensity for causing nephrotoxic effects, or both. Thus, they would have an increased safety margin compared with colistin and polymyxin B.

Pharmacokinetic studies in special patient populations

Landmark studies have been published reporting the population pharmacokinetics of colistin[35–37,43] and polymyxin B,[44,67] and these have allowed the proposition of scientifically based dose regimens.[35–37,44] Information is needed about the disposition of polymyxins in hitherto non-investigated groups of patients and definition of the subsequent implications for dosing. For example, obese patients have been poorly represented in population pharmacokinetic studies of colistin,[35–37] and polymyxin B.[44] Similarly, little information is available to guide dosing in paediatric patients, although some studies are underway.

Large-scale clinical studies with polymyxin B

Because of its wider global availability, most clinical studies aimed at optimisation of dosing have been focused on colistin.[35–37] Larger pharmacokinetic/pharmacodynamic and clinical studies of polymyxin B are urgently needed to develop improved dosing strategies with this drug. Because of the cross-resistance that occurs between the two polymyxins,[8,12] both drugs should be used such that their antibacterial effect is maximised, and nephrotoxic effects and emergence of resistance are minimised.

Colistin versus polymyxin B

Results from studies in critically ill patients identified that the patient factors affecting the disposition of colistin (administered as colistimethate)[35] differ from those for polymyxin B.[44] As discussed, although the two polymyxins have very similar antibacterial activity in vitro they differ substantially with regard to their behaviour in patients,[35,44] and this could affect their clinical benefit for various types of infections. Polymyxin B seems to be the better choice for treatment of infections that rely on the ability to rapidly and reliably achieve effective blood concentrations of active antibiotic, whereas colistimethate (colistin) could be the preferred polymyxin for urinary tract infections. No prospective head-to-head studies have been done that compared colistin with polymyxin B; however, these are needed. The source of infection should be thought about in the patient selection criteria, and the studies should examine both efficacy and toxicity endpoints. Such studies could be undertaken in those countries that have access to parenteral products of colistimethate and polymyxin B.

Combination versus monotherapy

For some patients, achievement of adequate plasma polymyxin concentrations with currently approved dose regimens might not be possible. Therefore, monotherapy with a polymyxin is unlikely to be reliably effective, especially for treatment of infections caused by pathogens with MICs near the current breakpoint.[35,44] Although many (largely empirically driven) preclinical and clinical studies have investigated polymyxin combinations,[16,68] the precise mechanisms of the synergy of bacterial killing and suppression of resistance for polymyxins and second antibiotics are largely unknown. Future preclinical research in this specialty should use molecular methods and genomics or transcriptomics[69] to measure bacterial responses to different combination regimens. Such studies can be done under well controlled conditions in in-vitro infection models[70,71] with the aim of identifying combination regimens associated with enhanced bacterial killing, and molecular signatures that are associated with optimum suppression of resistance. The most promising regimens can then be translated to the clinic for assessment in patients.

Polymyxin combinations have been used empirically in the clinic but their effectiveness is difficult to judge for reasons including an absence of appropriate controls, retrospective nature of studies, and few patients.[68,72–76] In a multicentre, randomised study (210 patients) of colistin plus rifampicin compared with colistin alone against infections (mainly ventilator-associated pneumonia) caused by MDR-*A baumannii*, there was a significantly higher rate of microbiological eradication in the combination group, but 30 day mortality was not reduced.[77] A loading dose of colistimethate was not given and the maximum daily maintenance dose of colistimethate was low (6 million IU equivalent to about 180 mg colistin base activity). Additionally, approximately two-thirds of patients in both groups received antibiotics other than those being investigated; more than 70% of the monotherapy group received other antibiotics (16% received meropenem).[77] Future studies comparing monotherapy with combination therapy should limit the use of other antimicrobials with in-vitro synergistic activity outside study drugs.

The benefit of polymyxin-based combination therapy compared with monotherapy needs to be better identified via well designed, randomised controlled trials (RCTs). To increase the probability of identifying a clinically useful combination, such studies should use appropriate doses of the polymyxin and other antibiotic, because suboptimum plasma concentrations of either antibiotic might conceal an otherwise beneficial combination. Two large RCTs (one in Europe and one in the USA [NCT01732250 and NCT01597973 at ClinicalTrials.gov]) are underway to examine colistin in combination with a carbapenem, versus colistin alone.

Nebulised polymyxins

Clinical studies have suggested that direct delivery of polymyxins to the lungs could have benefit for the treatment of pneumonia.[78–81] A clinical study has shown that, compared with intravenous administration, nebulisation of colistimethate results in substantially higher concentrations of colistin in sputum relative to those in plasma.[82] Results of preclinical studies have shown that administration of colistimethate into the

airways results in concentrations of colistin in lung epithelial-lining fluid that are very much higher than those in plasma.[83,84] Targeted delivery of polymyxins to the airways to maximise concentrations in lung fluids while minimising plasma concentrations (and potentially nephrotoxic effects) would be expected to be advantageous. The role of nebulised polymyxins alone or in combination with intravenous polymyxins for the treatment of pneumonia warrants further investigation in appropriate prospective studies. Careful selection of nebuliser type will be needed to optimise drug delivery to the lungs.[85]

Amelioration and management of nephrotoxic effects

Nephrotoxic effects are the major dose-limiting adverse effects of the polymyxins.[35,86] Future studies should be directed at means to ameliorate these toxic effects thereby widening the therapeutic window of the polymyxins. Management strategies are needed for patients who develop nephrotoxic effects while receiving polymyxins, recognising that the strategies might differ between colistimethate and polymyxin B. On one hand, decreasing the daily dose of colistimethate in a patient with declining kidney function could be tailored to maintain a pre-existing (desired), steady-state plasma concentration of formed colistin.[35] On the other hand, a reduction in the daily dose of polymyxin B in such a patient would lead to a lower steady-state plasma concentration,[44] with diminished antibacterial activity expected.

Infection control and antimicrobial stewardship programmes

In view of the last-line status of the polymyxins,[4–6,16–18,21,22] effective programmes for this specialty are urgently needed.[87–89] Infection control and antimicrobial stewardship programmes to prevent the spread of polymyxin-resistant organisms need to be developed and tested using rigorous methods. Optimum strategies for the use of active surveillance and rapid diagnostics to prevent the spread of polymyxin resistance need to be investigated. Management strategies to prevent the emergence and spread of polymyxin-resistant pathogens are needed at an individual patient level, and at institutional and public health levels.

Conclusions

The key areas identified as requiring attention (panel) provide a framework for action regarding the continued use of polymyxins, and are intended to help with the effective and safe use of these important antibiotics. Because of cross-resistance between colistin and polymyxin B, it is crucial to optimise the use of each of them to prolong their useful life as a class. Several key areas will need active cooperation and goodwill across several sectors—eg, regulators, manufacturers, journal editors, clinicians, and researchers. The proposed areas of preclinical, translational, and clinical research intensity are to address gaps in our understanding of how to best use the existing polymyxins; some of these studies will also help with the discovery of better, next-generation polymyxin-like antibiotics. In the battle against rapidly emerging Gram-negative superbugs with diminishing therapeutic options, we should pursue all possible approaches to increase the effectiveness of the last-line polymyxins and minimise resistance. We look forward to progress being made across all areas.

Panel: High-priority issues regarding use of polymyxins that need attention

Factors negatively affecting the safe and effective use of the polymyxins

- Multiple conventions used to describe doses of colistin
- Inadequate pharmacopoeial standards exist for polymyxins
- Outdated and diverse product information
- Low availability of colistin and polymyxin B in some parts of the world
- Inappropriate use of polymyxins
- Uncertainties relating to susceptibility testing and breakpoints
- Absence of therapeutic drug monitoring in routine clinical practice

Research needed to fill gaps in knowledge

- Prospective studies using therapeutic drug monitoring and adaptive feedback control
- Studies of mechanisms of action, resistance, and toxicity
- Pharmacokinetic studies in special patient populations
- Large-scale clinical studies with polymyxin B
- Colistin versus polymyxin B
- Combination versus monotherapy
- Nebulised polymyxins
- Amelioration and management of nephrotoxicity
- Infection control and antimicrobial stewardship programmes

Search strategy and selection criteria

We searched PubMed without language restrictions for articles published between Jan 1, 1945 and June 20, 2014. The search terms were "polymyxin", "colistin" or "polymyxin E", "colistin methanesulphonate" or "colistimethate", and "polymyxin B".

Contributors

Each author of this consensus statement was a member of the organising committee or an invited speaker at the 1st International Conference on Polymyxins held in Prato, Italy, or both. RLN did the literature search, and all authors contributed to the preparation of this report.

Declaration of interests

We declare no competing interests.

References

1 Bassetti M, Merelli M, Temperoni C, Astilean A. New antibiotics for bad bugs: where are we? *Ann Clin Microbiol Antimicrob* 2013; **12:** 22.

2 Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48:** 1–12.

3 Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infect Dis* 2006; **6:** 589–601.

4 Zavascki AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *J Antimicrob Chemother* 2007; **60:** 1206–15.

5 Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. *Clin Microbiol Rev* 2008; **21:** 449–65.

6 Couet W, Gregoire N, Marchand S, Mimoz O. Colistin pharmacokinetics: the fog is lifting. *Clin Microbiol Infect* 2012; **18:** 30–39.

7 Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin Infect Dis* 2005; **40:** 1333–41.

8 Gales AC, Jones RN, Sader HS. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006–09). *J Antimicrob Chemother* 2011; **66:** 2070–74.

9 Sader HS, Farrell DJ, Flamm RK, Jones RN. Antimicrobial susceptibility of Gram-negative organisms isolated from patients hospitalized in intensive care units in United States and European hospitals (2009-2011). *Diagn Microbiol Infect Dis* 2014; **78:** 443–48.

10 Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. *Int J Antimicrob Agents* 2005; **25:** 11–25.

11 Velkov T, Thompson PE, Nation RL, Li J. Structure-activity relationships of polymyxin antibiotics. *J Med Chem* 2010; **53:** 1898–916.

12 Velkov T, Roberts KD, Nation RL, Thompson PE, Li J. Pharmacology of polymyxins: new insights into an 'old' class of antibiotics. *Future Microbiol* 2013; **8:** 711–24.

13 Bergen PJ, Li J, Rayner CR, Nation RL. Colistin methanesulfonate is an inactive prodrug of colistin against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2006; **50:** 1953–58.

14 Nation RL, Li J, Cars O, et al. Consistent global approach on reporting of colistin doses to promote safe and effective use. *Clin Infect Dis* 2013; **58:** 139–41.

15 Bergen PJ, Landersdorfer CB, Lee HJ, Li J, Nation RL. 'Old' antibiotics for emerging multidrug-resistant bacteria. *Curr Opin Infect Dis* 2012; **25:** 626–33.

16 Bergen PJ, Landersdorfer CB, Zhang J, et al. Pharmacokinetics and pharmacodynamics of 'old' polymyxins: what is new? *Diagn Microbiol Infect Dis* 2012; **74:** 213–23.

17 Bergen PJ, Li J, Nation RL. Dosing of colistin-back to basic PK/PD. *Curr Opin Pharmacol* 2011; **11:** 464–69.

18 Nation RL, Li J. Optimizing use of colistin and polymyxin B in the critically ill. *Semin Respir Crit Care Med* 2007; **28:** 604–14.

19 Nation RL, Li J. Colistin in the 21st century. *Curr Opin Infect Dis* 2009; **22:** 535–43.

20 1st International Conference on Polymyxins. Prato, Italy, 2–4 May, 2013. http://monash.edu/pharm/about/events/polymyxins (accessed June 20, 2014).

21 Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* 2006; **10:** R27.

22 Falagas ME, Kasiakou SK, Tsiodras S, Michalopoulos A. The use of intravenous and aerosolized polymyxins for the treatment of infections in critically ill patients: a review of the recent literature. *Clin Med Res* 2006; **4:** 138–46.

23 Institute for Safe Medication Practices (ISMP). Warning! Dosing confusion with colistimethate for injection. 2011. http://www.ashp.org/DocLibrary/Policy/PatientSafety/NANAlert-Colistimethatesodium.aspx (accessed June 20, 2014).

24 He H, Li JC, Nation RL, et al. Pharmacokinetics of four different brands of colistimethate and formed colistin in rats. *J Antimicrob Chemother* 2013; **68:** 2311–17.

25 Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K. Stability of colistin and colistin methanesulfonate in aqueous media and plasma studied by high-performance liquid chromatography. *Antimicrob Agents Chemother* 2003; **47:** 1364–70.

26 Monographs for colistimethate sodium and colistin sulfate. In: United States Pharmacopeia 36. Rockville MD: The United States Pharmacopeial Convention, 2013: 3098–3100.

27 Monograph for polymyxin B sulfate. In: United States Pharmacopeia 36. Rockville MD: The United States Pharmacopeial Convention, 2013: 4826–4827.

28 Monographs for colistimethate sodium and colistin sulfate. In: European Pharmacopoeia 8.0. Strasbourg: Directorate for the Quality of Medicines and HealthCare of the Council of Europe, 2014: 1960–1962.

29 Monograph for polymyxin B sulfate. In: European Pharmacopoeia 8.0. Strasbourg: Directorate for the Quality of Medicines and HealthCare of the Council of Europe, 2014: 3055–3056.

30 Bode-Boger SM, Schopp B, Troger U, Martens-Lobenhoffer J, Kalousis K, Mailander P. Intravenous colistin in a patient with serious burns and borderline syndrome: the benefits of therapeutic drug monitoring. *Int J Antimicrob Agents* 2013; **42:** 357–60.

31 Spapen HD, Honore PM, Gregoire N, et al. Convulsions and apnoea in a patient infected with New Delhi metallo-beta-lactamase-1 *Escherichia coli* treated with colistin. *J Infect* 2011; **63:** 468–70.

32 Li J, Rayner CR, Nation RL, et al. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. *Antimicrob Agents Chemother* 2005; **49:** 4814–15.

33 Dudhani RV, Nation RL, Li J. Evaluating the stability of colistin and colistin methanesulphonate in human plasma under different conditions of storage. *J Antimicrob Chemother* 2010; **65:** 1412–15.

34 Theuretzbacher U. Product information for parenteral colistin varies substantially across Europe. *J Antimicrob Chemother* 2014; **69:** 1987–92.

35 Garonzik SM, Li J, Thamlikitkul V, et al. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* 2011; **55:** 3284–94.

36 Plachouras D, Karvanen M, Friberg LE, et al. Population pharmacokinetic analysis of colistin methanesulphonate and colistin after intravenous administration in critically ill patients with Gram-negative bacterial infections. *Antimicrob Agents Chemother* 2009; **53:** 3430–36.

37 Mohamed AF, Karaiskos I, Plachouras D, et al. Application of a loading dose of colistin methanesulphonate (CMS) in critically ill patients: population pharmacokinetics, protein binding and prediction of bacterial kill. *Antimicrob Agents Chemother* 2012; **56:** 4241–49.

38 Rolain JM, Parola P, Cornaglia G. New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? *Clin Microbiol Infect* 2010; **16:** 1699–701.

39 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:** 5046–54.

40 Luna CM, Aruj P, Niederman MS, et al. Appropriateness and delay to initiate therapy in ventilator-associated pneumonia. *Eur Respir J* 2006; **27:** 158–64.

41 Kumar A, Ellis P, Arabi Y, et al. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. *Chest* 2009; **136:** 1237–48.

42 Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; **34:** 1589–96.

43 Couet W, Gregoire N, Gobin P, et al. Pharmacokinetics of colistin and colistimethate sodium after a single 80-mg intravenous dose of CMS in young healthy volunteers. *Clin Pharmacol Ther* 2011; **89:** 875–79.

44 Sandri AM, Landersdorfer CB, Jacob J, et al. Population pharmacokinetics of intravenous polymyxin B in critically ill patients: Implications for selection of dosage regimens. *Clin Infect Dis* 2013; **57:** 524–31.

45 Oliveira MS, Prado GV, Costa SF, Grinbaum RS, Levin AS. Polymyxin B and colistimethate are comparable as to efficacy and renal toxicity. *Diagn Microbiol Infect Dis* 2009; **65:** 431–34.

46 Akajagbor DS, Wilson SL, Shere-Wolfe KD, Dakum P, Charurat ME, Gilliam BL. Higher incidence of acute kidney injury with intravenous colistimethate sodium compared with polymyxin b in critically ill patients at a tertiary care medical center. *Clin Infect Dis* 2013; **57:** 1300–03.

47 Phe K, Lee Y, McDaneld PM, et al. Comparison of nephrotoxicity rates associated with colistimethate versus polymyxin B therapy: in vitro assessment and a multicenter cohort study. *Antimicrob Agents Chemother* 2014; **58:** 2740–46.

48 Halaby T, Al Naiemi N, Kluytmans J, van der Palen J, Vandenbroucke-Grauls CM. Emergence of colistin resistance in *Enterobacteriaceae* after the introduction of selective digestive tract decontamination in an intensive care unit. *Antimicrob Agents Chemother* 2013; **57:** 3224–29.

49 de Smet AM, Kluytmans JA, Cooper BS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *N Engl J Med* 2009; **360:** 20–31.

50 European Medicines Agency. Use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health. Report EMA/755938/2012. 2013. http://www.ema.europa.eu/docs/en_GB/document_library/Report/2013/07/WC500146813.pdf (accessed June 20, 2014).

51 International Standards Organization. ISO 20776-1:2006 Clinical laboratory testing and in vitro diagnostic test systems–susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices–part 1: reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. Geneva: International Organization for Standardization, 2013.

52 Acar JF. Procedures for testing antibiotics in agar media: theoretical considerations. In: Lorian V, ed. Antibiotics in Laboratory Medicine, 1st edition. Baltimore: Williams and Wilkins, 1980: 124–54.

53 Albur M, Bowker NK, MacGowan A. Colistin MIC testing: the effect of different types of microtitre trays and surfactant Tween 80 in broth microdilution method. 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy; San Francisco, CA, USA; Sept 9–15, 2012. Abstract D-763.

54 Karvanen M, Malmberg C, Mohamed A, Lagerbäck P, Friberg LE, Cars O. Colistin is extensively lost during normal experimental conditions. 51st Interscience Conference on Antimicrobial Agents and Chemotherapy; Chicago, IL, USA; Sept 17–20, 2011. Abstract D-690.

55 Arhin FF, Sarmiento I, Belley A, et al. Effect of polysorbate 80 on oritavancin binding to plastic surfaces: implications for susceptibility testing. *Antimicrob Agents Chemother* 2008; **52:** 1597–603.

56 Rennie RP, Koeth L, Jones RN, et al. Factors influencing broth microdilution antimicrobial susceptibility test results for dalbavancin, a new glycopeptide agent. *J Clin Microbiol* 2007; **45:** 3151–54.

57 Hindler JA, Humphries RM. Colistin MIC variability by method for contemporary clinical isolates of multidrug-resistant Gram-negative bacilli. *J Clin Microbiol* 2013; **51:** 1678–84.

58 Sader HS, Rhomberg PR, Flamm RK, Jones RN. Use of a surfactant (polysorbate 80) to improve MIC susceptibility testing results for polymyxin B and colistin. *Diagn Microbiol Infect Dis* 2012; **74:** 412–14.

59 Clinical and Laboratory Standards Institute (CLSI). Development of in vitro susceptibility testing criteria and quality control parameters: approved guideline. Third edition. CLSI document M23-A3. Wayne, PA: 2008: 1–45.

60 Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. *Clin Microbiol Rev* 2007; **20:** 391–408.

61 European Committee on Antimicrobial Susceptibility and Testing: antimicrobial wild type distributions of microorganisms. 2013. http://mic.eucast.org/Eucast2/ (accessed June 20, 2014).

62 Mohamed AF, Cars O, Friberg LE. A pharmacokinetic/pharmacodynamic model developed for the effect of colistin on *Pseudomonas aeruginosa* in vitro with evaluation of population pharmacokinetic variability on simulated bacterial killing. *J Antimicrob Chemother* 2014; **69:** 1350–61.

63 Roberts JA, Abdul-Aziz MH, Lipman J, et al. Individualised antibiotic dosing for patients who are critically ill: challenges and potential solutions. *Lancet Infect Dis* 2014; **14:** 498–509.

64 Roberts JA, Norris R, Paterson DL, Martin JH. Therapeutic drug monitoring of antimicrobials. *Br J Clin Pharmacol* 2012; **73:** 27–36.

65 Jelliffe RW, Schumitzky A, Van Guilder M, et al. Individualizing drug dosage regimens: roles of population pharmacokinetic and dynamic models, Bayesian fitting, and adaptive control. *Ther Drug Monit* 1993; **15:** 380–93.

66 Azad MAK, Finnin BA, Poudyal A, et al. Polymyxin B induces apoptosis in kidney proximal tubular cells. *Antimicrob Agents Chemother* 2013; **57:** 4329–35.

67 Kwa AL, Lim TP, Low JG, et al. Pharmacokinetics of polymyxin B1 in patients with multidrug-resistant Gram-negative bacterial infections. *Diagn Microbiol Infect Dis* 2008; **60:** 163–67.

68 Petrosillo N, Ioannidou E, Falagas ME. Colistin monotherapy vs. combination therapy: evidence from microbiological, animal and clinical studies. *Clin Microbiol Infect* 2008; **14:** 816–27.

69 Henry R, Vithanage N, Harrison P, et al. Colistin-resistant, lipopolysaccharide-deficient *Acinetobacter baumannii* responds to lipopolysaccharide loss through increased expression of genes involved in the synthesis and transport of lipoproteins, phospholipids and poly-β-1,6-N-acetylglucosamine. *Antimicrob Agents Chemother* 2012; **56:** 59–69.

70 Velkov T, Bergen PJ, Lora-Tamayo J, Landersdorfer CB, Li J. PK/PD models in antibacterial development. *Curr Opin Microbiol* 2013; **16:** 573–79.

71 Bulitta JB, Landersdorfer CB, Forrest A, et al. Relevance of pharmacokinetic and pharmacodynamic modelling to clinical care of critically ill patients. *Curr Pharm Biotechnol* 2011; **12:** 2044–61.

72 Ostronoff M, Ostronoff F, Sucupira A, et al. Multidrug-resistant *Pseudomonas aeruginosa* infection in neutropenic patients successfully treated with a combination of polymyxin B and rifampin. *Int J Infect Dis* 2006; **10:** 339–40.

73 Sobieszczyk ME, Furuya EY, Hay CM, et al. Combination therapy with polymyxin B for the treatment of multidrug-resistant Gram-negative respiratory tract infections. *J Antimicrob Chemother* 2004; **54:** 566–69.

74 Falagas ME, Rafailidis PI, Kasiakou SK, Hatzopoulou P, Michalopoulos A. Effectiveness and nephrotoxicity of colistin monotherapy vs. colistin-meropenem combination therapy for multidrug-resistant Gram-negative bacterial infections. *Clin Microbiol Infect* 2006; **12:** 1227–30.

75 Kasiakou SK, Michalopoulos A, Soteriades ES, Samonis G, Sermaides GJ, Falagas ME. Combination therapy with intravenous colistin for management of infections due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. *Antimicrob Agents Chemother* 2005; **49:** 3136–46.

76 Aydemir H, Akduman D, Piskin N, et al. Colistin vs the combination of colistin and rifampicin for the treatment of carbapenem-resistant Acinetobacter baumannii ventilator-associated pneumonia. *Epidemiol Infect* 2013; **141:** 1214–22.

77 Durante-Mangoni E, Signoriello G, Andini R, et al. Colistin and rifampicin compared with colistin alone for the treatment of serious infections due to extensively drug-resistant *Acinetobacter baumannii*: a multicenter, randomized clinical trial. *Clin Infect Dis* 2013; **57:** 349–58.

78 Kwa AL, Loh C, Low JG, Kurup A, Tam VH. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2005; **41:** 754–57.

79 Michalopoulos A, Kasiakou SK, Mastora Z, Rellos K, Kapaskelis AM, Falagas ME. Aerosolized colistin for the treatment of nosocomial pneumonia due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. *Crit Care* 2005; **9:** R53–59.

80 Lu Q, Luo R, Bodin L, et al. Efficacy of high-dose nebulized colistin in ventilator-associated pneumonia caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Anesthesiology* 2012; **117:** 1335–47.

81 Kalin G, Alp E, Coskun R, Demiraslan H, Gundogan K, Doganay M. Use of high-dose IV and aerosolized colistin for the treatment of multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia: do we really need this treatment? *J Infect Chemother* 2012; **18:** 87–77.

82 Yapa S, Li J, Patel K, et al. Pulmonary and systemic pharmacokinetics of inhaled and intravenous colistin methanesulfonate in cystic fibrosis patients: targeting advantage of inhalational administration. *Antimicrob Agents Chemother* 2014; **58:** 2570–79.

83 Yapa S, Li J, Porter CJ, Nation RL, Patel K, McIntosh MP. Population pharmacokinetics of colistin methanesulfonate in rats: achieving sustained lung concentrations of colistin for targeting respiratory infections. *Antimicrob Agents Chemother* 2013; **57:** 5087–95.

84 Marchand S, Gobin P, Brillault J, et al. Aerosoltherapy with colistin methanesulfonate: a biopharmaceutical issue illustrated in rats. *Antimicrob Agents Chemother* 2010; **54:** 3702–07.

85 Daniels T, Mills N, Whitaker P. Nebuliser systems for drug delivery in cystic fibrosis. *Cochrane Database Syst Rev* 2013; **4:** CD007639.

86 Hartzell JD, Neff R, Ake J, et al. Nephrotoxicity associated with intravenous colistin (colistimethate sodium) treatment at a tertiary care medical center. *Clin Infect Dis* 2009; **48:** 1724–28.

87 Marchaim D, Chopra T, Pogue JM, et al. Outbreak of colistin-resistant, carbapenem-resistant *Klebsiella pneumoniae* in metropolitan Detroit, Michigan. *Antimicrob Agents Chemother* 2011; **55:** 593–99.

88 Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant *Enterobacteriaceae*: epidemiology and prevention. *Clin Infect Dis* 2011; **53:** 60–67.

89 Karageorgopoulos DE, Falagas ME. Current control and treatment of multidrug-resistant *Acinetobacter baumannii* infections. *Lancet Infect Dis* 2008; **8:** 751–62.