

Polymyxins: A Review of the Current Status Including Recent Developments

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Abstract

Introduction: Polymyxins have become the drug of choice for treatment of multidrug-resistant gram-negative bacilli infections in Singapore, simply because these pathogens are only susceptible to either aminoglycosides and polymyxins, or polymyxins only. Furthermore, there is no new antibiotic in the pipeline that targets these difficult-to-treat infections. **Materials and Methods:** All published literatures (up to end of February 2008) regarding polymyxins are included for review. **Results:** This review serves to give a summary of polymyxins from the current available literature, highlighting relevant clinical studies and information that help to guide informed prescription of polymyxins, should the need arise. **Conclusions:** However, there are substantial information gaps that needed to be filled urgently, to preserve the clinical utility of this very last line of antibiotic.

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Key words: *Acinetobacter baumannii*, Colistin, Multidrug resistance, Polymyxin B, *Pseudomonas aeruginosa*

Introduction

Polymyxins are polypeptide antibiotic that becomes available for clinical use in the 1960s, but was replaced in the 1970s by antibiotics considered less toxic. Presently, polymyxins have re-emerged as a “no choice” alternative for treatment of multidrug-resistant (MDR) gram-negative bacilli infections, which are not infrequent, in Singapore and Asia Pacific regions.¹ Gram-negative bacilli, particularly those with a high level of intrinsic resistance to many antibiotic classes and great ability to acquire resistance, such as *Pseudomonas aeruginosa* (PA) and *Acinetobacter baumannii* (AB), cause infections that are extremely difficult to treat. To make matters worse, no new antibiotic is in the drug development pipeline for multidrug-resistant gram-negative bacteria (unlike the circumstances for multidrug-resistant gram-positive bacteria). As a result, resurgence of old polymyxins is seen as a last resort for MDR gram-negative infections.

Polymyxin B was produced by the growth of *Bacillus polymyxa*, first made available for clinical use in 1947, and polymyxin E (colistin) was produced by the growth of *Bacillus polymyxa subsp. colistinus* in 1949.² We sought to

review the recent developments and evidence on polymyxins for use in clinical settings in view of multidrug-resistant gram-negative bacilli infections locally.

Physical Chemistry

Polymyxins, having a hydrophobic fatty acid moiety and a polar moiety of 5 unmasked α -amino groups, are amphipathic. Its basic pKa is approximately 10.

The only difference in the structure between polymyxin B and polymyxin E (colistin) lies in the amino acid components. Both polymyxins contain a mixture of D- and L-amino acids arranged as a cyclic heptapeptide ring with a tripeptide side chain, with the side chain covalently bound to a fatty acid via an acyl group. The presence of D-Leucine (highlighted in Fig. 1b) in the molecule of colistin distinguishes colistin from polymyxin B. D-Phenylalanine replaces Leucine in polymyxin B (Fig. 1a).

Existing and Future Commercial Formulations

For parenteral and inhalation administrations, polymyxin B is formulated as sulphate salt, while colistin is formulated as sodium salt of colistin methanesulphonate, which is an

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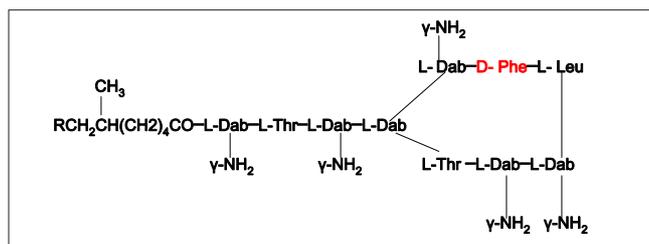


Fig. 1a. Polymyxin B.

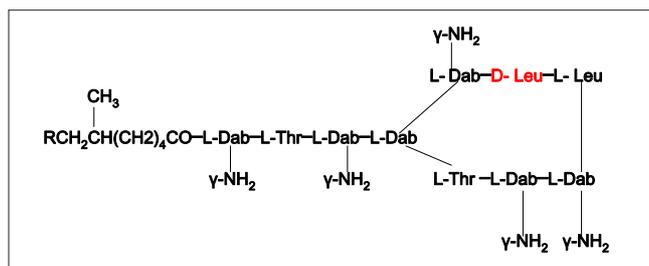


Fig. 1b. Colistin.

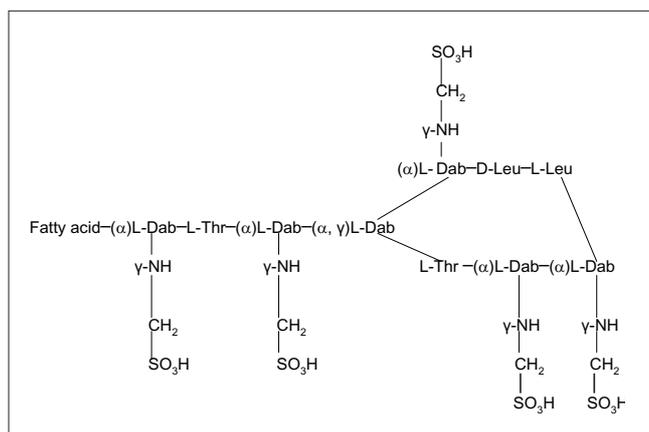


Fig. 1c. Colistimethate

inactive pro-drug that undergoes hydrolysis in vivo and in vitro to form the active colistin entity.³

Colistin is administered either orally or topically as colistin sulphate. Each milligram of pure polymyxin B base has an equivalency of 10,000 international units of polymyxin B. Each milligram of pure colistin base in colistin sulfate and in colistimethate sodium is equivalent to 30,000 international units and 12,500 units of colistin,

Table 3. Susceptibility Profile of Polymyxins

Most active against	Resistant to
Most gram-negative aerobic bacilli, including <i>Acinetobacter</i> species, <i>Pseudomonas aeruginosa</i> , <i>Klebsiella</i> species, <i>Enterobacter</i> species, <i>Escherichia coli</i> , <i>Salmonella</i> species, <i>Shigella</i> species, <i>Citrobacter</i> species, <i>Yersinia pseudotuberculosis</i> , <i>Haemophilus influenzae</i> , <i>Bordetella pertussis</i> , <i>Legionella pneumophila</i>	1. Gram-negative bacteria such as <i>Burkholderia cepacia</i> complex, <i>Burkholderia pseudomallei</i> , <i>Proteus</i> spp., <i>Providencia</i> spp., <i>Morganella morganii</i> and <i>Serratia marcescens</i> . ^{6,7} 2. All gram-positive bacteria, anaerobes, pathogenic <i>Neisseria</i> spp. (including meningococci and gonococci), <i>Moraxella catarrhalis</i> , <i>Helicobacter pylori</i> , <i>Vibrio</i> spp. and <i>Brucella</i> spp. ⁹

Table 1. Components of Polymyxin B

Polymyxin	M _r	Molecular formula
B1	1204	C ₅₆ H ₉₈ N ₁₆ O ₁₃
B2	1190	C ₅₅ H ₉₆ N ₁₆ O ₁₃
B3	1190	C ₅₅ H ₉₆ N ₁₆ O ₁₃
B1-I	1204	C ₅₆ H ₉₈ N ₁₆ O ₁₃

Sum of polymyxins B1, B2, B3 and B1-I: constitutes minimum 80.0% (Adapted from European Pharmacopoeia 5.08; 15_monographs_l-p ; polymyxin_b_sulphate; Polymyxin_B_sulphate_01-2006:0203 corrected 5.7)

Table 2. Components of Polymyxin E (Colistin)

Polymyxin	M _r	Molecular formula
E1	1170	C ₅₃ H ₁₀₀ N ₁₆ O ₁₃
E2	1155	C ₅₂ H ₉₈ N ₁₆ O ₁₃
E3	1155	C ₅₂ H ₉₈ N ₁₆ O ₁₃
E1-I	1170	C ₅₃ H ₁₀₀ N ₁₆ O ₁₃
E1-7MOA	1170	C ₅₃ H ₁₀₀ N ₁₆ O ₁₃

Sum of E1, E2, E3, E1-I and E1-7MOA: constitutes minimum 77.0% (Adapted from European Pharmacopoeia 5.08; 13_monographs_a-c; colistin_sulphate ; Colistin_sulphate_01-2006:0320 corrected 5.7)

respectively. Thus, the terms colistin and colistimethate are not interchangeable and formulations of colistin should be fully described in all clinical studies. Colistimethate sodium (CMS) is less potent and less toxic than colistin sulphate.

Table 1 illustrates the different molecular formula of the polypeptides that can be found in Polymyxin B. Table 2 illustrates the different molecular formula of the polypeptides that can be found in colistin sulfate, which is a mixture of sulphates of polypeptides. Hence, polymyxin B, colistin sulfate and colistimethate sodium vary in the ratio of their components from batch to batch commercially.

The development of different formulations of polymyxin B, to enhance their antimicrobial activities while minimising their toxic effects, is currently ongoing. A recent pilot study evaluated the antimicrobial effectiveness of liposomal polymyxin B against gram-negative resistant strains. The results of this study are promising, namely; 1) There is

spontaneous release of polymyxin B from the liposomal formulation, 2) Minimum inhibitory concentration (MIC) of liposomal polymyxin B maybe lower to that of free polymyxin B, 3) Penetration of polymyxin B into MDR PA is higher (when compared to conventional polymyxin B), following administration of liposomal formulation.⁴ Another study reported that lipid nanoparticles and nanoemulsion formulations are promising delivery vectors of antimicrobials like polymyxin B. Lipid nanoparticles could give an initial as well as sustained effect while the nanoemulsion was capable of exerting potent effect for a shorter period of time.⁵

Mechanism of Action

Polymyxins, which behave like detergents, are rapidly bactericidal. Their target site is bacterial outer cell membrane. Polymyxins increase permeability of the cell envelope, which lead to leakage of cell contents, and subsequently, cell death. It involves initial association of polymyxins through electrostatic interactions between cationic polypeptide (polymyxins) and anionic lipopolysaccharide (LPS) molecules in the membrane of gram-negative bacteria before leading to the derangement of cell membrane by displacing magnesium (Mg^{+2}) and calcium (Ca^{+2}) (stabilisers of LPS molecules) from the negatively charged LPS.

Polymyxins have potent anti-endotoxin activity. They bind to endotoxin, the lipid A portion of LPS molecules of gram-negative bacteria and neutralises LPS. However, the mechanism of septic shock prevention is unclear. It is thought that plasma endotoxin is immediately bound by LPS-binding protein, and the complex is quickly bound to cell-surface CD14.

Spectrum of Activity

The spectrum of activity of polymyxins is summarised in Table 3. *Acinetobacter* species and *P. aeruginosa* that are resistant to all the other classes of antibiotics, are still intrinsically susceptible to polymyxins.^{6,7} *Stenotrophomonas maltophilia* is usually susceptible to polymyxins, although some strains can be resistant.^{6,7} Polymyxin B is active against some species of *Aeromonas*, but *Aeromonas jandaei* is resistant and *Aeromonas hydrophilia* has inducible resistance.^{8,9} *Campylobacter* spp. vary in their susceptibility to polymyxin B and the susceptibility of *Bartonella* spp. is borderline.⁸

Susceptibility Testing, Breakpoints

The susceptibility testing methods and interpretation standards for both polymyxins have already been developed in Europe and the United Kingdom for a period of time, but are only published recently by the Clinical and Laboratory Standards Institute (CLSI) in the United States. The poor

correlation between different susceptibility test methods for polymyxins, is probably due to the inconsistent diffusion of polymyxins in agar and the fact that the in vitro activity of polymyxins is affected by cation concentrations in agar.^{6,7,10} Hence, broth dilutions, which are the reference methods in most susceptibility studies, may be the best susceptibility testing methods.

Polymyxin B sulfate is the adopted testing agent for polymyxin B. In the Kirby-Bauer method or disc susceptibility testing, a 300-unit (or 30 mcg) disc of polymyxin B is used. The resistance breakpoint for polymyxin B sulfate of ≥ 4 mg/L was last available in the Approved Standard M2-A2 S2 document provided by the CLSI (formerly National Committee on Clinical Laboratory Standards, NCCLS) in 1981;¹¹ however, with the very limited use of polymyxins, the published information was subsequently withdrawn until recently. CLSI's statement for the susceptibility testing of polymyxin B (and colistin) in 2007¹² are: 1) breakpoints for *P. aeruginosa* are: susceptibility, MIC ≤ 2 mg/L; intermediate, MIC = 4 mg/L; and resistance, MIC ≥ 8 mg/L; the zone diameter interpretative standards for the disc diffusion method were added: they are ≤ 1 mm indicating resistance and ≥ 12 mm, susceptibility; and 2) breakpoints for *Acinetobacter* spp. are: susceptibility, MIC < 4 mg/dL; resistance, MIC ≥ 4 mg/L. CLSI recommendations for *Enterobacteriaceae* do not exist currently.¹²

For colistin, colistin sulfate is the commonly adopted testing agent, despite the fact that it is more potent and is used less often clinically than colistimethate sodium. It is not known if the data from in vitro testing with the sulfate formulation are predictive of vivo activity of colistimethate sodium, but it should be noted that colistimethate sodium is converted in part to colistin base following administration. In Kirby-Bauer method or disc susceptibility testing, a 10-mcg disc of colistin sulfate is used. With respect to the breakpoints (for systemic use only) for susceptibility based on colistin sulfate, the British Society for Antimicrobial Chemotherapy has adopted ≤ 4 mg/L and ≥ 8 mg/L for susceptible and resistant strains, respectively. In contrast, the Société Française de Microbiologie has advocated ≤ 2 mg/L and ≥ 4 mg/L as the susceptibility and resistance breakpoints, respectively. The German Deutsches Institut für Normung adopts breakpoints that vary considerably with the rest: susceptible ≤ 0.5 mg/L, intermediate 1-2 mg/L, resistant ≥ 4 mg/L.

There is cross-resistance between polymyxin B and colistin. Bacteria can develop resistance to polymyxin B through the same mechanisms as those to colistin. These mechanisms mainly involve alterations of the outer membrane of the bacterial cell via reduction in LPS, reduced expression of specific outer membrane proteins,

reduction in cell envelope Mg⁺² and Ca⁺² contents and lipid alterations.¹³⁻¹⁵ An efflux pump/potassium system in *Yersinia* species has also been reported as a possible way to develop resistance to polymyxin B.¹⁶ While a strain of colistinase (an enzyme that inactivates colistin)-producing *Bacillus polymyxa* (subspecies *colistinus*) has been reported, no enzymatic resistance to polymyxin B has been mentioned in the literature.¹⁷

Modifications of lipid A, with 4-amino-4-deoxy-L-arabinose and/or phosphoethanolamine controlled by PmrA/PmrB, reducing the net charge of LPS are found in *P. aeruginosa*, *Salmonella enterica* serovar *Typhimurium*, *E. coli*, and *Yersinia pestis*, which are resistant to polymyxins.¹⁸⁻²¹ The presence of capsule in *K. pneumoniae* is needed for polymyxin resistance.²² In *S. Typhimurium*, the gene mig-14 is also involved in polymyxin resistance with the specific mechanism of action undefined.²⁰ In *Vibrio cholerae*, resistance to polymyxin is dependent on the outer-membrane porin, OmpU.²³

Heteroresistance and Resistance

Colistin heteroresistance has been reported among *Acinetobacter* isolates which are colistin susceptible.^{24,25} The proportion of cells exhibiting heteroresistance to colistin was significantly higher among isolates recovered from patients treated with colistin.²⁵

Recent data indicated that disk diffusion was an unreliable method to measure susceptibility to colistin, whereby high error rates and low levels of reproducibility were observed in the disk diffusion test.²⁶ The colistin Etest, agar dilution, and the VITEK 2 showed a high level of agreement with the broth microdilution reference method in the study.²⁶ Heteroresistance for colistin, observed in *Enterobacter cloacae* isolates and in *A. baumannii* isolate, could be detected in the broth microdilution, agar dilution, Etest or disk diffusion test.²⁶ The VITEK 2 displayed low sensitivity in the detection of heteroresistant subpopulations of *E. cloacae*.²⁶ The VITEK 2 colistin susceptibility test can be used to determine susceptibility to colistin in isolates of genera that are known not to exhibit resistant subpopulations. However, an alternative susceptibility testing method capable of detecting heteroresistance should be used in genera known to exhibit heteroresistance.²⁶

It was reported that the ability to form biofilm in the colistin-resistant *A. baumannii* was significantly lower than in the parent strains of colistin-susceptible AB.²⁷ Interestingly, the colistin-resistant strains had substantially increased susceptibility to most of the antibiotics that are usually inactive against gram-negative bacteria, namely, rifampicin, fusidic acid, erythromycin, teicoplanin and quinupristin-dalfopristin.²⁷ A majority of colistin-resistant strains showed increased susceptibility (at least 2 dilutions

in MICs), in the absence of colistin, to beta-lactam/beta-lactamase inhibitors, cephalosporins, carbapenems, fluoroquinolones and aminoglycosides.²⁷ However, novel combinations of antibiotics, additional pharmacokinetic and pharmacodynamic evaluations of such combinations are warranted before the agents are used clinically for treatment of infection due to colistin-resistant *A. baumannii*.

In a study that aimed to assess potential risk factors for the isolation of colistin-resistant *Klebsiella pneumoniae*, *A. baumannii* and *P. aeruginosa* from hospitalised patients, it was found that age, duration of intensive care unit (ICU) stay, duration of mechanical ventilation, surgical procedures, use of colistin, use of monobactams, and duration of use of third generation cephalosporins were significantly associated with the isolation of colistin-resistant isolates.²⁸ However, the use of colistin was identified as the only independent risk factor (adjusted odds ratio = 7.78, $P < 0.001$).²⁸ Thus, the use of polymyxins should be rational to decrease the rate of emergence of infections due to bacteria that are pan-drug resistant (PDR), i.e. resistant to all available antibiotics.

Dosage

The dosage of polymyxin B and colistimethate sodium that is widely used in clinical practice is described in Table 4a. The suggested renal dosing of intravenous polymyxin B, which is used locally, is described in Table 4b.

However, it should be noted that these dosing guidelines were developed without prior reliable pharmacokinetic studies.

Pharmacokinetics

The pharmacokinetics of colistin appear to be complex and have been reviewed in detail previously.²⁹ Colistin sulphate is used topically or via oral administration. It has negligible bioavailability. Colistimethate sodium is administered intravenously, intramuscularly or via inhalation. Approximately 31.2% of colistimethate sodium in human plasma is hydrolysed to sulfomethylated derivatives and colistin in 4 hours at 37°C. Sixty per cent of colistimethate sodium is excreted unchanged via glomerular filtration approximately.²⁹ Hence, half-life is expected to prolong in renal insufficiency. With anuria, half-life was approximately 48 to 72 hours. No biliary excretion has been reported.²⁹

Serum half-life of colistimethate sodium is approximately 1.5 hours following intravenous administration and 2.75 to 3 hours following intramuscular administration in healthy subjects. Peak serum levels after intravenous administration occur within 10 minutes and are higher but decline more rapidly than those achieved after intramuscular administration.³⁰ The specific serum levels of colistimethate

sodium and colistin were not known as the microbiological assay used in this study was unable to distinguish the relative contribution of antimicrobial activity by the parent compound (colistimethate sodium) administered, any of the partial derivatives or colistin.

The pharmacokinetics of colistimethate sodium and colistin were determined specifically in another study involving cystic fibrosis patients.³¹ Mean elimination half-life and volume of distribution of colistimethate sodium were reported to be 2.1 hours and 0.34 L/kg, respectively. In contrast, the mean elimination half-life of colistin was 4.2 hours. In a patient undergoing continuous venovenous hemodiafiltration (CVVHDF), conversion of colistimethate sodium to colistin was rapid, and the terminal half-lives of colistimethate sodium and colistin were 6.8 hours and 7.5 hours, respectively.³² Considering this and also the fact that colistin shows a very modest post-antibiotic effect,³³ extended dosing intervals of modest doses may place critically ill patients on CVVHDF at substantial risk of mortality due to inadequate therapy. Approximately 1 mg/h of colistin is removed from the body by peritoneal dialysis,³⁴ with an average of 16% of the total dose removed during a 2-hour peritoneal dialysis session.³⁵ Because of this poor clearance, it was recommended that the drug should be given only at a dose of 2 mg/kg/d during peritoneal dialysis.

Colistin is about 50% bound to human plasma.^{36,37} Colistimethate sodium is tightly bound to membrane lipids of cells of many body tissues, including liver, lung, kidney, brain, heart and muscles; hence, the release of tissue-bound drug is very slow. Sulfomethylation of colistin appears to decrease not only antibacterial activity but also membrane binding. Old reports have suggested that colistin is poorly distributed to the pleural cavity, lung parenchyma, bones and CSF (15% to 25%). Relevant data regarding using polymyxins for treatment of CNS infection have been reviewed recently.³⁸

Administration of polymyxins via inhalation has been adopted and recommended to improve lung parenchyma penetration in the adjunct treatment of MDR pneumonia.³⁹ No study has been done to assess the concentrations of polymyxins achieved in the pulmonary epithelial lining fluid, which is the target site for antibiotics, in the treatment of pneumonia. The closest study that was done to evaluate the pharmacokinetics of polymyxins post-inhalation was conducted by Ratjen et al.⁴⁰ Colistimethate sodium of a single dose of 2 million units was administered via inhalation to 30 cystic fibrosis patients to assess sputum, serum and urine concentrations in a multicentre study.⁴⁰ It was reported that the serum concentrations of colistin that reached their maximum at 1.5 h after inhalation and decreased thereafter, were well below those previously reported for systemic

administration. A mean of $4.3 \pm 1.3\%$ of the inhaled dose was detected in urine. Maximum sputum concentrations of at least 10 times higher than the MIC breakpoint for *P. aeruginosa* proposed by the British Society for Antimicrobial Chemotherapy were observed. Although sputum drug concentrations decreased after a peak at 1 hour, the mean colistin concentrations were still above 4 mg/L after 12 hours. Although the above data sounds promising, it is not known if this information can be extrapolated to non-cystic fibrosis patients with MDR nosocomial pneumonia, who display a different set of pharmacokinetics parameters in the handling of drugs. Hence, pharmacokinetic data regarding inhaled polymyxins in MDR nosocomial pneumonia in the critically ill is very much warranted at this point in time.

There is less information available on the pharmacokinetics of polymyxin B, but it appears to be less complex. Most pharmaceutical formulations contain polymyxin B sulfate. The only available data, which was also often cited, was from 30 years ago, after intramuscular administration; following a 50 mg (500,000 units) dose, peak concentration of 8 µg/mL was achieved in approximately 2 hours and serum half-life was approximately 6 hours.⁴¹ However, due to the limited nature in experimental setting and methodology, we should view such data cautiously. In a recent study involving a general patient population with multidrug-resistant infections, it was found that polymyxin B1 (major constituent of polymyxin B) has: 1) serum half-life of 13 hours, 2) high serum protein binding, 96.9% and 98.4% at 20°C and 37°C, respectively, 3) PK is satisfactorily described by a 1 compartment linear model, 4) volume of distribution at approximately 1.39 L/kg.⁴²

Pharmacodynamics

Rapid and concentration-dependent killing against *P. aeruginosa* and *A. baumannii* are exhibited by polymyxin B and colistin in time-kill studies.^{33,43,44} Extensive killing of heteroresistant *A. baumannii* was reported in the initial hours of administration of colistin regardless of the regimen (intermittent administration at 8 hours, 12 hours, 24 hours, or continuous infusion), with regrowth as early as 6 hours later and emergence of resistant subpopulation.⁴⁵ Regrowth of clinical isolates of *A. baumannii* at 24 hours can occur at concentrations up to 64 times MIC.⁴⁴ A lack of post-antibiotic effect had been reported for all clinical *A. baumannii* isolates studied recently and that could imply monotherapy with colistimethate sodium and long dosage interval maybe problematic for treatment of infections caused by heteroresistant *A. baumannii*.⁴⁴ Synergistic killing was observed when colistin was used in combination with ceftazidime given at a constant infusion (at 50 µg/mL) in an in-vitro infection model of MDR *P. aeruginosa* and *A.*

Table 4a. Dosage of Polymyxin B and Colistimethate Sodium

Colistimethate sodium (CMS)	Polymyxin B
For patients who are ≤ 60 kg and with normal renal function: IV or IM: 4 to 6 mg per kg/day CMS in 3 divided doses.	For adults and children older than 2 years with normal renal function: IV: 15,000 to 25,000 units/kg daily in 2 divided doses. IM: 25,000 to 30,000 units/kg daily in 4 or 6 divided doses.
For patients who are >60 kg and with normal renal function: IV or IM: 240 to 480 mg/day (UK) or up to 720 mg/day (USA) CMS in 3 divided dosing. 2.5 to 5.0 mg/kg/day colistin base in 2-4 divided doses (USA)	Intrathecal: 50,000 units once daily for 3 to 4 days, then 50,000 units once every other day for at least 2 weeks, after cultures of cerebrospinal fluid are negative and/or glucose normal.
Inhalation: 40 mg (500,000 units) every 12 hours for patients who are ≤ 40 kg. 80 mg (1,000,000 units) every 12 hours for patients who are >40 kg.	
For recurrent pulmonary infections, the dosage of aerosolised CMS can be increased to 160 mg (2 million units) every 8 hours.	
IV: intravenously, IM: intramuscularly, CMS: colistimethate sodium	

Table 4b. Renal Dosing of IV Polymyxin B

CCT 20-50 mL/min	75-100% of the total daily dose of 2.5 mg/kg (25,000 U/kg)
CCT 5 - 20 mL/min	50% of the total daily dose of 2.5 mg/kg (25,000 U/kg)
CCT <5 mL/min	15% of total daily dose of 2.5 mg/kg (25,000 U/kg)

baumannii.^{46,47} Combination therapy with intravenous colistin and other antimicrobial agents have been used with success in difficult-to-treat multidrug-resistant nosocomial gram-negative infections, e.g. osteomyelitis.⁴⁸⁻⁵² Recent data reported that colistimethate sodium is inactive; the observed activity was due to hydrolysis to colistin.⁵³

In *in vitro* *P. aeruginosa* infection model studies where the concentration of polymyxins fluctuates over time with linear elimination and repeated dosing, regrowth were readily observed after an initial decline in bacterial burden with polymyxin B monotherapy as well.⁴³ A dose fractionation study design (using identical daily dose but once, twice or three time daily administration) was used to explore if the frequency of dosing has an impact on the bactericidal activity of polymyxin B. Elevated dosing of polymyxin B (at 8x the most commonly used clinical dose) was also attempted; regrowth could be suppressed with a wild-type strain, but not with a clinical multidrug-resistant *P. aeruginosa* strain.⁴³ It was reported that the daily dose (but not dosing frequency) was the most important factor determining the antimicrobial activity of polymyxin B, and AUC/MIC (area under the curve/minimum inhibitory concentration) ratio appeared to be the pharmacodynamic parameter most closely linked to killing.

Polymyxin B is utilised as the main intravenous polymyxin in Singapore for multidrug-resistant gram-negative bacilli infection. Co-administration of polymyxin B with other antibiotics therapy against such infections is often observed locally. Table 5 is a summary of studies, conducted so far,

to evaluate the potential pharmacodynamic interaction (or potential synergism) between polymyxin B and other antibiotics, mostly against *A. baumannii*.

Available clinical data imply that commonly used dosing regimens, which are mostly based on product information, not supported by pharmacokinetics/pharmacodynamics studies, may be sub-optimal as monotherapy in immunosuppressed patients. With a better understanding of these agents, it is expected that optimal dosing regimens could be designed to maximise (prolong) their clinical utilities in our current limited armamentarium of antimicrobials for multidrug-resistant gram-negative infections.

Clinical Uses

Both polymyxin B and colistin sulphate have been widely used for the treatment of otic, ophthalmic, and skin infections.⁵⁴⁻⁵⁸ Recently, polymyxins have also been used for the treatment of critically ill patients with nosocomial infections caused by multidrug-resistant or polymyxin-only-sensitive gram-negative bacteria.⁵⁹⁻⁶⁹ It should be noted that there is limited clinical experience with intravenous polymyxin B, that is our local mainstay of polymyxin, when compared to colistimethate sodium, in the literature. There is no apparent reason except perhaps the fact that older studies reported a higher incidence of toxicity compared to colistimethate sodium.^{70,71}

In Table 6, we summarise the existing experience from recent clinical studies that evaluated the efficacy and safety

Table 5. In Vitro Pharmacodynamic Interactions Between Polymyxin B And Other Antibiotics Against MDR Gram-negative Bacteria

Papers	Polymyxin B Combined with	Organisms	Method	Pharmacodynamic Interaction/Outcome when combined with polymyxin B
Tascini et al (Ref 113)	Rifampicin	5 clonal unrelated MDR AB	chequerboard	Synergistic against 3 isolates; Additive against 2 isolated
Yoon et al (Ref 114)	Triple combination (at 25% of MIC) rifampicin + imipenem	8 unrelated clinical isolates AB (resistant to all antibiotics except polymyxin B)	3-dimensional chequerboard microtitre plate dilution and time-kill studies	All isolates killed within 24 h
Landman et al (Ref 115)	Azithromycin, imipenem, &/or rifampicin	10 MDR PA isolates of 7 unique ribotypes	Chequerboard & time-kill studies	Chequerboard: Azithromycin (4 mg/L): Synergistic against 6 isolates Imipenem (4 mg/L): Synergistic against 2 isolates Rifampicin (1 mg/L): Synergistic against 1 isolate Time-kill studies: Bactericidal for the following antibiotics when combined with polymyxin B: Imipenem plus rifampicin against all 10 isolates, Rifampicin in 9/10 isolates, Imipenem in 8/10 isolates Azithromycin in 4/10 isolates.
Bratu S et al (Ref 116)	Azithromycin	13 MDR PA	Time-kill studies	Addition of 4 mg/L azithromycin to the lower concentration of 2 mg/L polymyxin B produced a >2 log kill (synergistic) against most isolates & prevented regrowth in all but 2 isolates
Bratu S et al (Ref 117)	Rifampicin	16 <i>K. pneumoniae</i> which produced KPC-2 carbapenemase; comprised of 6 distinct strains & 10 isolates of another 2 different ribotypes	Time-kill studies	Combination of polymyxin B at 0.5 x MIC with rifampicin had synergic activity against 15/16 isolates, including 2 polymyxin-resistant strains. Combination of polymyxin B at 0.5 x MIC with imipenem had synergic bactericidal activity against 10/16 isolates, but was antagonistic for three isolates Imipenem (4 mg/L) + polymyxin B (0.5 x MIC) + rifampicin (1 mg/L) had no effect
Manikal et al (Ref 118)	Rifampicin or azithromycin	24 AB isolates, belonging to 4 distinct PFGE groups	Chequerboard	Azithromycin (4 mg/L): synergistic against 20 isolates including 2 polymyxin-resistant isolates; additive effects against remaining 4 isolates Rifampicin (1 mg/L): synergistic against 12 isolates and additive against the other 12 isolates
Wareham et al (Ref 119)	Imipenem, azithromycin, or rifampicin	5 unrelated MDR AB which encode OXA-23 carbapenemase; susceptible to polymyxins only	Etest agar dilution & combined Etest strip methods	Synergy was not observed with any one drug in combination with polymyxin B against 4 isolates. Borderline synergy was shown against 1 strain in combination with either imipenem or rifampicin, using the Etest agar dilution method only.
Petersen et al (Ref 120)	Tigecycline	Gram-negative organisms	Chequerboard & time-kill studies	Synergistic against 2 out of 9 AB isolates tested. No synergism observed in other gram-negative organisms. No antagonism observed for all strains tested.

of parenteral polymyxin B for the treatment of MDR gram-negative nosocomial infections. The clinical outcome of nosocomial pneumonia especially in the intensive care unit, after the intravenous use of polymyxin B, have been encouraging. Clinical response rate and mortality reported in most of these studies ranged from 52.7% to 95% and 20% to 51.4%, respectively. These observations were similar to those reported in the majority of trials that used

intravenous colistimethate sodium in similar clinical settings.^{59-61,72-74}

The clinical experience with the use of inhaled polymyxin B, colistin sulphate or colistimethate sodium are limited. Of interest, polymyxin B has not been examined in exacerbations of pulmonary infections in cystic fibrosis patients, unlike colistimethate sodium and colistin sulphate. Historically, polymyxin B via inhalation, had been utilised

Table 6. Characteristics and Clinical Outcomes of Recently Published Studies of Patients Who Received Polymyxin B for Infections Due to Multidrug-resistant Gram-negative Bacteria

Ref/Year patients	Setting	Number of	Drug administration	Dosage/duration of polymyxin B	Site of infection	Pathogen	Mortality	Clinical cure or improvement	Toxicity
Furtado/2007 Ref 69	ICU (100%)	74 total; 35 with favourable outcome and 39 with unfavourable outcome	IV	CrCL \geq 80 mL/min: 1.5-2.5 mg/kg/day; CrCL 30-80 mL/min: 2.5 mg/kg on Day 1, then 1-1.5 mg/kg/day thereafter. CrCL <30 mL/min: 2.5 mg/kg on Day 1, then 1-1.5 mg/kg given every 2-3 days thereafter. Anuric: 2.5 mg/kg on Day 1, then 1.0 mg/kg given every 5-7 days thereafter 5 to 34 days (median 16) in favourable gp2 to 38 days (median 6) in the unfavourable gp	Pneumonia	<i>P. aeruginosa</i>	In hospital mortality: 74.3% 51.4% died while receiving polymyxin B.	52.7% (39/74) had unfavourable response to polymyxin B	9.5% renal failure. No neuromuscular disorder observed
Pereira/2007 Ref 68	ICU (89%)	19 pts	IV and inhaled: 14 pts Inhaled only: 5 pts	Inhaled: 500,000 IU q12h Mean duration of inhaled: 14 d (4-25 d)	Pneumonia 74% Tracheobronchitis 26%	<i>P. aeruginosa</i> 84% <i>K. pneumoniae</i> , <i>A. xylosoxidans</i> , <i>Burkholderia</i> sp. 1 case each	47% of cases of pneumonia 0% of cases of tracheobronchitis	93% of cases of pneumonia 100% of cases of tracheobronchitis	Cough/ bronchospasm 21%
Ostromoff/2006 Ref 65	Hematology department	2 pts	IV	1 mg/kg q12h for 19 and 21 d	Bacteremia 1pt Cellulitis 1pt	<i>P. aeruginosa</i>	0%	Both pts were cured	No
Holloway/2006 Ref 66	ICU	33 pts	28 pts IV2 pts by nebulisation 3 pts both IV and by nebulisation	Median daily dose (IV): 1.3 MIU Median daily dose (by nebulisation): 2 MIU (27 of the 33 pts monotherapy)	VAP 50% BSI 43%	<i>A. baumannii</i>	27%	76%	Nephrotoxicity 21% Neurotoxicity 2%
Parchuri/2005 Ref 64	CAPD	1 pt	IV	150,000 IU q12h for 10 d (meropenem, amikacin)	CAPD-associated peritonitis	<i>K. pneumoniae</i>		The pt was discharged	No

Table 6. Contd.

Ref/Year patients	Setting	Number of patients	Drug administration	Dosage/duration of polymyxin B	Site of infection	Pathogen	Mortality	Clinical cure or improvement	Toxicity
Sobieszkyk/2004 Ref 62	ICU	25 pts/29 courses	29 courses: 21 IV 6 aerosol 2 combination CLcr (Aerosolised)	(IV) Loading dose 2.5-3 mg/kg and then according to estimated CLcr (Aerosolised) approximately 2.5 mg/kg/day ; Mean duration: 19 d (2-57 d)	Respiratory tract	<i>A. baumannii</i> 55% <i>P. aeruginosa</i> 41%	Overall mortality 48% End of treatment mortality 21%	76%	Nephrotoxicity 10% Neurotoxicity 7%
Sarria/2004 Ref 67	ICU	1 pt with septic shock receiving CVVHD	IV	Loading dose 2.5-3 mg/kg followed by 2 doses of 1 mg/kg on days 4 and 8, then 0.8 mg/kg daily; Duration 24 d	Catheter-related bacteraemia	<i>A. baumannii</i>		The pt was discharged	No
Ouderkerk/2003 Ref 63	Critically ill	60 pts	Parenterally	Mean daily dose: 1.1 MIU Mean duration: 13.5 d	Lung 65% Blood 8% Abdomen 5% Urine 3% Bone 3%	<i>A. baumannii</i> 77% <i>P. aeruginosa</i> 3% Both isolates 3%	Overall mortality 20%	Microbiological cure 88%	Nephrotoxicity 14%

to decrease the incidence of nosocomial pneumonia by eradicating colonisation in the lungs of critically ill patients. These studies, which were reported in the early 1970s, displayed conflicting results.⁷⁵⁻⁷⁸ There are also a few studies that assessed the effectiveness and safety of aerosolised polymyxin B for the treatment of MDR gram-negative nosocomial pneumonia.^{62,66,68,79,80}

The largest study reported clinical cure and improvement of 93% and 100% in patients with pneumonia and tracheobronchitis, mainly in ICU, respectively. This study included 14 patients with pneumonia and 5 patients with tracheobronchitis caused mainly by *P. aeruginosa* (84% of patients). In pneumonia cases, intravenous and inhaled polymyxin B were administered, while in tracheobronchitis cases only inhaled polymyxin B was used. Dosage of inhaled polymyxin B was 500,000 IU twice a day for a mean duration of 14 days (range, 4 to 25 days). No serious adverse events requiring discontinuation of treatment were observed. All-cause mortality occurred in 64% of episodes of nosocomial pneumonia.⁶⁸

Aerosolised colistin (for a mean duration of 16.4 days) as adjunctive treatment of ventilator-associated pneumonia due to multidrug-resistant gram-negative bacteria had been used with favourable response bacteriologically and clinically in 50 out of 60 patients.⁸¹ All-cause hospital mortality was 25% while mortality attributable to ventilator-associated pneumonia was 16.7%.⁸¹ The intrathecal and intraventricular use of polymyxin B for the treatment of central nervous system infections has been thoroughly mentioned in recent reviews.^{38,82}

Polymyxin B immobilised fibre column (PMX-F), whereby polymyxin B is bound and immobilised to polystyrene fibres, has been used in sepsis and septic shock.⁸³⁻⁸⁵ It aims to disrupt the inflammatory response cascade leading to sepsis by absorbing circulating bacterial endotoxin (LPS of gram-negative and lipoteichoic acid of gram-positive bacteria).⁸⁶⁻⁸⁸ By haemoperfusing septic patients directly with PMX-F, various mechanisms such as the inhibition of neutrophil reactive oxygen species, the absorption of anandamide (an intrinsic cannabinoid that induces hypotension in septic shock), the reduction of circulating neutrophil elastase, the improvement of pulmonary oxygenation, the decrease of mediators such as TNF- α , IL-6, IL-8, IL-10, and plasminogen activator inhibitor-1, are effected.^{84,89,90-92} The exact mechanism of action is not fully understood. A recent systematic review of effectiveness of PMX-F suggests beneficial outcome may be observed in septic patients, with statistically significant increase in mean arterial pressure, mean PaO₂/FiO₂, decrease in dopamine/dobutamine dose requirement, and a positive effect on mortality compared to the conventional treatment.⁹³ However, the weakness of this review is the inclusion of low quality clinical trials.

Toxicity

Parenteral administration of polymyxins is associated with nephrotoxicity partly due to their D-amino acid content and fatty acid component. Polymyxin B, being the most potent polymyxin, was also thought to be the most nephrotoxic compound compared to colistin sulfate and colistimethate sodium. However, the less potent colistimethate sodium is required in larger doses than polymyxin B for effectiveness. Thus the rate of nephrotoxicity equals that of polymyxin B.⁹⁴ The mechanisms by which polymyxin B induces acute renal failure is via increasing membrane permeability, resulting in an increased influx of cations, anions and water, leading to cell swelling and lysis.^{95,96} Polymyxins also increased the transepithelial conductance of the urinary bladder epithelium, whereby the magnitude of the conductance's increase depended on the concentration and length of exposure to polymyxins as well as the divalent cation concentration.⁹⁷ Renal toxicity associated with the use of polymyxins is considered to be dose dependent. Haematuria, proteinuria, cylindruria or oliguria, in addition to the usual increase in serum creatinine, may also be manifestation of nephrotoxicity with the parenteral administration of polymyxins. Acute tubular necrosis could also develop;⁷⁰ however, the basement membrane and the glomeruli remain intact.^{98,99}

A review had concluded that the incidence of nephrotoxicity in recently published reports of experiences with polymyxins were less common and severe compared to the studies in the 1970s.¹⁰⁰ It maybe partly due to 1) Improvement in supportive treatment provided to critically ill patients, 2) A better understanding of adverse effects, hence, close monitoring of renal function and of factors that affect renal function, 3) Avoidance of co-administration of other agents with known nephrotoxicity, 4) Different formulations of colistin, containing a proportion of colistin sulfate that is more toxic than the recommended form of colistimethate sodium for parenteral use might have been used in old studies. Michalopoulos et al⁵⁹ and Markou et al⁶⁰ reported incidences of nephrotoxicity of 18.6% and 14.3% respectively in their intensive care unit studies which utilised 9 million units of colistimethate sodium per day. Falagas et al⁶¹ reported incidence of nephrotoxicity of 8% in their medical ICU study which utilised a dose of 4.5 million units colistimethate sodium per day. A mouse model, which was employed to mimic regimens of twice- and once-daily dosing of a clinically relevant dose of colistimethate sodium in humans, revealed that more severe renal lesions histologically, with the regimen corresponding to once-daily dosing, indicating the potential for renal toxicity may be greater with extended-interval dosing.¹⁰¹

It should be noted that most studies assessing toxicities of polymyxins were conducted with colistimethate sodium

and they may not represent polymyxin B toxicity. Evaluation of nephrotoxicity caused by polymyxin B is shown in Table 5. In addition, a recent study in New York reported a nephrotoxicity rate of 14%, which was an overestimate as multiple nephrotoxic agents were administered concurrently, with microbiological eradication in 88% of the patients.⁶³ We also previously reported a polymyxin B-related nephrotoxicity rate of 0% in 26 patients who received polymyxin B for multidrug-resistant gram-negative infections in Singapore General Hospital.¹⁰² The incidence of nephrotoxicity was lower than previous reports which ranged from 17% to 100%.¹⁰³⁻¹⁰⁵

Parenteral administration of polymyxins is associated with neurotoxicity via their interactions with neurons, which have high lipid content. A biphasic mechanism for neurotoxicity has been put forth: a short phase of competitive blockade caused by pre-synaptic action of polymyxins that interferes with the receptor site and blocks the release of acetylcholine to the synaptic gap and followed by a prolonged phase of depolarisation associated with calcium depletion.¹⁰⁶⁻¹⁰⁸ Neurological toxicity can be manifested as dizziness, weakness, facial, peripheral paresthesia, vertigo, visual disturbances, confusion, ataxia, and it also includes neuromuscular blockade, which can lead to respiratory failure or apnoea. Incidence of colistin-associated neurotoxicity in non-cystic fibrosis patients, reported in earlier literature was approximately 7%, with paresthesias constituting the main neurotoxic adverse event.¹⁰⁹ We reported a possible case of paresthesia in our case study of 26 patients and no case of neuromuscular blockade leading to respiratory paralysis.¹⁰² Hypersensitivity reactions (skin rash, urticaria, generalised itching, drug fever, mild gastrointestinal disorders) related to colistimethate sodium use happened at an estimated incidence of 2%.¹⁰⁹ The use of polymyxin B via inhalation has also been associated with a higher incidence of bronchoconstriction (use of salbutamol nebuliser before polymyxins inhalation can help alleviate) compared to colistimethate sodium. All these hypersensitivity reactions are results of the irritative effects of the active forms of polymyxins⁷⁰ and their histamine-releasing action or IgE-mediated, especially observed in higher incidences with polymyxin B.¹⁰⁰ The recent report of a cystic fibrosis patient who died of acute respiratory distress syndrome (ARDS) reiterated that the pro-drug colistimethate sodium should be reconstituted just before administration in order to avoid excessive conversion to biologically active colistin, which can cause airway or alveolar injury.¹¹⁰

Intraventricular or intrathecal administration of polymyxins, especially in high doses, may lead to convulsions and signs of meningismus. Polymyxin B was also found to cause electrolyte abnormalities, such as

hypokalaemia, hyponatraemia, hypochloreaemia, and a negative anion gap previously.^{111,112}

Conclusion

The use of polymyxins is likely to continue to increase globally, as there is no new drug for gram-negative bacilli in the pipeline. Unfortunately, there are substantial gaps of knowledge in polymyxins' pharmacology. Optimal dosing that maximises efficacy, suppresses resistance and minimises toxicities is not known. The current normal, renal and dialysis dosages are not based on solid pharmacokinetic studies. The recent clinical reports are not without major drawbacks (including limited sample size, lack of control arm and co-administration of other antibiotics) that hinder definitive conclusions to be drawn. Therefore, further investigations are very much warranted in the pharmacokinetics, pharmacodynamics, toxicodynamics, and its efficacy alone and in combination with other antibiotics, to help guide the treatment of increasing prevalence of polymyxin-only-susceptible infections locally and globally.

At this point in time, we would encourage use of systemic polymyxins, especially in the case of polymyxin B (where its use is mostly systemic in Singapore):

- 1) as part of the combination therapy with other antibiotics (e.g. rifampicin) for the treatment of multi-resistant gram-negative bacilli infections in view of the heteroresistance exhibited by *A. baumannii*.
- 2) for treatment of documented multi-resistant gram-negative infection and NOT for prophylactic or unjustified empiric indication to preserve its use.

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