

Polymyxin B Revisited: Structural Insights, Clinical Resurgence, and Emerging Strategies Against Multidrug-Resistant Gram-Negative Infections.

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Abstract

The global resurgence of Polymyxin B underscores its pivotal role as a last-line defence against multidrug-resistant (MDR) Gram-negative bacterial infections. As resistance to conventional antibiotics escalates, Polymyxin B has reemerged as a vital therapeutic agent, distinguished by its unique membrane-disruptive mechanism and consistent pharmacokinetic profile. This review synthesizes current knowledge surrounding the structural and functional attributes of Polymyxin B, including its biosynthetic origins, antimicrobial spectrum, and pharmacological advantages over colistin. Recent advances in novel drug delivery platforms such as liposomes, nanoparticles, and inhalation therapies are examined alongside emerging strategies like combination regimens and therapeutic drug monitoring, which aim to enhance efficacy while minimizing toxicity. Special attention is given to the evolving role of pharmacists in individualized dosing, antimicrobial stewardship, and implementation of precision medicine approaches. The integration of pharmacodynamic insights with clinical practice is critical for optimizing therapeutic outcomes. As new resistance mechanisms emerge, a deeper understanding of Polymyxin B's pharmacology, innovations, and challenges is essential to extend its clinical utility in the era of antimicrobial resistance.

Keywords

Polymyxin B, multidrug resistance, Gram-negative bacteria, pharmacokinetics, antimicrobial stewardship, therapeutic drug monitoring, nephrotoxicity, drug delivery systems, combination therapy, resistance mechanisms

1. Introduction

Polymyxins are a class of antimicrobial agents known for their potent bactericidal activity against Gram-negative pathogens. Chemically classified as cyclic cationic polypeptides, Polymyxins exert their antibacterial effect by targeting the outer membrane of Gram-negative bacteria. They achieve this by binding to lipopolysaccharides (LPS) in the bacterial outer membrane, particularly the lipid A component, disrupting membrane integrity and causing intracellular leakage, ultimately leading to cell death. Among the Polymyxins, Polymyxin B and Polymyxin E (commonly called colistin) are the two most widely used compounds in clinical practice.

The resurgence of Polymyxins in recent decades is primarily attributed to the global rise in multidrug-resistant (MDR) Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae*.¹ These pathogens often

display resistance to most other antibiotics, including carbapenems, leaving Polymyxins as a last-resort treatment option. Despite historical concerns regarding their toxicity, Polymyxins have thus reemerged as indispensable agents in managing severe infections caused by MDR organisms.

Polymyxin B sulfate is the sulfate salt form of Polymyxin B, recognized for its broad-spectrum activity against Gram-negative bacteria. It is commonly administered through intravenous, topical, or intrathecal routes, depending on the clinical scenario. A key advantage of Polymyxin B over colistin is that it is delivered in its active form, whereas colistin is administered as colistimethate sodium, an inactive prodrug that requires *in vivo* conversion to its active metabolite. This distinction provides Polymyxin B with more predictable pharmacokinetics and consistent serum levels, which is critical for achieving optimal therapeutic concentrations, particularly in critically ill patients.²⁻³

Clinical evidence suggests that Polymyxin B may also be less nephrotoxic compared to colistin, a factor that has contributed to its preferential use in certain healthcare settings.⁴ However, the difference in toxicity profiles remains an area of ongoing research and debate. The pharmacokinetic characteristics of Polymyxin B also make it a favourable choice in patients with impaired renal function, as the drug is primarily eliminated via non-renal pathways. Nevertheless, the narrow therapeutic window of Polymyxin B necessitates careful dosing and monitoring to prevent toxicity and ensure efficacy.

Polymyxins were first discovered in 1947, isolated from *Bacillus polymyxa* (later reclassified as *Paenibacillus polymyxa*). Their clinical introduction occurred during the 1950s, with both Polymyxin B and colistin gaining recognition for their ability to treat Gram-negative bacterial infections. However, systemic use of Polymyxins declined in the 1970s due to significant concerns over nephrotoxicity and neurotoxicity. This led to a preference for less toxic antibiotics when treating Gram-negative infections.⁵

The rapid emergence of antibiotic-resistant bacteria in the past two decades has revived interest in Polymyxins, particularly Polymyxin B, as a critical component in antimicrobial therapy. One of the pivotal moments in the history of Polymyxin B was the establishment of the International Standard in 1954. This standard, developed from highly purified Polymyxin B sulfate and tested in collaboration with the World Health Organization (WHO), provided a global reference for dosage and quality control.⁶

In addition to its direct antimicrobial applications, Polymyxin B has found innovative uses in extracorporeal therapies. In Japan, a hemoperfusion device known as Terramycin was developed using Polymyxin B-immobilized fibres to remove endotoxins from the blood of patients with sepsis and septic shock. Approved in 1994, this device has since been used in over 100,000 clinical cases and is available in several countries. While some randomized trials have reported mixed results regarding its efficacy, meta-analyses have demonstrated improvements in hemodynamic parameters and potential reductions in mortality among septic patients, highlighting its continued relevance in clinical care.⁷

Pharmacists play an indispensable role in optimizing the use of Polymyxin B sulfate, given its narrow therapeutic index and potential for serious adverse effects. Their responsibilities extend beyond dispensing to encompass individualized dosing based on patient-specific factors such as weight, renal function, and severity of infection. Pharmacists also contribute significantly to therapeutic drug monitoring (TDM), using advanced analytical methods like high-performance liquid chromatography (HPLC) to measure serum drug concentrations and adjust doses to achieve optimal therapeutic levels while minimizing toxicity.⁸

In addition, pharmacists are integral to antimicrobial stewardship programs, where they guide clinicians in selecting appropriate antibiotic combinations and treatment durations to prevent resistance development and improve clinical outcomes.⁴ Given Polymyxin B's role as a last-line agent against MDR pathogens, stewardship interventions are critical to preserving its effectiveness. Pharmacists also play a key role in educating healthcare professionals and patients about the appropriate use of Polymyxin B and in implementing policies that promote safe and effective antibiotic practices.

Recent research has explored novel drug delivery systems, including microencapsulation with polysaccharides such as alginate and cyclodextrin, as well as formulations involving liposomes and spray-dried particles. These innovations aim to enhance drug stability, prolong release profiles, and improve efficacy against biofilm-associated infections.⁹ Pharmacists are at the forefront of evaluating and incorporating these emerging technologies into clinical practice to improve patient outcomes and reduce toxicity.¹⁰

Given the alarming rise in carbapenem-resistant Enterobacteriaceae (CRE) and other MDR organisms, Polymyxin B is frequently employed as part of combination therapy. Meta-analytical studies have demonstrated that when used in combination regimens, often with

agents like carbapenems, tigecycline, or Fosfomycin, Polymyxin B is associated with a significant reduction in 28-day mortality compared to monotherapy or alternative treatments.¹¹ These findings underscore the necessity of combination strategies to enhance bactericidal activity, limit the emergence of resistance, and improve overall clinical outcomes.

However, combination therapy and dose optimization are not without challenges. Both underdosing and overdosing can lead to treatment failure or increased toxicity. Thus, the incorporation of TDM, evidence-based dosing protocols, and interdisciplinary collaboration involving pharmacists, physicians, and microbiologists is vital to ensuring effective use of Polymyxin B.

Polymyxin B sulfate remains a cornerstone in the treatment of MDR Gram-negative infections. Its reintroduction into clinical practice has provided a valuable tool against pathogens resistant to other antibiotics. Nevertheless, challenges persist, including the risks of nephrotoxicity and neurotoxicity, dosing complexities, and the potential for resistance development. Ongoing research is focused on understanding its biosynthesis, structural variations, pharmacokinetics, and pharmacodynamics to develop next-generation analogues with improved safety and efficacy profiles.

This review will comprehensively explore the multifaceted aspects of Polymyxin B sulfate, including its discovery, chemical structure, mechanism of action, pharmacokinetics, clinical applications, resistance mechanisms, and innovations in drug delivery. By synthesizing current knowledge and highlighting emerging strategies, this review aims to provide insights into optimizing Polymyxin B use in an era of escalating antimicrobial resistance^{9, 12}

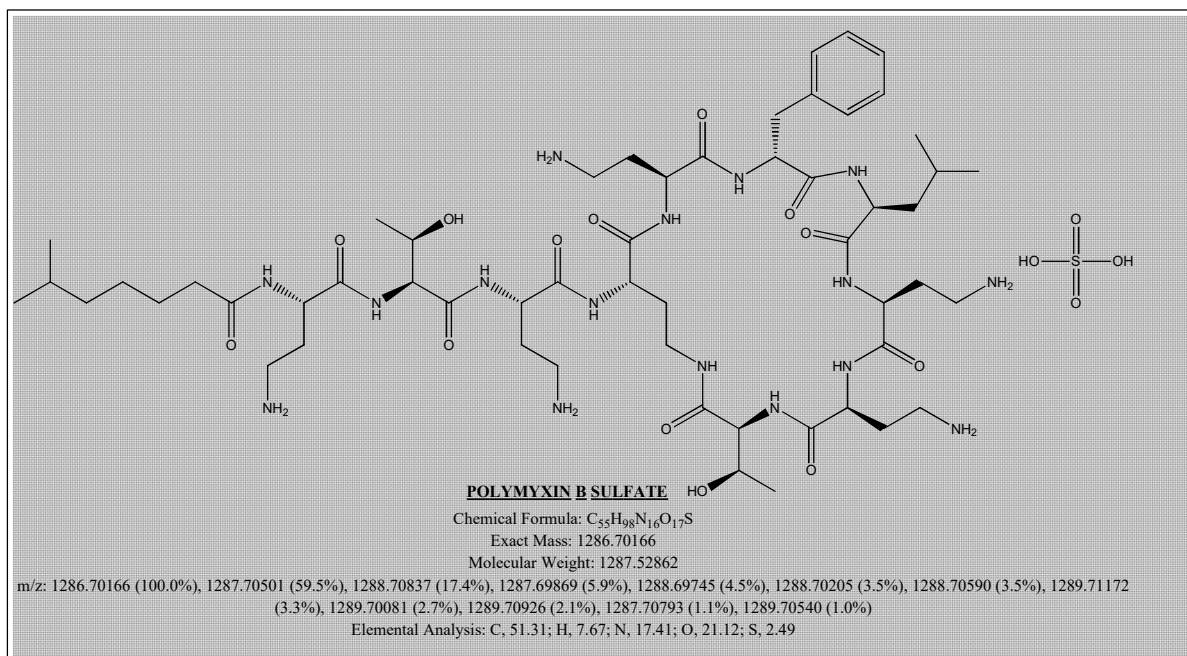
2. Chemical Structure

2.1 Chemical Composition

Polymyxins, such as Polymyxin B and colistin (Polymyxin E), are cationic cyclic lipopeptides produced by *Paenibacillus polymyxa*. They are characterized by a decapeptide structure comprising a cyclic heptapeptide core and a linear tripeptide chain linked to a fatty acyl tail at the N-terminus. The fatty acyl chain, typically consisting of (S)-6-methyloctanoic acid or (S)-6-methylheptanoic acid, is critical for their antibacterial activity and toxicity profile.¹³ The Polymyxin molecule is enriched in L- α,γ -diamino butyric acid (Dab) residues,

which contribute to its polycationic nature. These positively charged residues interact electrostatically with the negatively charged phosphate groups in the lipid A component of lipopolysaccharides (LPS) in the bacterial outer membrane.¹⁴

Figure 1: Chemical properties of Polymyxin B



The amphipathic nature of Polymyxins is integral to their membrane-disrupting properties. The hydrophobic face, formed by the fatty acyl tail and hydrophobic amino acids (e.g., D-Phe6, L-Leu7), facilitates membrane insertion, while the polar face, comprising Dab residues, mediates interactions with LPS.¹⁴ Structural studies using NMR have demonstrated that Polymyxins fold in a manner that separates these hydrophobic and polar regions, enhancing their interaction with bacterial membranes¹⁴

Modification studies have explored structural variants, such as Polymyxin B nonapeptide (PMBN), which lacks the terminal Dab and fatty acyl tail. PMBN retains LPS binding but lacks the hydrophobic tail essential for membrane permeabilization, resulting in reduced antibacterial activity.¹⁵ Research has also demonstrated that altering the fatty acid moiety or the peptide sequence can affect the potency and toxicity of Polymyxin derivatives, highlighting the importance of the exocyclic FA-Dab1-Thr2-Dab3 motif in Polymyxin B for optimal antibacterial activity.¹³

Recent research has further highlighted the significance of Polymyxin's chemical structure, particularly the roles of the fatty acyl chain and cyclic peptide regions, in mediating both antibacterial activity and nephrotoxicity. Ongoing efforts aim to develop analogues with

modified structures to enhance selectivity, broaden activity spectra, and reduce adverse effects.¹⁴

2.2 Mechanism of Action on the Bacterial Membrane

Polymyxins exert their bactericidal effects by targeting the outer membrane of Gram-negative bacteria. The initial step involves electrostatic binding of the cationic Dab residues to the negatively charged lipid A of LPS in the bacterial outer membrane, displacing divalent cations like calcium and magnesium that stabilize the membrane.¹⁴ This interaction leads to destabilization of the outer membrane and insertion of the Polymyxin's hydrophobic tail into the lipid bilayer, disrupting membrane integrity.¹⁶

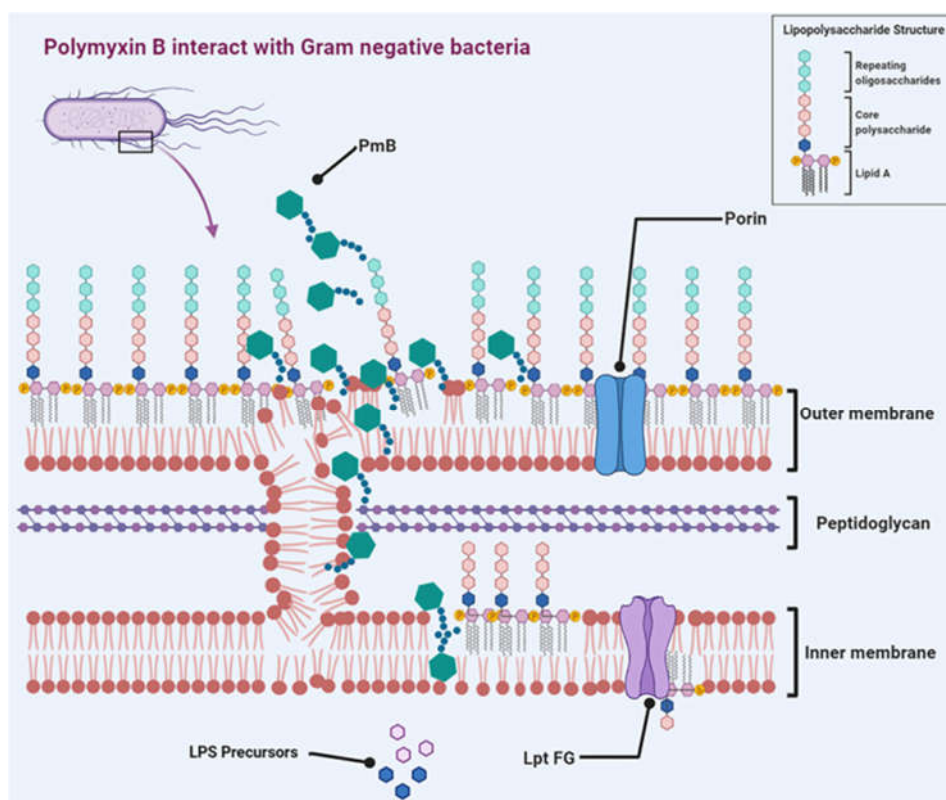


Figure 2: Schematic illustration of lipid scramble, membrane phase separation, and peptide translocation mechanism model.¹⁷

Recent molecular dynamics simulations and atomic force microscopy studies have provided a more detailed understanding of this process. Polymyxins have been shown to reorganize LPS into ordered hexagonal crystalline structures, increasing membrane stiffness and mechanical stress, ultimately causing membrane rupture.¹⁶ This crystalline arrangement is

absent in Polymyxin-resistant strains, indicating its direct role in the antibiotic's mechanism of action.¹⁶

Further research has proposed a model where Polymyxin binding induces lipid scrambling and membrane phase separation. Binding of Polymyxins disrupts the balance between the inner and outer leaflets of the outer membrane, causing phospholipids to migrate to the outer leaflet and forming phase-separated domains rich in either LPS or phospholipids. These structural defects lower the energy barrier for Polymyxins to translocate across the membrane.¹⁸ Once inside the periplasm, Polymyxins interact with the inner membrane, causing further disruptions that lead to leakage of cytoplasmic contents and bacterial cell death.¹⁸

Additionally, studies have revealed that Polymyxins can induce membrane thinning and osmotic imbalance by mediating contacts and lipid exchange between the inner and outer membranes, leading to cell lysis.¹⁸ However, bacteria have evolved resistance mechanisms, most notably the MCR-1 enzyme, which modifies lipid A by adding phosphoethanolamine. This reduces the negative charge on LPS, weakening the initial electrostatic interactions and thus reducing Polymyxin efficacy.¹⁹ Structural studies of MCR-1 have shed light on its function and potential for targeted inhibition to restore Polymyxin activity against resistant strains.¹⁹

Innovative strategies to enhance Polymyxin efficacy include the development of nanocomposites combining Polymyxins with graphene oxide (GO). These composites leverage the physical membrane-penetrating properties of GO and the chemical membrane-disrupting properties of Polymyxins, significantly enhancing antibacterial activity.²⁰ The synergistic interaction increases membrane permeability, reduces bacterial viability, and lowers the minimum inhibitory concentration of Polymyxin B against resistant strains.²⁰

The mechanism of action of Polymyxins involves an intricate process starting with electrostatic interactions and progressing through membrane disruption, lipid scrambling, and translocation. These actions ultimately compromise the integrity of both the outer and inner bacterial membranes, leading to cell death. Resistance mechanisms, such as MCR-1-mediated

modifications, highlight the need for continued research into structure-function relationships and novel therapeutic strategies.^{16, 18-20}

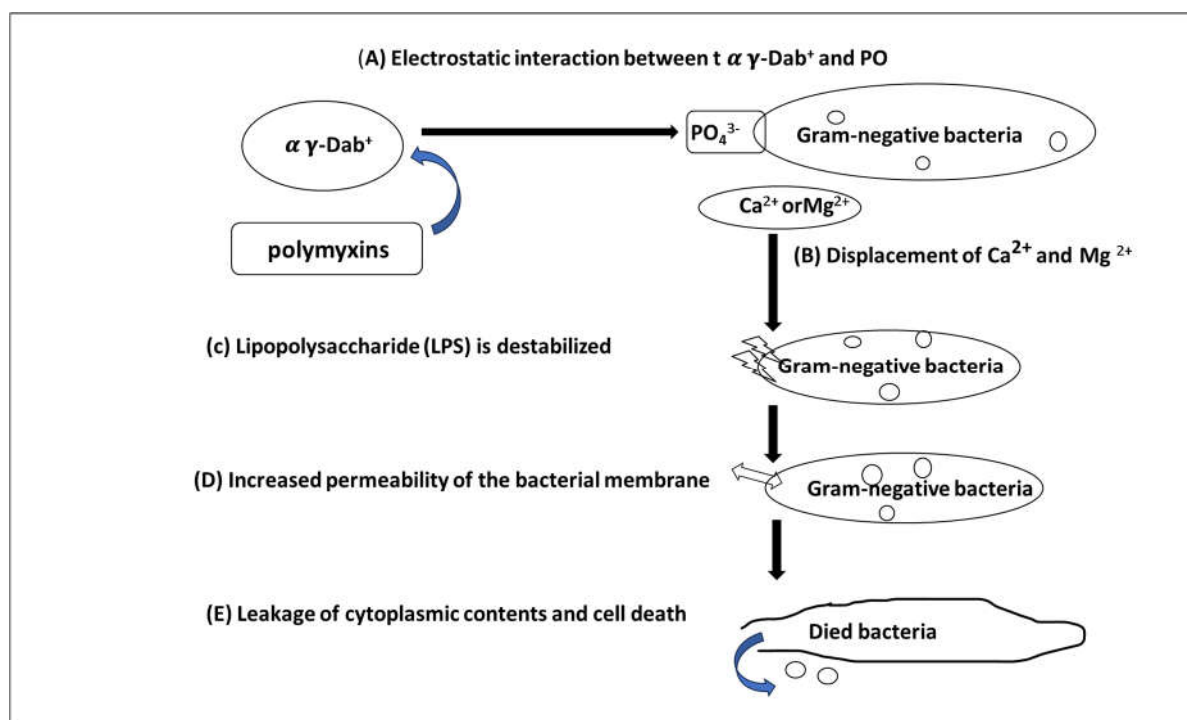


Figure 3: Mechanism of action of polymyxins.²¹

2.3 Comparison with Other Polymyxins

Polymyxins represent a unique class of cyclic lipopeptide antibiotics, rediscovered in clinical settings due to the rising prevalence of multidrug-resistant (MDR) Gram-negative pathogens. While Polymyxin B and colistin (Polymyxin E) are the most widely used, other Polymyxins such as A, C, and D have also been investigated for their antimicrobial potential and distinctive pharmacological profiles. This section critically compares the pharmacodynamics, antimicrobial efficacy, toxicity, and biosynthetic aspects of these Polymyxins, drawing from recent experimental and clinical findings.

2.3.1 Comparative Nephrotoxicity: Colistin vs Polymyxin B

One of the primary concerns in Polymyxin therapy is nephrotoxicity. In a prospective clinical study comparing colistin and Polymyxin B, Aggarwal and Dewan (2018) observed a significantly higher incidence of nephrotoxicity in patients treated with colistin (39.3%) compared to Polymyxin B (11.8%). Notably, the onset of kidney injury occurred within the first 3.8 to 4.2 days of therapy, and renal recovery was documented in the majority of cases within one week. The findings suggest that colistin toxicity is dose-dependent and predominantly reversible. Furthermore, colistin was found to be more nephrotoxic even when

administered according to current dosing protocols, emphasizing the relatively safer renal profile of Polymyxin B under similar clinical conditions²²

2.3.2 Polymyxin A: Enhanced Activity and Reduced Cytotoxicity

Polymyxin A, derived from *Paenibacillus dendritiformis*, remains less explored compared to Polymyxins B and E. Jangra et al. (2018) characterized two components—Polymyxin A1 and A2 (referred to as P2 and P1, respectively)—and evaluated their antibacterial activity against MDR clinical isolates. Remarkably, Polymyxin A2 (P1) showed superior activity in vitro, being two to four times more potent than Polymyxin B and colistin against several strains. Importantly, cytotoxicity assays demonstrated that P1 had significantly lower toxicity on human monocyte (THP-1) cells while maintaining low toxicity in kidney epithelial (HEK-293) cells, comparable to colistin. These findings suggest Polymyxin A, particularly the A2 variant, as a promising alternative due to its favourable safety and efficacy profile.²³

2.3.3 Polymyxin D: Biosynthetic Diversity and Therapeutic Potential

Polymyxin D, primarily produced by *Paenibacillus polymyxa* ATCC 10401, features distinct amino acid modifications, including D-serine at position 3 and L-threonine at position 7, differentiating it structurally from Polymyxins B and E. Galea et al. (2017) mapped the biosynthetic gene cluster (pmxA, pmxB, pmxE) responsible for Polymyxin D production and identified two natural forms, Polymyxin D1 and D2. These variants exhibited potent antimicrobial activity against Gram-negative pathogens, including *Klebsiella pneumoniae* and *Acinetobacter baumannii*, in a mouse model. Moreover, the study demonstrated the capacity to generate novel Polymyxin analogues through precursor amino acid supplementation, although the engineered variants were less effective than the natural forms. The work highlights the therapeutic promise of Polymyxin D and the potential for further optimization through biosynthetic engineering.²⁴

2.3.4 Structural and Pharmacological Differences between Colistin and Polymyxin B

Kwa et al. (2007) conducted a detailed review comparing the physicochemical and pharmacological properties of Polymyxin B and colistin. Structurally, these two agents differ only at position 6; colistin contains D-leucine, while Polymyxin B has D-phenylalanine. Despite their similar antimicrobial mechanisms and spectrum, notable differences exist in their formulations and pharmacokinetics. Polymyxin B is administered directly in its active sulfate form, whereas colistin is typically given as colistimethate sodium, a prodrug requiring in vivo conversion. This difference results in more predictable plasma concentrations for Polymyxin

B and possibly contributes to its lower nephrotoxicity. Additionally, Polymyxin B exhibits greater chemical stability and is not influenced by renal function to the same extent as colistin.²⁵

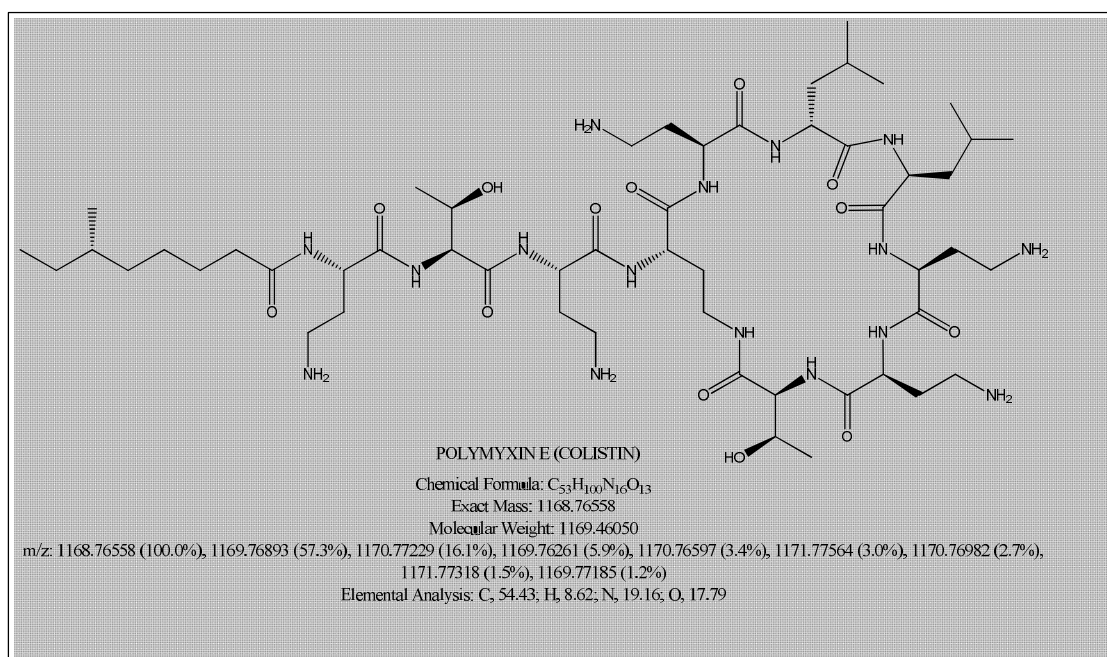


Figure 4: Chemical properties of Polymyxin E (Colistin)

2.3.5 Non-Antimicrobial Role of Polymyxin B: Inhibition of PKC

Interestingly, Polymyxin B also exhibits biological activity beyond its antimicrobial role. Reymann et al. (1988) demonstrated that Polymyxin B can inhibit protein kinase C (PKC), an enzyme involved in neuronal signalling pathways. In rat hippocampal slices, Polymyxin B application disrupted the maintenance phase of long-term potentiation (LTP), a neural correlate of memory. These findings indicate that Polymyxin B may affect central nervous system (CNS) functions through its action on intracellular signalling pathways. While this property is not directly related to its antibacterial effect, it raises concerns about potential off-target neurological effects during systemic therapy.²⁶

Future directions should emphasize in vivo validation of these lesser-known Polymyxins, structural modifications to enhance selectivity, and pharmacokinetic profiling to support clinical translation

Table 1: Comparison between the types of Polymyxins

Polymyxin	Key Differences	Antimicrobial Activity	Toxicity Profile	Clinical Relevance	Reference
Polymyxin B	D-Phe at position 6	High	Moderate (less renal)	Widely used	Aggarwal et al. ²²

Colistin (E)	D-Leu at position 6	High	Higher nephrotoxicity	Widely used	Kwa et al. ²⁵
Polymyxin A2	Better LPS binding	Higher than B & E	Least toxic to THP-1	Promising candidate	Jangra et al. ²³
Polymyxin D	D-Ser at position 3	Excellent in vitro & in vivo	Not fully studied	Experimental	Galea et al. ²⁴
Polymyxin C	PKC inhibition	Not widely evaluated	CNS-related concerns	Neuro research	Reymann et al. ²⁶

3. Spectrum of activity

Polymyxins, particularly Polymyxin B and colistin (Polymyxin E), are last-line antibiotics predominantly active against multidrug-resistant Gram-negative pathogens. Their antibacterial efficacy is largely attributed to their unique interaction with bacterial membranes, allowing them to effectively target certain bacterial species while being ineffective against others. This section outlines the antimicrobial spectrum of Polymyxins, focusing on their strong activity against specific Gram-negative bacteria and their ineffectiveness against Gram-positive organisms and anaerobes.

3.1 Predominant Activity Against Gram-Negative Bacteria Such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*

Polymyxins are particularly effective against Gram-negative bacilli due to their unique structure and mechanism of action. These antibiotics interact with the lipopolysaccharide (LPS) component of the outer membrane, leading to membrane disruption and subsequent bacterial death. This mechanism is specific to Gram-negative bacteria, which possess LPS as a major component of their outer membrane.

Studies demonstrate Polymyxin B's potent bactericidal action against major Gram-negative pathogens, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*.²⁷ A global assessment involving over 54,000 clinical isolates found Polymyxin B to be highly active against these species, with resistance rates below 3% for most strains, including carbapenem-resistant and multidrug-resistant variants.²⁷

Additional comparative studies confirm that Polymyxin B generally shows lower minimum inhibitory concentrations (MICs) than colistin against these same pathogens, suggesting slightly superior in vitro activity. This was evident across clinical isolates of *K.*

pneumoniae, *A. baumannii*, and *P. aeruginosa*, further reinforcing Polymyxin B's therapeutic value in infections caused by these bacteria.²⁸

Moreover, recent research has focused on synthesizing new Polymyxin analogues with broader or more potent activity. These compounds have been engineered to optimize hydrophobic interactions with the LPS layer, enhancing their activity against even colistin-resistant Gram-negative bacteria.²⁹ Some of these modified Polymyxins show increased antimicrobial activity and reduced toxicity, offering promising leads for future clinical development.²⁹

Liposomal formulations of Polymyxin B have also been explored to enhance targeted drug delivery and reduce nephrotoxicity. For instance, Polymyxin B-modified liposomes loaded with Fosfomycin exhibited improved efficacy in treating *A. baumannii* infections and showed significant bacterial targeting in both in vitro and in vivo models.³⁰

In addition, Polymyxin B nonapeptide (PMBN), a derivative lacking the fatty acyl tail, although not independently bactericidal, enhances the activity of other antibiotics by permeabilizing the outer membrane of Gram-negative bacteria, making them more susceptible to co-administered agents. This has been proposed as a strategy to reduce resistance development and eradicate persisted cell populations.³¹

Combination therapies are being increasingly adopted in clinical practice to maximize Polymyxin efficacy and prevent resistance development. For example, combining Polymyxin B with resveratrol has shown synergistic antibacterial and anti-biofilm activity against multidrug-resistant *P. aeruginosa* in both planktonic and biofilm-associated states.³²

Furthermore, genome mining studies have identified *Paenibacillus polymyxa* as a rich natural producer of Polymyxin B, with high biosynthetic potential. When cultivated under optimal conditions, the yield of Polymyxin B increases, and the compound shows robust activity against *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* with MICs ranging between 1–4 µg/mL.³³⁻³⁴

Overall, Polymyxins are a cornerstone in treating Gram-negative bacterial infections, especially where resistance to other antibiotic classes limits treatment options.

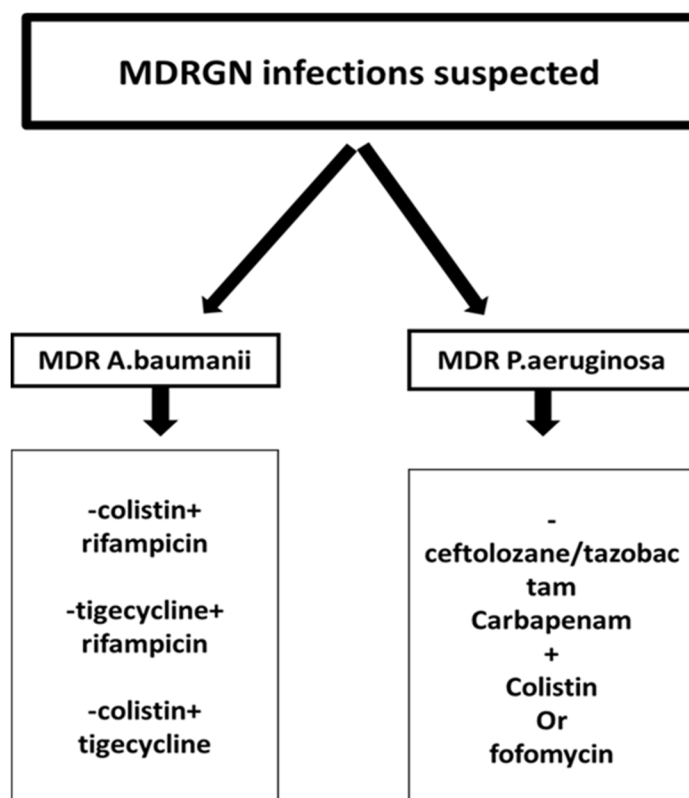


Figure 5. Empirical treatment for suspected multidrug-resistant Gram-negative infections.³⁵

3.2 Lack of Activity Against Gram-Positive Bacteria and Anaerobic Organisms

While Polymyxins are highly effective against Gram-negative organisms, they are largely ineffective against Gram-positive bacteria and anaerobes. This limitation is due to structural differences in the cell walls of these bacterial classes. Gram-positive bacteria lack the outer membrane and LPS layer that Polymyxins target, preventing the antibiotic from binding and disrupting the cell wall.³⁶

Anaerobic bacteria such as *Bacteroides* and *Clostridium* species are also intrinsically resistant to Polymyxins. These organisms either lack the specific LPS target altogether or possess modified outer membranes that do not interact with Polymyxins. Additionally, reduced oxygen availability in anaerobic environments affects the uptake and action of many antibiotics, including Polymyxins.^{28, 31}

Even with advancements in Polymyxin derivative design, their spectrum has not extended significantly into Gram-positive or anaerobic domains. The core mechanism involving LPS binding remains unchanged, and without this target, the compound is rendered ineffective. This fundamental limitation confines the clinical use of Polymyxins primarily to Gram-negative infections.

To address these limitations in broader-spectrum infections, Polymyxins are often combined with other antibiotics that are effective against Gram-positive and anaerobic organisms. This strategy ensures that Polymyxins target the Gram-negative component while companion drugs address other pathogens.³⁰

Moreover, structure-function studies have suggested that removing or modifying certain regions of the Polymyxin molecule can slightly reduce toxicity without broadening its spectrum significantly. For example, lipopeptides that lack one of the Dab (diamino butyric acid) residues may retain activity against some Gram-negative strains but still show no effect on Gram-positive bacteria.¹³

In conclusion, the activity of Polymyxins is strongly restricted by bacterial cell envelope structure. This selective spectrum is a double-edged sword: it provides precise targeting of problematic Gram-negative pathogens with minimal impact on beneficial flora, but also limits their standalone use in polymicrobial or anaerobic infections.

Table 2: Genetic Engineering Effects on Polymyxin Production in *Bacillus subtilis*

Strain	Genetic Modification	Effect on Polymyxin Production	Relative Production Level	Reference
BSK4	<i>ectB</i> gene inserted at <i>srfC</i> locus	Baseline production (normalized to 1.0)	1.0	Park et al. ³⁷
BSK4-0A	<i>spo0A</i> knockout	Production completely inhibited	0.0	Park et al. ³⁷
BSK4-rB	<i>abrB</i> knockout	2.3-fold increase over baseline	2.3	Park et al. ³⁷
BSK4-0A-rB	Double knockout of <i>spo0A</i> and <i>abrB</i>	1.7-fold increase over baseline	1.7	Park et al. ³⁷

Efficient Production of Polymyxin in the Surrogate Host *Bacillus subtilis* by Introducing a Foreign *ectB* Gene and Disrupting the *abrB* Gene ³⁷

- The table highlights the impact of specific genetic modifications on Polymyxin production, demonstrating the complex regulatory interactions of key genes.
- The **BSK4 strain**, which serves as the baseline, was engineered by inserting the *ectB* gene at the *srfC* locus. This insertion provides the foundational level of Polymyxin production, set as the reference value of 1.0. The *ectB* gene is likely involved in a pathway enhancing or enabling Polymyxin synthesis, although it alone does not dramatically increase production.
- In **BSK4-0A**, the *spo0A* gene was disrupted. *spo0A* is a master regulator that controls the initiation of sporulation and influences various secondary metabolic processes, including antibiotic production. Its deletion completely halted Polymyxin production, indicating that *spo0A* activates or is essential for the expression of genes directly or indirectly involved in Polymyxin biosynthesis.
- The **BSK4-rB** strain has a knockout of *abrB*, a global transcriptional repressor that suppresses multiple stationary-phase and stress-response genes, including those for antibiotic synthesis. The deletion of *abrB* lifted this repression, resulting in a 2.3-fold increase in Polymyxin production compared to the baseline. This demonstrates that *abrB* negatively regulates the Polymyxin biosynthetic pathway, and its removal unleashes greater expression of the necessary genes.
- In the **BSK4-0A-rB** double knockout strain, both *spo0A* and *abrB* were inactivated. While *spo0A* deletion alone stopped production, the additional *abrB* knockout restored Polymyxin synthesis to 1.7 times the baseline. This suggests that in the absence of *spo0A*, *abrB*'s repressive effect becomes more pronounced, but removing *abrB* can partially compensate for the loss of *spo0A*. This indicates a hierarchical and interdependent regulatory relationship where *spo0A* may activate Polymyxin genes, and *abrB* represses them, but removal of the repressor (*abrB*) can still permit some gene expression even without *spo0A*.
- The data indicate that *spo0A* acts as an activator, while *abrB* functions as a repressor of Polymyxin biosynthesis. Their interplay determines the overall production level of Polymyxin in the strains examined.

4. Pharmacokinetics and pharmacodynamics

The renewed use of Polymyxins, especially Polymyxin B and colistin, in response to the rise in multidrug-resistant (MDR) Gram-negative bacterial infections highlights the importance of understanding their pharmacokinetics (PK) and pharmacodynamics (PD). These agents, once abandoned due to toxicity, have re-emerged as vital treatment options in severe infections where other antibiotics fail. This section outlines their pharmacokinetic properties, bactericidal activity, and the role of various routes of administration.

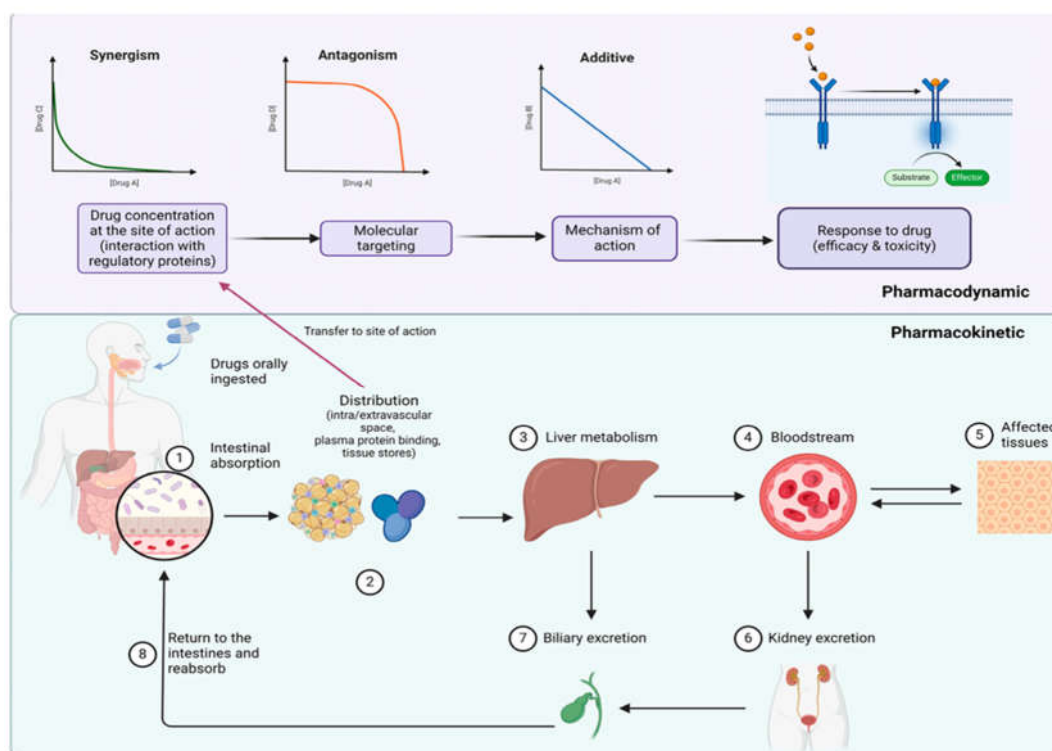


Figure 6 : Pharmacokinetic and pharmacodynamic Drug-Drug interactions³⁸

4.1 Treatment of MDR Infections: Absorption, Distribution, Metabolism, and Excretion (ADME)

Polymyxins are large, cationic polypeptides that are not absorbed via the gastrointestinal tract, making them unsuitable for oral therapy. They are thus administered parenterally in most clinical settings. Colistin is commonly delivered as colistin methane sulfonate (CMS), an inactive prodrug that is converted in vivo into the active compound, whereas Polymyxin B is administered in its active form, simplifying its pharmacokinetics and resulting in less variability in serum concentrations.³⁹⁻⁴⁰

Upon intravenous administration, Polymyxins exhibit a limited volume of distribution (approximately 0.2–0.5 L/kg), which suggests confinement mainly to extracellular fluids. Studies report that Polymyxin B has a distribution volume ranging from 12.7 to 34.3 L, depending on patient condition.¹⁻⁴¹⁻⁴² Tissue penetration is variable and generally limited, with poor penetration into cerebrospinal fluid (CSF), necessitating alternative administration routes for central nervous system infections.¹⁻⁴³

Regarding metabolism, CMS undergoes hydrolysis to form active colistin, though this conversion is incomplete and inconsistent. Polymyxin B, however, is not significantly metabolized and remains in its active form post-administration. This difference partly accounts for the more predictable PK profile of Polymyxin B compared to colistin.³⁴⁻⁴³

Elimination also differs between the two. CMS and colistin are primarily excreted via the kidneys, making dose adjustments essential in renal impairment. Polymyxin B, conversely, is eliminated largely by non-renal routes. Multiple studies, including trials involving patients with varying degrees of renal function and those undergoing haemodialysis, indicate that Polymyxin B clearance is only marginally influenced by renal status, although some variability still exists.⁴⁴⁻⁴⁶

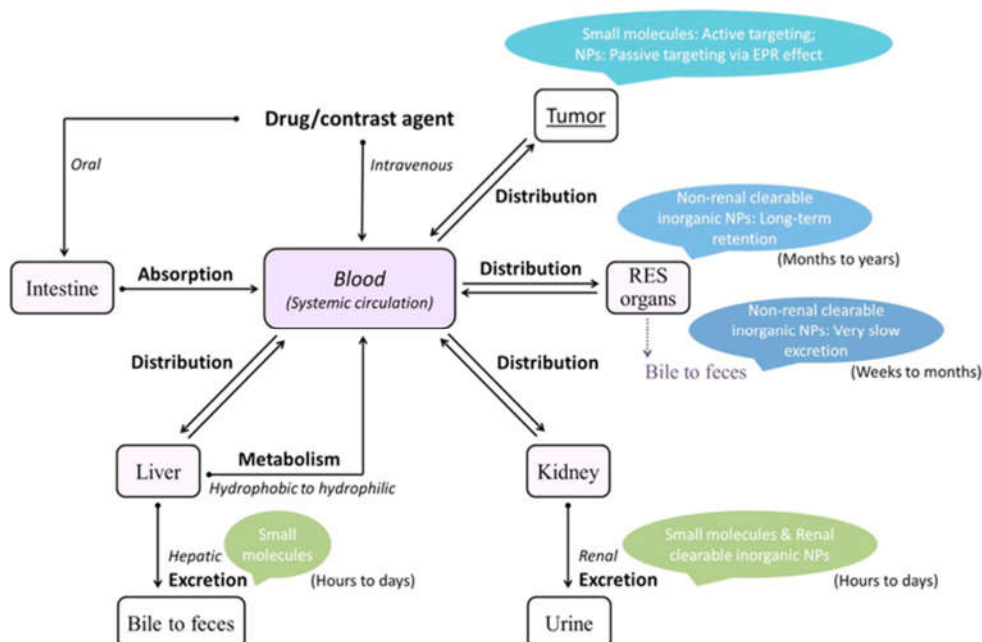


Figure 7: Pharmacokinetics includes absorption, distribution, metabolism, and excretion (ADME) of a drug ⁴⁷

4.2 Tissue Penetration and Bioavailability

Polymyxin B sulfate (PMB) demonstrates significant potential as an antimicrobial agent, but its therapeutic success largely depends on its capacity to penetrate tissues and maintain bioavailability at infection sites. This characteristic is crucial, especially in critically ill patients where infections may localize in hard-to-reach tissues. Recent physiologically-based pharmacokinetic (PBPK) modeling and clinical studies have provided valuable insights into PMB's tissue distribution profiles. For instance, Wu et al. highlighted moderate penetration into pulmonary tissue, with lung tissue-to-plasma concentration ratios in simulations and animal models ranging between 1.93 and 3.38, suggesting that while PMB reaches pulmonary tissues effectively, its penetration remains limited.⁴⁸

Furthermore, tissue penetration varies across different organs. In heart tissue, the penetration ratio is close to 1, indicating similar levels in plasma and tissue, while skin penetration ratios are generally lower (1.46–1.53).⁴⁸ This variability is clinically relevant when treating deep-seated infections, such as endocarditis or osteomyelitis, where achieving therapeutic concentrations is essential.

Oral bioavailability of PMB is notably poor due to its high molecular weight and hydrophilic nature, limiting systemic absorption from the gastrointestinal tract. To address this, advanced drug delivery systems such as liposomes formed using non-ionic surfactants have been developed to enhance solubility and protect PMB from enzymatic degradation. Chauhan and Bhatt demonstrated that optimized liposome formulations achieved higher drug entrapment, improved AUC, and extended half-life compared to conventional forms.⁴⁹

Other innovative approaches, including aerosolized delivery systems, offer enhanced drug delivery directly to the lungs, bypassing systemic circulation and increasing local drug concentrations. These methods are particularly beneficial for nosocomial pneumonia, where PMB's systemic penetration may be inadequate.⁵⁰

Collectively, these findings underscore the importance of optimizing PMB delivery strategies to enhance bioavailability and achieve effective tissue penetration, especially in patients with compromised organ function or in infection sites where drug access is limited.

4.3 Pharmacokinetics and Pharmacodynamic Target for Efficiency

The pharmacokinetics (PK) and pharmacodynamics (PD) of PMB are critical to its therapeutic optimization. PMB is administered in its active form, unlike colistin, and this distinction offers more predictable pharmacokinetic behaviour and consistent plasma concentrations, especially in critically ill patients.⁵⁰

Key PK parameters, including volume of distribution, clearance, and half-life, have been extensively studied. Zuo et al. reported that, in critically ill patients with extensively drug-resistant Gram-negative pneumonia, PMB showed a mean maximum plasma concentration (C_{max}) of approximately 8.3 µg/mL, clearance of 1.55 L/h, a volume of distribution of 30.44 L, and a terminal half-life near 19.56 hours.⁵¹ Importantly, the area under the concentration-time curve (AUC) over 24 hours emerged as a reliable predictor of treatment outcomes, with a threshold of 77.27 h·µg/mL associated with clinical efficacy.

The pharmacodynamic parameter most closely associated with PMB's antibacterial efficacy is the ratio of free drug AUC over 24 hours to the minimum inhibitory concentration (fAUC/MIC). Research indicates that an fAUC/MIC of at least 8.2 is necessary for optimal efficacy, particularly against pathogens like *Acinetobacter baumannii*.⁵² Additionally, Monte Carlo simulations have confirmed that a target AUC_{ss,0–24h} of 50–100 h·µg/mL achieves over 90% probability of target attainment (PTA).⁵⁰

However, PMB's relatively low penetration into the epithelial lining fluid (ELF), estimated at only 15.69%, highlights a limitation for treating pulmonary infections. This challenge has prompted investigations into adjunctive therapies such as nebulization or combination regimens.⁵⁰ Moreover, inflammatory biomarkers like interleukins IL-6 and IL-10 have been found to correlate with altered PK parameters, indicating that systemic inflammation can influence PMB's disposition and may necessitate personalized dosing adjustments.⁵⁰

Population pharmacokinetic models, including those by Manchandani et al. and Sandri et al., have proposed dosing regimens for PMB that include an initial loading dose followed by maintenance dosing (e.g., 100 mg loading, then 50–100 mg every 12 hours), though interpatient variability, especially in critically ill individuals undergoing continuous renal replacement therapy (CRRT), underscores the need for therapeutic drug monitoring (TDM).⁵⁰

Biomarker-driven pharmacokinetic approaches further enhance clinical decision-making by integrating patient-specific inflammatory markers and pharmacokinetic parameters to optimize dosing and minimize toxicity.^{48, 50}

5: Dosing and Administration

5.1 Standard Dosing Regimens – In-depth Analysis

Polymyxin B, a last-resort antibiotic for multidrug-resistant Gram-negative infections, has undergone a resurgence in clinical use due to the increase in antimicrobial resistance. Its reintroduction has necessitated a deeper understanding of its dosing regimens, particularly because of its narrow therapeutic window and the balance required between efficacy and toxicity. This section summarizes and analyzes standard dosing regimens across different populations, based on recent pharmacokinetic/pharmacodynamic (PK/PD) studies, clinical trials, and Monte Carlo simulations, as detailed in the referenced literature.

5.1.1. General Principles and Rationale for Dosing

Polymyxin B is administered as its active form, bypassing the prodrug conversion necessary for colistin. This simplifies its pharmacokinetics (PK) and makes it a preferred choice for treating bloodstream and severe systemic infections. The primary PK/PD index correlating with efficacy is the 24-hour area under the concentration-time curve (AUC₂₄) to minimum inhibitory concentration (MIC) ratio, with a target AUC₂₄/MIC ≥ 50 –100 mg·h/L for optimal bacterial killing.^{43, 53-54}

The FDA-approved dosing regimen generally recommends 1.5–2.5 mg/kg/day, divided every 12 hours. However, fixed dosing regimens and adaptive strategies are increasingly being considered due to variable pharmacokinetics, especially in critically ill or renal-compromised patients.

5.1.2. Population-Based Variations in Dosing

a. Adults with Normal and Impaired Renal Function

Recent population PK models indicate that Polymyxin B clearance is not significantly influenced by renal function, contrasting earlier assumptions and label recommendations. Studies have demonstrated a weak or negligible correlation between creatinine clearance (CrCL) and Polymyxin B clearance.⁴⁸⁻⁵⁵ For example, Sandri et al. and Thamlikitkul et al. concluded that Polymyxin B dosing should not necessarily be reduced in renal impairment, challenging FDA recommendations.⁵⁵

However, newer models suggest slight but clinically relevant associations, particularly in extreme cases of renal insufficiency. Monte Carlo simulations propose a moderate dose reduction (e.g., 75 mg loading and 50 mg maintenance) to reduce nephrotoxicity risk while maintaining efficacy in such patients.⁵⁶

b. Paediatric Patients

Dosing in paediatric populations presents unique challenges. A two-compartment PK model in children showed that weight significantly influences Polymyxin B clearance. Children dosed between 1.5–3.0 mg/kg/day achieved >90% probability of target attainment (PTA) when MIC was ≤ 0.5 mg/L.⁵⁷ Nevertheless, exposures in many paediatric patients remained below the adult therapeutic target, prompting calls for revised weight-based regimens or therapeutic drug monitoring (TDM).

c. Renal Transplant Patients

In renal transplant patients, Polymyxin B pharmacokinetics showed considerable variability. A one-compartment model demonstrated that higher CrCL was associated with increased clearance, and dose adjustments were necessary to avoid subtherapeutic exposures or toxicity. A regimen of 75 mg loading followed by 50 mg maintenance showed optimal PTA while reducing nephrotoxicity risks.⁵⁶

5.1.3. Optimisation Techniques

a. Monte Carlo Simulations

Monte Carlo simulations have been extensively employed to simulate various dosing regimens under different MIC scenarios. For pathogens with MIC ≤ 1 mg/L, most standard and high-dose regimens achieved PTA >90%. However, at MIC = 2–4 mg/L, even high doses (e.g., 1.5–2.5 mg/kg q12h) often failed to achieve the target, indicating a need for combination therapy or alternative agents in such scenarios.⁵⁸

b. Adaptive Feedback Control Algorithms

Personalized dosing using adaptive feedback control (AFC) has shown promise. With as few as one PK sample, AFC algorithms can individualize dosing to keep AUC_{0–24} within the optimal range (50–100 mg·h/L). Studies using Bayesian estimators and sparse sampling have achieved >95% PTA, significantly improving over fixed-dose regimens.⁵⁴

5.1.4. High-Dose Regimens and Associated Risks

High-dose Polymyxin B regimens (e.g., $\geq 30,000$ IU/kg/day or ≥ 200 mg/day) have been associated with increased bacterial clearance, especially against organisms with MIC ≤ 2 mg/L.

However, this comes at a cost. Studies report nephrotoxicity in 40–60% of patients receiving such regimens, especially when used in combination with other nephrotoxic agents.⁵⁹

Therefore, the potential benefits of aggressive dosing must be carefully balanced against risks, with close monitoring of renal function. Strategies to mitigate toxicity include avoiding concurrent nephrotoxins, shortening treatment duration, and incorporating therapeutic drug monitoring.

5.1.5. Special Populations: Cystic Fibrosis

In cystic fibrosis patients, especially adults, PMB pharmacokinetics may differ due to altered body composition and renal clearance. A fixed-dose regimen (75 mg q12h) without loading was sufficient to achieve the target exposure ($AUC_{24} = 50\text{--}100 \text{ mg}\cdot\text{h/L}$), though neurotoxicity was common. This highlights the importance of individualized therapy and monitoring in this population.⁴³

5.1.6. Current Challenges and Future Directions

Despite substantial progress, Polymyxin B dosing remains controversial in several aspects:

- Renal function's precise role in clearance is still debated.
- Lack of universal consensus on fixed versus weight-based dosing.
- Insufficient large-scale prospective trials to validate PK/PD models.
- Need for validated paediatric and transplant-specific guidelines.

Future efforts should focus on integrating real-time TDM and population-based adaptive dosing algorithms into routine clinical practice. Continued collection of pharmacokinetic data, especially in vulnerable subgroups, and updating labelling to reflect contemporary evidence are also essential.

5.2 Route-Specific Administration (IV, IM, Topical, Ophthalmic, Inhaled)

The administration route of Polymyxin B (PMB) significantly affects its pharmacokinetics, therapeutic efficacy, and safety. Given the increasing use of PMB in treating infections caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria, tailoring the route of administration is essential for optimal clinical outcomes.

5.2.1 Intravenous (IV) Administration

Intravenous delivery remains the most widely used route for PMB, particularly for systemic infections in critically ill patients. According to a comprehensive meta-analysis, the all-cause mortality in patients treated with intravenous PMB was approximately 41.2%, with nephrotoxicity reported in 40.7% of cases. These figures are comparable to those observed with colistin use, but with a slightly better renal safety profile for PMB. The study suggests that intravenous PMB continues to be a critical option in the antimicrobial arsenal, especially for severe systemic infections.⁶⁰

5.2.2 Inhaled Administration

The inhalation route is especially relevant for patients with ventilator-associated pneumonia (VAP). A retrospective study involving 111 VAP patients treated with PMB compared the efficacy of intravenous (IV), inhaled (IH), and combination IV+IH therapies. The results showed similar clinical outcomes across groups, but inhalation therapy led to faster bacterial clearance and reduced nephrotoxicity compared to IV use alone ($p = 0.025$).⁶¹ Another pharmacokinetic study found that aerosolized PMB achieved significantly higher concentrations in the epithelial lining fluid (ELF) than in plasma, confirming its value in targeting pulmonary infections more effectively.⁶²

5.2.3 Topical Administration

Topical use of compound PMB ointment has demonstrated therapeutic benefits in patients with chronic, non-healing wounds. In a study of 111 patients with conditions such as diabetic foot ulcers and pressure injuries, those treated with PMB ointment experienced significantly faster wound healing and lower infection rates than the control group treated with silver sulfadiazine. Notably, the PMB-treated group had better outcomes by the 21st day, highlighting its efficacy in topical applications.⁶³

5.2.4 Ophthalmic Administration

Ocular delivery presents unique challenges due to rapid drug clearance from the eye surface. To address this, researchers developed a mucoadhesive nanoemulsion containing dexamethasone acetate and PMB. This formulation, designed to enhance ocular retention and bioavailability, showed no cytotoxicity in vitro and maintained suitable physical properties for ophthalmic use. The combination effectively addressed inflammation and bacterial infection in eye disorders such as conjunctivitis and blepharitis.⁶⁴

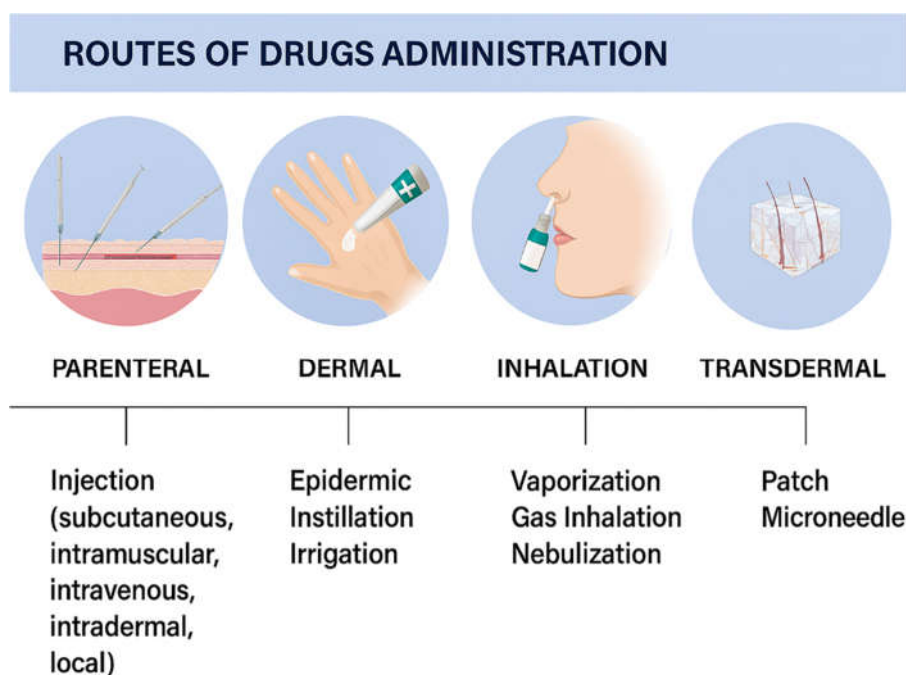


Figure 8. Main drug administration routes.⁶⁵

5.3 Dosage Adjustments and TDM Consideration

Polymyxin B exhibits a narrow therapeutic window, necessitating careful dosing and therapeutic drug monitoring (TDM) to balance efficacy and nephrotoxicity. This is especially important in critically ill patients or those with renal impairment, where pharmacokinetics can be highly variable.

5.3.1 Importance of TDM

TDM plays a vital role in optimizing PMB treatment. A clinical study focusing on nosocomial pneumonia found that achieving a steady-state area under the curve over 24 hours ($AUC_{ss,24h}$) within the range of 50–100 mg·h/L was associated with improved clinical outcomes. Patients who reached this target were significantly more likely to experience successful treatment (OR = 13.15, $p = 0.015$).⁶⁶

A multicentre randomized trial (PMB-CROS) further evaluated the role of TDM in patients with sepsis caused by carbapenem-resistant Gram-negative bacteria. It showed that a high-dose PMB regimen led to a higher proportion of patients achieving the target AUC and improved long-term survival. However, elevated AUC levels were also associated with a higher incidence of acute kidney injury (AKI), emphasizing the delicate balance between effective dosing and toxicity.⁶⁷

5.3.2 National Guidelines and Clinical Standards

To streamline the clinical use of PMB, Chinese experts developed consensus guidelines on TDM. These guidelines include recommendations on target plasma concentrations, sample collection, timing, and interpretation of results. They also emphasize multidisciplinary collaboration among physicians, pharmacists, and microbiologists to ensure accurate and effective use of TDM in clinical settings.⁶⁸

5.3.3 Special Considerations in Paediatric and Renal Patients

In paediatric patients undergoing continuous renal replacement therapy (CRRT), PMB clearance is significantly increased due to extracorporeal removal. A case report involving two paediatric patients demonstrated that the standard dosing regimen resulted in subtherapeutic plasma concentrations during CRRT. When the dose was adjusted to 2 mg/kg every 12 hours based on TDM findings, therapeutic levels were achieved, and infections were controlled. This case underscores the necessity of individualized dosing and the impact of CRRT on PMB pharmacokinetics.⁶⁹

Table 3: Comparison of Polymyxin B Concentrations in Plasma vs. Epithelial Lining Fluid (ELF)

Route of Administration	Plasma Concentration (mg/L)	ELF Concentration (mg/L)	Reference
Intravenous (IV) Only	1.19 – 5.16	0.8 – 4.0	Liu et al. 62
Inhaled (IH) Only	1.00 – 2.00	20.6 – 97.6	Liu et al. 62
Intravenous + Inhaled (IV + IH)	2.5 – 4.0	40.0 – 80.0	Liu et al. 62

Table 3 compares Polymyxin B concentrations in plasma and epithelial lining fluid (ELF) based on different administration routes. The data are derived from a pharmacokinetic study by Liu et al., which investigated Polymyxin B levels in patients with ventilator-associated

pneumonia (VAP) receiving treatment through intravenous, inhaled, or combined administration routes.⁶²

Key Insights:

- **Inhaled Polymyxin B** achieved significantly higher drug levels in the ELF, ranging from **20.6 to 97.6 mg/L**, while maintaining relatively low plasma levels (**1.00–2.00 mg/L**). This indicates efficient drug delivery directly to the lungs, the primary site of infection in VAP.⁶²
- In contrast, **intravenous administration alone** resulted in **lower ELF concentrations**, between **0.8 and 4.0 mg/L**, despite higher systemic levels (**1.19–5.16 mg/L**). This demonstrates that systemic administration may be less effective in targeting pulmonary infections due to limited drug penetration into the lung tissues.⁶²
- The **combination of IV and inhaled routes** provided both sufficient systemic coverage (**2.5–4.0 mg/L** in plasma) and high local drug concentrations (**40.0–80.0 mg/L** in ELF), suggesting that this strategy could be beneficial in severe or resistant infections requiring optimal lung exposure and systemic protection.⁶²

6. Drug Interaction and Compatibility

6.1 Interaction with Other Nephrotoxic/Neurotoxic Drugs

Polymyxin B has re-emerged as a crucial agent in the treatment of infections caused by multidrug-resistant (MDR) Gram-negative bacteria, particularly *Acinetobacter baumannii*. Despite its therapeutic importance, Polymyxin B's clinical use is constrained by its well-documented nephrotoxic and neurotoxic effects. This concern is further amplified when Polymyxin B is used in combination with other drugs that carry similar toxicity risks. In current clinical settings, especially in critical care, transplant, and immunocompromised populations, polypharmacy is common. Thus, understanding how Polymyxin B interacts with other nephrotoxic and neurotoxic drugs is essential for improving therapeutic outcomes and minimizing adverse events.

6.1.1 Nephrotoxic Drug Interactions

One of the major risks associated with Polymyxin B therapy is nephrotoxicity, which is particularly pronounced in vulnerable populations such as critically ill or renal transplant patients. In a study involving 62 critically ill patients with nosocomial pneumonia, the incidence of acute kidney injury (AKI) was reported to be approximately 45.2%. The risk was

further amplified in patients with low baseline creatinine clearance and those receiving loop diuretics concurrently. Therapeutic drug monitoring (TDM) demonstrated that achieving optimal drug exposure within the therapeutic window significantly impacted treatment outcomes and minimized nephrotoxic complications.⁶⁶

In renal transplant recipients, nephrotoxicity presents an even greater concern. A prospective study showed that although only 5.5% developed AKI, a substantial proportion experienced other dose-dependent adverse effects, including neurotoxicity and skin hyperpigmentation. The study identified high cumulative doses and pre-existing renal impairment as independent risk factors. Additionally, co-administration of other nephrotoxic agents likely exacerbated renal complications in this population.⁷⁰

6.1.2 Neurotoxic Drug Interactions

Neurotoxicity, while historically underreported, is now recognized as a significant and frequent adverse effect of Polymyxin B therapy. Recent findings suggest that up to 63.4% of renal transplant patients receiving Polymyxin B developed symptoms of neurotoxicity, including dizziness, muscle weakness, hallucinations, and neuromuscular blockade.⁷⁰ A particularly illustrative case involved a 62-year-old male who developed neuromuscular dysfunction and respiratory failure shortly after initiation of Polymyxin B for the treatment of MDR *E. coli* bacteraemia. Following the discontinuation of Polymyxin B, the patient's condition improved markedly, indicating that prompt drug withdrawal can reverse neurotoxic effects. This case underscores the importance of early recognition and intervention in managing Polymyxin-induced neurotoxicity.⁷¹

6.1.3 Clinical Evidence from Combination Therapies

Several studies have evaluated the efficacy and safety of Polymyxin B when used in combination with other antibiotics, particularly in the treatment of resistant *A. baumannii* infections. One study demonstrated that the combination of ciprofloxacin and Polymyxin B, delivered via inhalable dry powders, provided a synergistic antibacterial effect while limiting resistance development. Although promising, such combinations still carry inherent toxicity risks due to the nephrotoxic and neurotoxic profiles of both agents.⁷² Additional research evaluated Polymyxin B in combination with carbapenems (imipenem, meropenem), sulbactam, rifampicin, and tigecycline. These combinations showed favourable in vitro and in vivo outcomes, particularly against carbapenem-resistant *A. baumannii* (CRAB). Among these, the

Polymyxin B and rifampicin pairing demonstrated the most substantial synergistic effect in both bacterial reduction and reduction of inflammatory markers such as IL-6 and TNF- α in murine models. However, the improved efficacy did not significantly reduce the incidence of toxicity, suggesting that synergistic efficacy must be weighed carefully against safety risks.⁷³ Moreover, metabolomic profiling provided insight into the mechanism of synergy in triple-drug therapy involving Polymyxin B, meropenem, and sulbactam. This study observed rapid disruption of bacterial membrane biosynthesis and central metabolism within minutes of administration. Despite the potent antimicrobial effect, such aggressive combinations may increase toxicity, especially when administered to patients with existing renal impairment or neurological vulnerability.⁷⁴

6.1.4 Special Considerations in High-Risk Populations

Renal transplant recipients and ICU patients represent high-risk populations where Polymyxin B-induced toxicities are especially concerning. Immunosuppressed individuals often receive multiple nephrotoxic or neurotoxic drugs simultaneously, such as calcineurin inhibitors or aminoglycosides, which compounds the potential for adverse outcomes. In transplant patients, neurotoxicity and skin hyperpigmentation were not only more common but also more severe, often requiring discontinuation of therapy (Document 6.2.1).⁷⁰ Similarly, critically ill patients with hospital-acquired pneumonia were more likely to develop renal injury when Polymyxin B was co-administered with other nephrotoxic agents like loop diuretics or in the presence of septic shock. The study highlighted the value of TDM in such settings, as it helped achieve optimal pharmacokinetics without crossing the toxicity threshold.⁶⁶

6.1.5 Recommendations and Mitigation Strategies

To safely use Polymyxin B alongside other nephrotoxic or neurotoxic drugs, clinicians should adopt several precautionary measures:

- **Therapeutic Drug Monitoring (TDM):** Regular plasma level assessments help maintain drug concentrations within the therapeutic window while minimizing toxicity.⁶⁶
- **Avoidance of Toxic Drug Combinations:** Drugs such as aminoglycosides, vancomycin, and calcineurin inhibitors should be used cautiously or avoided, if possible, in patients on Polymyxin B therapy.

- **Close Clinical Monitoring:** Early signs of neurotoxicity, such as ptosis, paresthesias, and confusion, should prompt immediate evaluation and possible drug discontinuation.⁷¹
- **Dose Adjustments:** Particularly necessary in patients with altered renal function or those on renal replacement therapies.
- **Patient-Specific Risk Assessment:** High-risk individuals, especially those with renal transplants or ICU admissions, should be carefully evaluated before initiating Polymyxin-based combination therapy.

6.2 Physical and Chemical Compatibility in IV Solutions

The 4 key considerations for physical and chemical compatibility are: Hazard, pH value, inorganic/organic nature, and solid/liquid state.⁷⁵

- **Hazard:** This refers to the potential for dangerous reactions, such as explosions, fires, or toxic fumes, when different chemicals are mixed.⁷⁵
- **pH Value:** pH is a measure of acidity or alkalinity, and different chemicals can react differently depending on their pH.⁷⁵
- **Inorganic/Organic:** This distinction helps determine how different chemicals will interact with each other, as inorganic and organic substances can have different compatibility characteristics.⁷⁵
- **Solid/Liquid:** The physical state of a substance can affect its compatibility with other substances. For example, solids may react differently with liquids than they would with other solids.⁷⁵

The intravenous administration of Polymyxin B (PMB), especially in critically ill patients, requires a thorough understanding of its physical and chemical stability in various infusion solutions. This knowledge is essential to ensure consistent therapeutic outcomes, minimize risks of infusion-related complications, and support antimicrobial efficacy during storage and use.

A study by Lim et al. investigated the physicochemical stability of PMB when diluted in different intravenous solutions, including 0.9% saline, 5% dextrose, 0.45% saline/5% dextrose, and 0.225% saline/5% dextrose. PMB retained more than 95% of its initial concentration when stored at 25°C and 30°C for 24 hours and demonstrated near-total stability for up to 168 hours at 4°C. The pH and osmolarity remained within acceptable ranges throughout the testing period, confirming its compatibility with commonly used diluents in intensive care settings.⁷⁶

Complementing this, Taylor et al. conducted a stability study under varied stress conditions using a validated HPLC method. They found that PMB exhibited considerable chemical stability at lower temperatures and near-neutral pH, but its degradation rate increased significantly at elevated temperatures and alkaline pH levels above 7.⁷⁷ These findings collectively support the safe and effective preparation of PMB in various infusion solutions, provided that appropriate temperature and pH conditions are maintained during storage and administration.

7. Clinical Applications

Polymyxin B has resurged as a pivotal antimicrobial agent, especially in the context of increasing multidrug-resistant (MDR) Gram-negative bacterial infections. Its applications encompass systemic therapy, localized topical treatments, and synergistic combination regimens aimed at maximizing therapeutic success while curbing resistance development.

7.1 Treatment of MDR Infections

The global escalation in resistance among Gram-negative organisms such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* has necessitated the reliance on Polymyxin B as a last-line therapeutic option. This antibiotic operates by binding to lipid A of the lipopolysaccharide layer in the bacterial outer membrane, disrupting membrane integrity and resulting in cell death.⁷⁸

A clinical study by Xia and Jiang involving 181 patients with carbapenem-resistant infections demonstrated a bacterial eradication rate of 42% and an overall clinical response rate of nearly 50%. Notably, early administration of Polymyxin B (within 24 hours of bacterial isolation) significantly enhanced treatment outcomes.⁷⁹ Similarly, a comparative analysis conducted by Wang et al. found that while colistin sulfate achieved higher microbiological clearance, the overall clinical success rate between colistin and Polymyxin B groups did not differ significantly.⁸⁰

Polymyxin B is often favoured over colistin methanesulfonate due to its superior pharmacokinetic profile, including faster systemic availability and more predictable drug levels, making it more suitable for severe infections such as sepsis and bloodstream infections.¹

Despite its efficacy, Polymyxin B is not devoid of adverse effects. Nephrotoxicity and neurotoxicity are the most significant limitations, necessitating careful monitoring of renal function and appropriate dose adjustment, especially in critically ill patients.⁸¹

7.2 Topical Applications (e.g., Eye and Ear Infections)

In addition to systemic use, Polymyxin B is widely utilized in topical formulations for treating localized infections of the eyes and ears. Its topical use minimizes systemic absorption, thus reducing the risk of toxicity while achieving high local concentrations. It is commonly included in combination products such as the triple antibiotic ointment with bacitracin and neomycin for bacterial conjunctivitis and otitis externa.⁷⁸

Polymyxin B has shown excellent efficacy in these formulations, particularly due to its stability and ability to penetrate local tissues. It is often preferred over colistin for otic preparations because of its favourable pharmacological properties, including a reduced degradation rate and sustained antibacterial action.⁷⁸

7.3 Use in Combination Therapies

Combining Polymyxin B with other antibiotics has emerged as a strategic approach to combat MDR pathogens and enhance therapeutic efficacy. These combinations often demonstrate synergistic effects, improve bacterial killing, and lower the risk of resistance development.⁷⁸

For instance, pairing Polymyxin B with agents such as carbapenems, tigecycline, Fosfomycin, or rifampicin has resulted in improved clinical outcomes. Studies have reported that combinations like Polymyxin B with rifampicin significantly increase bacterial membrane disruption and bactericidal activity, especially against *Acinetobacter baumannii*.¹

Moreover, novel adjuvants such as guanidine-based compounds and melatonin have been explored in combination with Polymyxin B. These agents enhance drug uptake by increasing membrane permeability and generating reactive oxygen species, effectively restoring activity against resistant strains like mcr-1-positive *Escherichia coli*.¹

Ongoing clinical trials are evaluating the effectiveness of Polymyxin B in combination with agents like doripenem, tigecycline, and Fosfomycin for conditions such as ventilator-associated pneumonia and bacteraemia.¹ These combination strategies not only broaden the antibacterial spectrum but also mitigate toxicity through dose-sparing effects. Nevertheless, careful selection of companion drugs is essential, taking into account factors like pharmacokinetic compatibility, toxicity, and site-specific penetration.

8. Recent advances and research

The resurgence of interest in Polymyxins as a last-line treatment for infections caused by multidrug-resistant (MDR) Gram-negative pathogens has catalyzed a wave of innovative research. This section reviews recent developments, including novel drug delivery systems, combination therapies aimed at overcoming resistance, and global trends in resistance surveillance.

8.1 New Delivery Systems (e.g., Liposomes, Nanoparticles)

Traditional formulations of Polymyxins like Polymyxin B and colistin suffer from considerable limitations, notably nephrotoxicity and poor pharmacokinetics. To overcome these challenges, nanotechnology-based delivery systems have been widely explored. Liposomes, polymeric nanoparticles, and surfactant-based carriers have demonstrated the potential to enhance the efficacy and safety profile of Polymyxins.

Nanocarriers such as liposomes improve drug stability, enable targeted delivery, and reduce systemic toxicity. For example, the integration of Polymyxin B with exogenous pulmonary surfactant (EPS) like Curosurf has been shown to enhance surfactant function while retaining antimicrobial activity, offering a promising dual therapy for pulmonary infections in neonates and adults alike.⁸² Moreover, experimental data suggest that this combination mitigates the disruptive effects of lipopolysaccharide (LPS) on surfactant layers, potentially reducing inflammation and mechanical injury in lung tissues.

Polymeric particles and conjugates have also gained attention for parenteral, oral, and inhalational delivery routes. These systems protect the drug from enzymatic degradation and support sustained release at infection sites, thus reducing the required dosage and associated toxicity.⁷⁸ Notably, nanoparticles containing Polymyxin have been successfully administered intratracheally in animal models, leading to significant antibacterial activity against *Pseudomonas aeruginosa* without inducing acute toxicity.⁸²

Additionally, recent research highlights the thermodynamic compatibility and structural integration of Polymyxin B with lung surfactants, supporting its clinical use as a component of inhalable formulations for respiratory infections.⁸²

8.2 Combination Therapies to Overcome Resistance

To address the increasing resistance to monotherapy, combination treatments involving Polymyxins and other antimicrobial or non-antibiotic agents have become a key focus. These

combinations aim to enhance efficacy through synergistic mechanisms and mitigate the development of resistance.

Combining Polymyxins with β -lactam/ β -lactamase inhibitors, such as ceftazidime-avibactam or meropenem-vaborbactam, has shown encouraging results. These combinations have broadened the spectrum of activity and improved clinical outcomes, particularly in infections caused by carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*.⁸³ For instance, plazomicin and ceftolozane-tazobactam have demonstrated efficacy against Polymyxin-resistant strains, reducing the reliance on Polymyxins as monotherapy.

New-generation Polymyxins such as SPR206, MRX-8, and QPX9003 are under development to reduce toxicity and restore efficacy against resistant pathogens. These agents maintain strong bactericidal activity but demonstrate lower nephrotoxic and neurotoxic effects compared to conventional Polymyxins.⁸⁴

Other strategies include pairing Polymyxins with non-antibiotic adjuvants. For instance, PBT2, a zinc ionophore, has been shown to reverse colistin resistance and restore activity in resistant strains.⁸⁴ Such synergistic interactions are especially valuable in regions with high endemic resistance.

While combination therapies offer hope, clinical evidence is mixed. Some trials show no additional benefit of combination over monotherapy for certain infections, such as CRAB (carbapenem-resistant *Acinetobacter baumannii*) pneumonia. Hence, such regimens are currently recommended mainly in cases where newer agents are unavailable or resistance profiles necessitate combination use.⁸⁴

8.3 Surveillance Data and Resistance Trends

Surveillance systems play a pivotal role in guiding the clinical use of Polymyxins and in detecting emerging resistance patterns. Global programs such as the WHO's GLASS and regional networks like China's CHINET have contributed valuable data on resistance trends.

Infections due to carbapenem-resistant Gram-negative bacilli (CR-GNB), particularly CRAB, remain a significant concern. According to CHINET, Polymyxins and tigecycline are among the few agents that retain activity against CRAB, with resistance rates as low as 1.1% and 2.3%, respectively.⁸⁵ These findings underscore the critical role of Polymyxins as salvage therapy.

Comparative studies have assessed the efficacy and safety of Polymyxin B versus colistimethate sodium (CMS) in real-world ICU settings. While both agents display similar clinical success and mortality rates, Polymyxin B is often associated with higher rates of reversible neurotoxicity, such as paresthesias and neuromuscular blockade.⁸⁶⁻⁸⁷ These adverse events emphasize the importance of therapeutic drug monitoring and dosage adjustment, especially in patients with renal impairment. Pharmacokinetic studies further show that age, total body weight (TBW), and baseline renal function significantly influence Polymyxin exposure. Elderly patients, in particular, are at heightened risk for nephrotoxicity, highlighting the need for individualized dosing strategies based on patient-specific parameters.⁸⁸

Efforts to integrate antimicrobial surveillance with pharmacokinetic/pharmacodynamic (PK/PD) modeling are underway. These systems can guide dose optimization and help curb the spread of resistance by ensuring effective yet safe Polymyxin exposure. Surveillance data are also instrumental in shaping treatment guidelines and stewardship protocols aimed at preserving the efficacy of existing antimicrobials.¹

9. Conclusion

Polymyxin B has reemerged as a critical weapon in the global fight against multidrug-resistant (MDR) Gram-negative bacterial infections. Its potent bactericidal action, relatively predictable pharmacokinetics, and advantages over colistin, particularly regarding nephrotoxicity and bioavailability, have solidified its role in contemporary antimicrobial therapy. Advances in drug delivery systems, such as liposomal encapsulation and inhalation therapies, have further enhanced their clinical applicability while mitigating toxicity. Moreover, therapeutic drug monitoring and individualized dosing regimens, especially in critically ill and paediatric populations, are key to optimizing outcomes and minimizing adverse effects. Despite these strengths, challenges remain. The narrow therapeutic index, potential for neurotoxicity, and growing concerns about resistance development necessitate cautious and judicious use. Pharmacists and clinicians must work collaboratively through antimicrobial stewardship programs to preserve the efficacy of this last-resort antibiotic. Emerging research into novel Polymyxin analogues and combination therapies offers hope for expanding the utility of Polymyxins while addressing current limitations.

In conclusion, Polymyxin B remains a cornerstone in the treatment of MDR Gram-negative infections. Continued innovation in its clinical use, pharmacological optimization, and

resistance management will be crucial to ensuring its effectiveness in the face of evolving microbial threats.

10. References

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