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# Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review

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**Polymyxins have re-emerged in clinical practice owing to the dry antibiotic development pipeline and worldwide increasing prevalence of nosocomial infections caused by multidrug-resistant (MDR) Gram-negative bacteria. Polymyxin B and colistin (polymyxin E) have been ultimately considered as the last-resort treatment of such infections. Microbiological, pharmacokinetic, pharmacodynamic and clinical data available for polymyxin B are reviewed in this paper. Polymyxin B has rapid *in vitro* bactericidal activity against major MDR Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Acquired resistance to this agent is still rare among these pathogens. However, optimized dosage regimens are not known yet. Good clinical outcomes have been observed in the majority of the patients treated with intravenous polymyxin B in recent studies. However, these studies failed to provide definitive conclusions due to limitations of study design and additional clinical trials are required. Although combination therapy may be an attractive option based on some currently available *in vitro* data, clinical data supporting such recommendations are lacking. Since polymyxins will be increasingly used for the treatment of infections caused by MDR bacteria, clinical pharmacokinetic, pharmacodynamic and toxicodynamic studies underpinning the optimal use of these drugs are urgently required.**

Keywords: polymyxins, antimicrobial cationic peptides, multiple bacterial drug resistance

## Introduction

Emergence of nosocomial bacterial pathogens with acquired resistance to almost all available antimicrobial agents, namely 'superbugs', has severely threatened therapeutic choices in the last decade.[1] Although the emergence of multidrug-resistant (MDR) Gram-positive bacteria has been a public health issue, a handful of novel antibiotics have been developed and recently approved for the treatment of infections caused by these organisms.[2–5] A major challenge has arisen, however, regarding the treatment of infections caused by Gram-negative bacilli, particularly those with high-level intrinsic resistance to many antibiotic classes and extreme ability to acquire resistance, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[6,7] With the exception of tigecycline, a relatively recently approved antibiotic active against MDR *A. baumannii* but not *P. aeruginosa*,[4] no new antibiotic is even in the drug development pipeline for MDR Gram-negative bacteria.[8] Consequently, there has been the resurgence of old antibiotics, such as the polymyxins, as the last resort for the treatment of infections caused by MDR Gram-negative pathogens which are resistant to all the other currently available antibiotics.[9–12]

Although knowledge of the pharmacokinetics (PK) and pharmacodynamics (PD) of polymyxins is very limited due to the lack of use in the last 50 years, intravenous (iv) administration of these drugs has substantially increased in the last decade. Of very significant concern, resistance to polymyxins, including hetero-resistance,[13] has emerged recently.[12] This highlights the urgency of obtaining knowledge on their pharmacology to optimize their clinical use and minimize potential for development of resistance.

Polymyxin B and colistin (also known as polymyxin E) are the two polymyxins used clinically; colistin is more widely used and most recent clinical experience with polymyxins is with it.[12,14–16] Knowledge on the PK and PD of polymyxin B is extremely limited[17] and most was obtained before the 1980s.[9,12]

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The polymyxins were never subjected to the drug development processes required for compliance with contemporary regulatory requirements.[12] It should be noted that the PK and PD information in the Product Information required to underpin prescribing recommendations is sadly lacking.[18]

Knowledge on the pharmacology and clinical use of colistin has been reviewed recently.[11,12,15] Since iv polymyxin B has also been increasingly prescribed in many parts of the world, we review recent progress on its PK, PD and clinical experience in this paper.

## Literature review

Data for this review were obtained from publications identified by systematically searching PubMed (1966–June 2007) with 'polymyxin B', 'polymyxins', 'polymyxin E', 'colistin', 'antimicrobial cationic peptides' and 'multiple bacterial drug resistance' in combination with '*P. aeruginosa*', '*Acinetobacter*', '*Klebsiella pneumoniae*' and 'Gram-negative bacterial infections'.

## Chemistry

Polymyxin B is a lipopeptide antibiotic isolated from *Bacillus polymyxa*. Its basic structure (Figure 1) consists of a polycationic peptide ring and a tripeptide side chain with a fatty acid tail.[19] Polymyxin B contains five primary amine groups and is a polycation at physiological pH. Polymyxin B is a mixture of at least four closely related components, polymyxin $B_1$ to $B_4$, with polymyxin $B_1$ and $B_2$ being the two major components.[20,21] The four components differ from each other only in the fatty acid moiety.[20,21] Polymyxin B is available for parenteral use as the sulphate salt, and batch-to-batch variation exists in the ratio of different components.[10] There is only one amino acid difference between polymyxin B (Figure 1) and colistin.[12] Another important difference between polymyxin B and colistin is that the former is administered parenterally as the sulphate salt, whereas the latter is administered as the sodium salt of colistin methanesulphonate, an inactive prodrug that undergoes hydrolysis *in vivo* and *in vitro* to form the active entity colistin.[22]

## Mechanism of action

Both polymyxin B and colistin are rapid-acting bactericidal agents, with a detergent-like mechanism of action.[9,10,17] Polymyxins interact with lipopolysaccharide (LPS) of the outer membrane of Gram-negative bacteria and are subsequently taken up via the 'self-promoted uptake' pathway.[19] The polycationic peptide ring binds to the outer membrane displacing the calcium and magnesium bridges that stabilize the LPS.[9,10] Because the peptides have affinities for LPS that are at least three orders of magnitude higher than the divalent cations $Ca^{2+}$ or $Mg^{2+}$, they competitively displace these ions and consequently disrupt the outer membrane.[19] The fatty acid side chain further interacts with the LPS, contributing to the insertion of polymyxins into the outer membrane. Polymyxins produce a disruptive physicochemical effect, leading to permeability changes in the outer membrane.[10] The affected membrane is thought to develop transient 'cracks' which permit passage of a variety of molecules, including hydrophobic compounds and small proteins, and, more importantly, promote the uptake of the perturbing peptide itself and lead to cell death[10] (hence the term 'self-promoted uptake').[19]

## Spectrum of activity

Polymyxin B has no activity against Gram-positive bacteria and anaerobes,[9,10] but is active against a variety of Gram-negative bacilli, including most clinically relevant Enterobacteriaceae and non-fermentative species.[9,10,23–26] Its spectrum of activity is nearly identical to colistin.[24] *P. aeruginosa* and *Acinetobacter* spp. are intrinsically susceptible, including most of the isolates that are resistant to all the other classes of antibiotics.[23] *Stenotrophomonas maltophilia* is usually susceptible although some strains are resistant.[23,24] *Burkholderia cepacia* complex and *Burkholderia pseudomallei* are resistant to polymyxin B.[23,24] Among Enterobacteriaceae, *Escherichia coli*, *Enterobacter* spp., *Citrobacter* spp., *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. are usually susceptible.[23,24] *Proteus* spp., *Providencia* spp., *Morganella morganii* and *Serratia marcescens* are resistant.[23,24] Polymyxin B is active against some species of *Aeromonas*, but *Aeromonas jandaei* is resistant and *Aeromonas hydrophila* has inducible resistance.[15,25] Studies with colistin have demonstrated that polymyxins are also active against *Haemophilus influenzae*, *Bordetella pertussis* and *Legionella pneumophila*.[15] The pathogenic *Neisseria* spp. (including meningococci and gonococci), *Moraxella catarrhalis*, *Helicobacter pylori*, *Vibrio* spp. and *Brucella* spp. are intrinsically resistant.[25] *Campylobacter* spp. vary in their susceptibility to polymyxin B and the susceptibility of *Bartonella* spp. is borderline.[15]

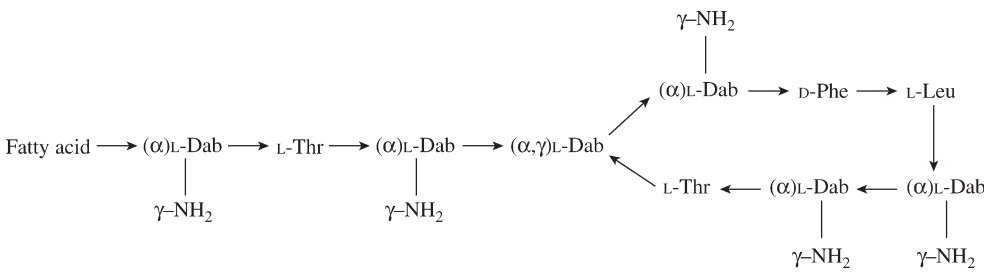


**Figure 1.** Structure of polymyxin B. Fatty acid: 6-methyloctanoic acid for polymyxin $B_1$, 6-methylheptanoic acid for $B_2$, octanoic acid for $B_3$ and heptanoic acid for $B_4$. Thr, threonine; Leu, leucine; Dab, α,γ-diaminobutyric acid; Phe, phenylalanine; where α and γ indicate the respective amino group involved in the peptide linkage.



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## Resistance

### *Susceptibility tests*

In the 1970s, the NCCLS (now the CLSI) published the breakpoints of susceptibility for colistin and polymyxin B.[24] However, at that time the procedures for standardization of susceptibility testing, the establishment of interpretative breakpoints and the definition of quality control strain guidelines were less rigorous.[24] Such breakpoint criteria for polymyxins in the 1981 NCCLS Approved Standard M2-A2 S2 were withdrawn in the 1990s.[24]

Some recent studies have demonstrated a poor correlation between different susceptibility test methods for polymyxins possibly due to the poor diffusion of polymyxins in agar.[24,27] Any resistance obtained with a diffusion test should be confirmed by broth dilution methods.[24] Additionally, *in vitro* activity of the polymyxins may be affected by cation concentrations in agar.[23] Nonetheless, a more recent multicentre study has provided initial quality control ranges for polymyxin B and colistin.[28] All proposed ranges incorporated >97.9% of study-generated zone diameters and MICs without significant occurrence of inter-laboratory variation or medium quality issues.[28]

In 2007, the CLSI has again provided guidance for the susceptibility testing of polymyxins.[29] Current polymyxin B breakpoints for *P. aeruginosa* are: susceptibility, MIC $\leq$ 2 mg/L; intermediate, MIC = 4 mg/L; and resistance, MIC $\geq$ 8 mg/L. For *Acinetobacter* spp., an MIC $\geq$4 mg/L is considered resistant.[29] The zone diameter interpretative standards for the disc diffusion method were added for *P. aeruginosa* only: they are $\leq$11 mm indicating resistance and $\geq$12 mm, susceptibility. Currently, there are no CLSI recommendations for Enterobacteriaceae.[29] The BSAC has never provided breakpoints for polymyxin B, possibly because this drug is not available in the UK for systemic administration. Nevertheless, colistin MIC breakpoints are provided for Enterobacteriaceae and *P. aeruginosa* (susceptible $\leq$4 mg/L and resistant >4 mg/L for both).[30] These breakpoints are different from those of colistin and polymyxin B proposed by the CLSI for *P. aeruginosa*: i.e. susceptible, $\leq$2 mg/L; intermediate, 4 mg/L; and resistant, $\geq$8 mg/L.[29] It is very important to note that in susceptibility tests colistin (in the form of its sulphate salt) must be used, and not colistin methanesulphonate (sodium salt); the latter is an inactive prodrug which undergoes hydrolysis to colistin during incubation *in vitro*, potentially to varying extents from laboratory to laboratory.[22]

### *Mechanisms*

Several molecular mechanisms of resistance have been characterized in various bacterial species with the majority of those studies focusing on *P. aeruginosa*. There is cross-resistance between polymyxin B and colistin.[9]

An initial and critical step in polymyxin action on Gram-negative bacteria is the electrostatic interaction between the positively charged peptide and the negatively charged LPS.[31] The majority of the mechanisms of resistance to polymyxins are based on modifications to LPS, which stop or reduce this initial interaction (Table 1). Numerous species have developed different mechanisms for the modification of lipid A by reducing its net negative charge. In *P. aeruginosa*, *Salmonella enterica* serovar Typhimurium and *E. coli*, the modification of lipid A with 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/or phosphoethanolamine (PEtn) reduces the net negative charge of LPS thereby increasing resistance to polymyxins.[32,40,41] The LPS modification causing polymyxin resistance is also mediated by $Fe^{3+}$ concentrations and low pH.[42,43]

Modification of LPS is not the only mechanism of resistance to polymyxins. In *K. pneumoniae*, the presence of capsule is critical for polymyxin resistance.[35] In *S.* Typhimurium, the gene *mig-14* is involved in polymyxin resistance.[34] While the specific mechanism of action is undefined, it is not related to LPS modification.[34] In *Vibrio cholerae*, resistance to polymyxin is dependent on the outer-membrane porin, OmpU.[39]

**Table 1.** Summary of major resistance mechanisms to polymyxins in Gram-negative bacteria

| Bacterium | Mechanism(s) of resistance to polymyxin | Ref. |
|---|---|---|
| *P. aeruginosa* | lipid A modifications with L-Ara4N controlled by PmrA/PmrB | 32 |
| *S. enterica* serovar Typhimurium | lipid A modification with both L-Ara4N and PEtn controlled by PmrA/PmrB | 33 |
| | the gene *mig-14* is required for resistance but resistance does not involve LPS modification | 34 |
| *E. coli* | lipid A modification with both L-Ara4N and PEtn controlled by PmrA/PmrB | 33 |
| *K. pneumoniae* | increased production of capsule polysaccharide. | 35 |
| *Burkholderia cenocepacia* | a complete LPS inner core oligosaccharide is required | 36 |
| *H. pylori* | lipid A modification | 37 |
| *Yersinia pestis* | lipid A modification with L-Ara4N controlled by PmrA/PmrB | 38 |
| *V. cholerae* | presence of outer membrane protein OmpU regulated by ToxR | 39 |

### *Epidemiology*

Acquired resistance to polymyxins in MDR Gram-negative bacilli is not common currently, probably due to the infrequent usage of these agents over the last 50 years. However, polymyxin-resistant bacteria have been identified and sporadic cases have been increasingly reported in the last five years.[44–47] It should be noted that in one report[45] the MICs were determined using Etest, which has been shown to present poor concordance with the broth microdilution method with colistin, also believed to be related to the poor diffusion of polymyxins in agar and a possible interference of cation levels in agar, particularly in extreme dilutions with higher MICs noted by the Etest method.[48] The contemporary activity and spectrum of polymyxin B against a worldwide collection of Gram-negative bacilli in the SENTRY antimicrobial surveillance programme has been recently evaluated and the results of this study are summarized in Table 2.[23] Fortunately, resistance to polymyxin B is low in *P. aeruginosa*, *Acinetobacter* spp. and *K. pneumoniae*,



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**Table 2.** Antimicrobial activity of polymyxin B against non-fermentative Gram-negative bacteria and Enterobacteriaceae isolates[a]

| Organism (number of isolates) | MIC (mg/L) | | | % resistant |
|---|---|---|---|---|
| | 50% | 90% | range | |
| Non-fermentative Gram-negative bacteria | | | | |
| *Acinetobacter* spp. (2621) | ≤1 | 2 | ≤1 to >8 | 2.1 |
| *Aeromonas* spp. (368) | ≤1 | >8 | ≤1 to >8 | 28.3 |
| *Alcaligenes* spp. (121) | 2 | >8 | ≤1 to >8 | 36.4 |
| *B. cepacia* (153) | >8 | >8 | 0.5 to >8 | 88.2 |
| *P. aeruginosa* (8705) | ≤1 | 2 | ≤1 to >8 | 1.3 |
| *Pseudomonas* spp. (non-*aeruginosa*; 282) | ≤1 | 4 | ≤1 to >8 | 11.7 |
| *S. maltophilia* (1256) | 1 | 8 | ≤0.12 to >8 | 27.6 |
| other non-enteric Gram-negative bacilli (302) | 4 | >4 | ≤1 to >8 | 55.6 |
| Enterobacteriaceae | | | | |
| *Citrobacter* spp. (895) | ≤1 | ≤1 | ≤1 to >8 | 0.9 |
| *Enterobacter* spp. (4693) | ≤1 | >8 | ≤1 to >8 | 16.7 |
| *E. coli* (18 325) | ≤1 | ≤1 | ≤1 to >8 | 0.5 |
| *Klebsiella* spp. (8188) | ≤1 | ≤1 | ≤1 to >8 | 1.8 |
| indole-positive *Proteus* spp. etc. (895)[b] | >8 | >8 | ≤1 to >8 | 98.7 |
| *Proteus mirabilis* (1931) | >8 | >8 | ≤1 to >8 | 99.3 |
| *Salmonella* spp. (2909) | ≤1 | 4 | ≤1 to >8 | 24.0 |
| *Shigella* spp. (828) | ≤1 | ≤1 | ≤1 to >8 | 1.0 |
| *Serratia* spp. (1919) | >8 | >8 | 0.25 to >8 | 94.6 |
| other enteric Gram-negative bacilli (340) | ≤1 | 8 | ≤1 to >8 | 24.1 |

[a]Data from SENTRY antimicrobial surveillance programme, 2001–04.[23]
[b]Includes: *M. morganii* ($n = 507$), *Proteus* spp. ($n = 64$), *Proteus vulgaris* ($n = 179$), *Providencia alcalifaciens* ($n = 1$), *Providencia rettgeri* ($n = 41$), *Providencia* spp. ($n = 18$) and *Providencia stuartii* ($n = 85$).

three 'superbugs' listed by the Infectious Diseases Society of America (IDSA), which require the most urgent attention from the pharmaceutical industry.[1] Although the influence of the emergence of polymyxin B and colistin resistance in MDR Gram-negative bacteria on clinical outcomes has not been assessed so far, it is very likely to have major public health implications, in particular with no new antibiotic against Gram-negative bacteria in the current drug development pipeline.

## Pharmacokinetics

There are no solid PK data available in the literature regarding iv administration of polymyxin B in humans. Even in the Product Information of Polymyxin B for Injection (Bedford Laboratories™),[18] no values for $C_{max}$ or half-life were provided. Since there is only one amino acid difference between polymyxin B and colistin, it might be expected that polymyxin B has similar protein binding as colistin which is ~50% bound in human plasma.[9,10] It should be noted that most PK data were obtained from studies conducted more than 30 years ago involving intramuscular administration.[10] The PK information for polymyxin B from old studies reported in recent reviews should be viewed with caution because of the limited nature of the data and the descriptions of the experimental conditions. Therefore, clinical PK studies are urgently required for polymyxin B.

The current recommended dose of iv polymyxin B for patients with normal renal function is 1.5–2.5 mg/kg/day in two divided doses administered as a 1 h infusion.[18] Although dosage recommendations have been specified for adults and infants in the Product Information (Bedford Laboratories™) for polymyxin B administered by iv, im and intrathecal routes, no clinical studies could be located in the literature to support these recommendations. Continuous iv infusion administration has also been recommended.[9] These doses and regimens appear to have been proposed on a totally empirical base.[49] Therefore, well-designed PK/PD studies are required urgently to define the optimal use of polymyxin B.

Dose adjustments for patients with renal impairment, including decreasing daily dose and extending administration intervals have been suggested.[9,50] Unfortunately, the recommendations do not appear to be based on solid PK data. Considering that colistin shows a very modest post-antibiotic effect only after exposure to high concentrations[51] and low exposure to colistin after administration of 400 mg of colistin methanesulphonate (i.e. 150 mg of colistin base activity) every 48 h in a critically ill patient on continuous venovenous haemodiafiltration,[52] it has been suggested that extended dosing intervals may place patients at substantial risk.[52] However, owing to the lack of PK data, it is not known if the same situation applies to polymyxin B and there are no recommended dosage regimens for polymyxin B in patients on haemodialysis, peritoneal dialysis or continuous renal replacement therapy.[50] Sarria *et al.*[50] have suggested that even if some drug can be cleared via dialysis, the amount eliminated is not high enough to warrant the administration of a supplemental dose after dialysis. In that study,[50] polymyxin B was administrated as a loading dose of 2.5 mg/kg, followed by two doses of 1.0 mg/kg on days 4 and 8, then 0.8 mg/kg daily to



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complete a 24 day course. Polymyxin B was not detected in the dialysate fluid (volume not described) by a broth dilution assay using *E. coli* strain ATCC 25922 from days 13 to 17 of therapy, despite its presence in serum.[50] Serum concentrations of polymyxin B ranged from 6.25 to 50 mg/L.[50] The concentrations at the upper end of this range appear to be high, and care is required when considering such dosage schedules. Definitive dosage recommendations of polymyxin B for various categories of patients will not be available until more experience is gained from modern PK and clinical studies.

## Pharmacodynamics

Most investigations on the PD of polymyxins have focused on colistin.[12,15] So far, there is only one study examining the PD of polymyxin B,[17] which has shown concentration-dependent killing similar to colistin.[51] In an *in vitro* static system, polymyxin B was rapidly bactericidal at super-MIC concentrations against four *P. aeruginosa* strains (MICs of polymyxin B were 0.5 mg/L for one strain and 1 mg/L for the others).[17] In these time–kill studies, regrowth was noted after the initial rapid reduction in bacterial burden at all tested concentrations (1–16 mg/L).[17] In addition, the killing effect of polymyxin B was subject to inoculum effect.[17] The post-antibiotic effect of polymyxin B was not assessed in this study.[17] Using an *in vitro* hollow-fibre PD infection model with a simulated dose of 2.5 mg/kg/day and half-life of 6 h, polymyxin B showed an initial rapid bacterial killing of *P. aeruginosa*, but regrowth occurred after 24 h irrespective of the dosing interval employed (once daily, every 12 h or every 8 h).[17] The lack of difference in bacterial killing with the same daily dose may indicate that the antibacterial effect of polymyxin B was most closely related to the ratio of area under the concentration–time curve to MIC (AUC/MIC).[17] Certainly, further PK/PD experiments are required to determine which PK/PD index (i.e. AUC/MIC, $C_{max}$/MIC and $\%t > \text{MIC}$) is best correlated to the efficacy of polymyxin B. Despite rapid initial killing, the emergence of resistance over a 4 day treatment period was observed in the hollow-fibre model experiment.[17] This resistance was demonstrated to be adaptive, since susceptibility reversal was observed upon serial passaging on drug-free medium plates over 20 days.[17] These results have raised some concern regarding the use of monotherapy with polymyxin B, particularly in immunocompromised patients.[17] The potential for the emergence of resistance during therapy seems also to occur with colistin, as demonstrated by two recent *in vitro* studies on *A. baumannii*,[53,54] although resistance to colistin was not reversed after a 10 day serial passage on drug-free medium plates.[53]

## Combination of polymyxin B with other antibiotics

*In vitro* synergism of colistin combined with other antimicrobials has been investigated recently and reviewed elsewhere.[7,12] Overall, synergistic or additive activity was shown with combinations of colistin with several other agents compared with any agent alone. So far, seven studies have evaluated the potential synergistic activity of polymyxin B with other antibiotics, most of them against *A. baumannii*.[55–61]

Tascini *et al.*[55] assessed the combination of polymyxin B and rifampicin against five clonally unrelated MDR *A. baumannii* isolates with the chequerboard method. The combination was synergistic against three isolates [fractional inhibitory concentration index (FICI) $\leq 0.5$] and additive (FICI between 0.5 and 1) against the other two isolates.[55] Yoon *et al.*[56] studied the double and triple combinations of polymyxin B, imipenem and rifampicin against eight unrelated clinical *A. baumannii* isolates resistant to all commonly used antibiotics except polymyxins through three-dimensional chequerboard microtitre plate dilution and time–kill studies at one-fourth of the MICs of these drugs. The triple combination of polymyxin B–rifampicin–imipenem was synergistic against all isolates (synergy was defined as an FICI $< 1.0$).[56] Time–kill curves using 0.25 mg/L polymyxin B, 0.5 mg/L rifampicin and 8 mg/L imipenem showed that all isolates were killed within 24 h, a result that was not achieved with each antibiotic alone.

Landman *et al.*[57] analysed synergism of polymyxin B with imipenem, azithromycin and rifampicin against 10 MDR *P. aeruginosa* isolates, comprising 7 unique ribotypes. Chequerboard studies revealed synergy of polymyxin B combined with 4 mg/L azithromycin for six isolates, with 4 mg/L imipenem for two and with 1 mg/L rifampicin for one.[57] In the time–kill studies, the combinations of polymyxin B with either rifampicin or imipenem were bactericidal against most of the isolates, and the three-drug combination against all isolates. The three-drug combination was most rapidly bactericidal.[57] The same group of authors also investigated the combinations with time–kill method against 13 MDR *P. aeruginosa* isolates.[58] The addition of 4 mg/L azithromycin to the lower concentration of polymyxin B (2 mg/L) produced a $>2$ log kill against most isolates and prevented regrowth in all but two isolates.[58]

Another study examined the combination of polymyxin B and rifampicin against 16 *K. pneumoniae* which produced KPC-2 carbapenemase; these isolates comprised 6 distinct strains and 10 isolates representative of another 2 different ribotypes.[59] The combination of 1 mg/L polymyxin B plus 1 mg/L rifampicin was synergistic against 15 out of the 16 isolates. For a polymyxin B-resistant isolate (MIC of 16 mg/L), a decrease of $\sim$2 log cfu/mL was observed with the combination of subinhibitory polymyxin B and rifampicin. The combination of polymyxin B (0.5× MIC) with 4 mg/L imipenem was synergistic against 10 out of 16 isolates but antagonistic for three isolates. The addition of 4 mg/L imipenem to the combination of polymyxin B (0.5× MIC) and 1 mg/L rifampicin had no effect.[59]

Manikal *et al.*[60] investigated the combinations of polymyxin B and azithromycin or rifampicin using chequerboard studies against 24 *A. baumannii* isolates, belonging to four distinct PFGE groups. The combination of 4 mg/L azithromycin with polymyxin B showed synergy (FICI range $\leq 0.18-0.5$) against 20 isolates, including two polymyxin-resistant isolates, and additive effect against the remaining 4 (FICI range $0.52-1.0$). The combination of 1 mg/L rifampicin and polymyxin B demonstrated synergy against half of the isolates (FICI range, $\leq 0.18-0.5$) and an additive effect (FICI range, $0.52-1.0$) against the remainder.[60] In another study, combinations of polymyxin B with imipenem, azithromycin or rifampicin were assessed using Etest agar dilution and combined Etest strip methods against five unrelated MDR *A. baumannii* isolates, which encoded OXA-23 carbapenemase and were only susceptible to polymyxins.[61] Synergy was not observed with



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polymyxin B in combination with any drug against four of the isolates. Borderline synergy (FICI = 0.5) was shown against one strain with polymyxin B in combination with rifampicin or imipenem.[61]

Although combination therapy of polymyxin B with other antibiotics seems to be an attractive option, there are no clinical data showing superiority of this strategy over polymyxin B monotherapy. Nevertheless, given that no new antibiotics will be available in the next few years for MDR Gram-negative bacteria, in particular *P. aeruginosa* and *A. baumannii*, novel combinations of the currently available antibiotics have to be investigated.

## Clinical use for treatment of MDR Gram-negative infections

Polymyxin B sulphate is the form available for iv administration and each milligram of polymyxin B base is equivalent to ~10 000 IU.[9,10] Compared with colistin (methanesulphonate), there is very limited clinical experience with polymyxin B in the literature. There are no well-designed clinical trials evaluating the efficacy of iv polymyxin B for treatment of infections caused by MDR Gram-negative bacteria, or comparing its clinical efficacy with colistin. There are only three studies investigating the use of polymyxin B for treatment of infections caused by MDR Gram-negative bacilli, mostly *Acinetobacter* spp. and *P. aeruginosa*.[62–64] Additional to these studies, we investigated iv use of polymyxin B in a subgroup from a cohort of patients with infections caused by metallo-β-lactamase-producing *P. aeruginosa*.[65,66] Pereira *et al.*[67] reported the concomitant use of inhaled and iv polymyxin B for pneumonia due to MDR Gram-negative bacilli after treatment failure with the latter. Unfortunately, all these studies (Table 3) were limited by small sample sizes and lack of standardized definitions of outcomes among them.

In a recent clinical study on polymyxin B,[62] 60 patients with nosocomial infections, mostly due to *A. baumannii*, were investigated. The majority of the patients were mechanically ventilated and had pulmonary infections. The iv polymyxin B dose was adjusted according to the estimated creatinine clearance: 20–50 mL/min, 75% of the total daily dose of 2.5 mg/kg; <20 mL/min, 33% of the total daily dose. The overall mortality of these patients was 20%. Bacteria were cleared in 88% of the patients; however, susceptibility testing revealed that the bacteria persisting in other patients remained susceptible to polymyxin B. A major drawback in both clinical efficacy and microbiological endpoint analyses is that up to 90% of patients received combination therapy with another agent active against *P. aeruginosa* and *A. baumannii*.[62]

In another study, only patients who received combination therapy were included.[63] Twenty-nine treatments from 25 patients were analysed. Ninety-two per cent of the patients were from intensive care units and 88% were mechanically

**Table 3.** Studies assessing clinical efficacy of polymyxin B against multidrug-resistant Gram-negative bacteria

| Demographics | Infections and treatments | | Outcomes | | |
|---|---|---|---|---|---|
| *n*/mean age (years)/% males | pathogens (%) | daily dosage, mean (range)[a]/duration of treatment, mean days (range) | mortality[b] % | nephrotoxicity % | Ref. |
| 60/61/65 | *A. baumannii* (77); *P. aeruginosa* (3); both (3); none identified (17) | 1.1 (0.12–2.25)[c]/13.5 (1–56) | 20 | 14[d] | 62 |
| 25 (29 episodes of infection)/55/52 | *A. baumannii* (55); *P. aeruginosa* (41); *A. xylosoxidans* (3)[e] | day 1: 2.5–3 mg/kg/day[f]/19 (2–57) | 48; 21[g] | 10[h] | 63 |
| 33/41/78 | MDR *A. baumannii* (100) | 1.3 (0.186–3.0)[i]/NA | 27 | 21[j] | 64 |
| 13/51/NA | MBL-producing *P. aeruginosa* (100) | 1.92 mg/kg/day (1.66–2.12)[k] | 54 | 0[l] | 65 |
| 14/69/79 | *P. aeruginosa* (79); *K. pneumoniae* (7); *A. xylosoxidans* (7); *Burkholderia* spp. (7) | NA[m] | 64 | NA | 67 |

MBL, metallo-β-lactamase; MDR, multidrug-resistant; VAP, ventilator-associated pneumonia; NA, not available.
[a]Dose $\times 10^6$ U, unless otherwise indicated.
[b]Overall in-hospital mortality, unless otherwise indicated.
[c]All patients were co-administered with other antibiotics. When ampicillin/sulbactam and/or amikacin were active (90% and 80%, respectively), they were added to polymyxin B therapy.
[d]Doubling of serum creatinine to a value of ≥2.0 mg/dL.
[e]Seven isolates of *A. baumannii* and five isolates of *P. aeruginosa* were reported resistant to all available antibiotics except polymyxin B.
[f]Subsequent doses were determined by estimated creatinine clearance and adjusted accordingly during therapy as proposed by Evans *et al.*[9] Intravenous therapy only in 21 (72%) patients, aerosol in 6 (21%) and both in 2 (7%). All patients received combination therapy with polymyxin B: imipenem or meropenem, 19 (65%); amikacin, 8 (28%); tobramycin, 3 (10%); cefepime, 3 (10%); quinolone, 2 (7%); ampicillin/sulbactam, 3 (10%); aztreonam, 1 (3%).
[g]End-of-treatment mortality.
[h]Doubling of serum creatinine during therapy. Thirty-eight per cent of the patients also received aminoglycosides.
[i]Twenty-eight patients received iv therapy only, two aerosolized and three received both. Monotherapy with polymyxin B was used in 27 patients.
[j]Increase in serum creatinine of 0.5 mg/dL or ≥50% over the baseline value, or a reduction of ≥50% in the calculated creatinine clearance.
[k]Dose adjustment for renal function was not described. Six received monotherapy of polymyxin B, five received polymyxin B with a β-lactam for which MBL-producing *P. aeruginosa* were resistant and two received polymyxin B with aztreonam for which MBL-producing *P. aeruginosa* was susceptible.
[l]Defined as the need to discontinue iv polymyxin B therapy due to renal toxicity.
[m]All patients were concomitantly treated with inhaled polymyxin B.



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ventilated. All patients had respiratory tract infections caused by *A. baumannii* (55%), *P. aeruginosa* (41%) and *Alcaligenes xylosoxidans* (3%). Only seven *A. baumannii* and five *P. aeruginosa* isolates were resistant to all available antibiotics except polymyxin B. Since all patients were treated with another antibiotic, efficacy analysis of polymyxin B was compromised.[63] The overall discharge mortality was 48%. Follow-up cultures were available in 22 cases, of which 9 achieved microbiological clearance but were associated with a longer duration of therapy. Resistance to polymyxin B was not observed during the therapy.[63]

Holloway *et al.*[64] recently published their experience with the treatment of 37 patients with infections due to polymyxin-only-susceptible *A. baumannii*, of whom 33 received polymyxin B therapy. Monotherapy with polymyxin B was used in 27 patients. Most infections were ventilator-associated pneumonia. Nine (27%) patients died after treatment with polymyxin B.[64] Microbiological cure was achieved in 17 (81%) of 21 patients evaluated for this outcome.[64]

In our recent study on the treatment of 13 patients with iv polymyxin B against infections caused by MDR metallo-β-lactamase-producing *P. aeruginosa*,[65] 8 patients had pneumonia, of whom 4 were ventilator-associated.[66] Overall in-hospital mortality was 54%.[65] Of six patients with ventilator-associated pneumonia treated with polymyxin B, four (67%) died within 30 days after initial treatment with polymyxin B.[66]

Pereira *et al.*[67] described clinical features and outcomes of 19 patients treated with inhaled polymyxin B. Fourteen of them had nosocomial pneumonia (11 were ventilator-associated pneumonia) and were concomitantly treated with iv polymyxin B. *P. aeruginosa* was the aetiological agent in 11 of these 14 patients. Nine (64%) of the 14 patients died during hospitalization, although 13 (93%) of them were described as having a good clinical outcome of the pneumonia.[67] Interestingly, most of the selected patients for this study (not precisely described) had previously presented failure with iv polymyxin B therapy.[67] This highlights the urgency to investigate the PK of polymyxin B after iv administration and inhalation in pneumonia patients.

In addition, Ostronoff *et al.*[68] described two cases of successful treatment of cellulitis, caused by MDR *P. aeruginosa* (one complicated with bacteraemia) in neutropenic patients, with polymyxin B in combination with rifampicin. Polymyxin B was administered at a dose of 1.0 mg/kg iv every 12 h for both patients. No renal toxicity was observed between 19 and 21 days of treatment.[68]

Although these studies suggest that iv polymyxin B has acceptable effectiveness for the treatment of severe infections by MDR Gram-negative bacteria, such a conclusion must be taken with caution due to the lack of a comparative group, and also co-administration of other antibiotics in most of the patients. As noted above, it is not known if any potential differences in clinical efficacy and outcomes exist between polymyxin B and colistin (methanesulphonate).

## Toxicity

Nephrotoxicity and neurotoxicity are the most common potential toxicities with parenteral administration of polymyxins.[12,69] However, the toxicity observed in early clinical studies with colistimethate sodium was almost certainly due to a lack of understanding of its pharmacokinetics, pharmacodynamics and toxicodynamics, and the use of inappropriate doses.[12] It should be noted that most studies assessing toxicities of polymyxins were conducted with colistin methanesulphonate and they may not necessarily represent polymyxin B toxicity. In a systematic review of the old literature and very limited recent studies,[69] Falagas *et al.*[69] concluded that the incidence of nephrotoxicity in recently published experience with polymyxins is less common and severe compared with the studies in the 1970s. Incidences of renal toxicity in recent studies ranged from 0% to 37%.[69] Evaluations of polymyxin B nephrotoxicity are shown in Table 3. Clinicians should be alert to the potential for nephrotoxicity, adjust the dose according to renal function, avoid concomitant administration of other potentially nephrotoxic drugs where possible and undertake appropriate monitoring to detect deterioration in renal function.[69]

Neurotoxicities of polymyxins are considerably less frequent than nephrotoxicity, and they are usually mild and resolve after prompt discontinuation of therapy.[69] Neurotoxicities were also less frequent in recent studies compared with older ones.[69] Dizziness, generalized or not muscle weakness, facial and peripheral paraesthesia, partial deafness, visual disturbances, vertigo, confusion, hallucinations, seizures and ataxia have been associated with the use of polymyxins, although most studies reporting such effects were with colistin (methanesulphonate).[69] No severe toxicity, such as neuromuscular blockade or apnoea induced by polymyxins, has been reported over the last 15 years.[69] Seizures and neuromuscular weakness possibly related to polymyxin B have been reported in two cases.[63] Holloway *et al.*[64] observed a new-onset altered mental status in one (3%) patient and distal paraesthesias in another (3%) associated with iv polymyxin B.

Other adverse reactions include rash, pruritus, dermatitis and drug fever, probably resulting from the histamine-releasing action of polymyxin B.[69] A recent case of rhabdomyolysis potentially associated with iv colistin (methanesulphonate) has been described,[70] but definitive relation warrants further investigation.

A substantial risk of congenital abnormalities in the infants of women who are treated with parenteral polymyxin B during pregnancy is unlikely.[71] However, this assessment was based on a single clinical study. Overall, there is extremely limited teratological data for polymyxin B in experimental animals and further examination is required.

## Conclusions

Polymyxin B has re-emerged in medical practice in recent years and its use will likely continue to increase since new drugs for the treatment of infections caused by MDR Gram-negative bacteria are beyond a distant horizon. Unfortunately, there are very substantial gaps in the knowledge of polymyxin B pharmacology. As a result, optimal dosage regimens with maximal efficacy but minimal toxicities and potential for the development of resistance are still not known. The current recommendations for dose adjustment in renal insufficiency and dialysis are not based on solid PK data. Furthermore, although recent clinical reports suggest that polymyxin B has reasonable efficacy, there are major drawbacks in these studies, including limited sample



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sizes, absence of a control group and co-administration of other antibiotics, which impair definitive conclusions. Therefore, further investigations on the pharmacokinetics, pharmacodynamics and toxicodynamics of polymyxin B and its efficacy alone and in combination with other antibiotics are urgently required. The need for these studies is heightened by the rapidly increasing prevalence of nosocomial infections caused by polymyxin-only-susceptible pathogens and the absence of novel antibiotics in the drug discovery and development pipeline.

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## Transparency declarations

None to declare.

## References

**1.** Infectious Diseases Society of America. *Bad Bugs, No Drugs*. http://www.idsociety.org/pa/IDSA_Paper4_final_web.pdf (18 June 2007, date last accessed).

**2.** Grundmann H, Aires-de-Sousa M, Boyce J *et al*. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 2006; **368**: 874–85.

**3.** Livermore DM. Quinupristin/dalfopristin and linezolid: where, when, which and whether to use? *J Antimicrob Chemother* 2000; **46**: 347–50.

**4.** Pankey GA. Tigecycline. *J Antimicrob Chemother* 2005; **56**: 470–80.

**5.** Steenbergen JN, Alder J, Thorne GM *et al*. Daptomycin: a lipopeptide antibiotic for the treatment of serious Gram-positive infections. *J Antimicrob Chemother* 2005; **55**: 283–8.

**6.** Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2006; **43** Suppl 2: 49–56.

**7.** Rahal JJ. Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. *Clin Infect Dis* 2006; **43** Suppl 2: 95–9.

**8.** Livermore DM. The need for new antibiotics. *Clin Microbiol Infect* 2004; **10** Suppl 4: 1–9.

**9.** Evans ME, Feola DJ, Rapp RP. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. *Ann Pharmacother* 1999; **33**: 960–7.

**10.** Hermsen ED, Sullivan CJ, Rotschafer JC. Polymyxins: pharmacology, pharmacokinetics, pharmacodynamics, and clinical applications. *Infect Dis Clin North Am* 2003; **17**: 545–62.

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

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

**13.** Li J, Rayner CR, Nation RL *et al*. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2006; **50**: 2946–50.

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

**15.** Li J, Nation RL, Milne RW *et al*. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. *Int J Antimicrob Agents* 2005; **25**: 11–25.

**16.** Owen RJ, Li J, Nation RL *et al*. In vitro pharmacodynamics of colistin against *Acinetobacter baumannii* clinical isolates. *J Antimicrob Chemother* 2007; **59**: 473–7.

**17.** Tam VH, Schilling AN, Vo G *et al*. Pharmacodynamics of polymyxin B against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005; **49**: 3624–30.

**18.** Bedford Laboratories. Polymyxin B for injection (package insert). Bedford, OH 44146. Bedford Laboratories; 2004.

**19.** Hancock REW. Peptide antibiotics. *Lancet* 1997; **349**: 418–22.

**20.** Kang JW, Van Schepdael A, Orwa JA *et al*. Analysis of polymyxin B sulfate by capillary zone electrophoresis with cyclodextrin as additive. Method development and validation. *J Chromatogr A* 2000; **879**: 211–8.

**21.** Orwa JA, Govaerts C, Busson R *et al*. Isolation and structural characterization of polymyxin B components. *J Chromatogr A* 2001; **912**: 369–73.

**22.** Bergen PJ, Li J, Rayner CR *et al*. Colistin methanesulfonate is an inactive prodrug of colistin against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2006; **50**: 1953–8.

**23.** Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001–2004). *Clin Microbiol Infect* 2006; **12**: 315–21.

**24.** Gales AC, Reis AO, Jones RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. *J Clin Microbiol* 2001; **39**: 183–90.

**25.** Storm DR, Rosenthal KS, Swanson PE. Polymyxin and related peptide antibiotics. *Annu Rev Biochem* 1977; **46**: 723–63.

**26.** Catchpole CR, Andrews JM, Brenwald N *et al*. A reassessment of the in-vitro activity of colistin sulphomethate sodium. *J Antimicrob Chemother* 1997; **39**: 255–60.

**27.** Hogardt M, Schmoldt S, Gotzfried M *et al*. Pitfalls of polymyxin antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients. *J Antimicrob Chemother* 2004; **54**: 1057–61.

**28.** Jones RN, Anderegg TR, Swenson JM *et al*. Quality control guidelines for testing gram-negative control strains with polymyxin B and colistin (polymyxin E) by standardized methods. *J Clin Microbiol* 2005; **43**: 925–7.

**29.** Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement M100-S17*. CLSI, Wayne, PA, USA, 2007.

**30.** British Society for Antimicrobial Chemotherapy. *BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing, Version 6.1*. http://www.bsac.org.uk/_db/_documents/version_6.1.pdf (6 August 2007, date last accessed).

**31.** Clausell A, Garcia-Subirats M, Pujol M *et al*. Gram-negative outer and inner membrane models: insertion of cyclic cationic lipopeptides. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* 2007; **111**: 551–63.

**32.** Moskowitz SM, Ernst RK, Miller SI. PmrAB, a two-component regulatory system of *Pseudomonas aeruginosa* that modulates



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resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. *J Bacteriol* 2004; **186**: 575–9.

**33.** Winfield MD, Groisman EA. Phenotypic differences between *Salmonella* and *Escherichia coli* resulting from the disparate regulation of homologous genes. *Proc Natl Acad Sci USA* 2004; **101**: 17162–7.

**34.** Brodsky IE, Ernst RK, Miller SI *et al*. mig-14 is a *Salmonella* gene that plays a role in bacterial resistance to antimicrobial peptides. *J Bacteriol* 2002; **184**: 3203–13.

**35.** Campos MA, Vargas MA, Regueiro V *et al*. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. *Infect Immun* 2004; **72**: 7107–14.

**36.** Loutet SA, Flannagan RS, Kooi C *et al*. A complete lipopolysaccharide inner core oligosaccharide is required for resistance of *Burkholderia cenocepacia* to antimicrobial peptides and bacterial survival *in vivo*. *J Bacteriol* 2006; **188**: 2073–80.

**37.** Tran AX, Whittimore JD, Wyrick PB *et al*. The Lipid A 1-phosphatase of *Helicobacter pylori* is required for resistance to the antimicrobial peptide polymyxin. *J Bacteriol* 2006; **188**: 4531–41.

**38.** Winfield MD, Latifi T, Groisman EA. Transcriptional regulation of the 4-amino-4-deoxy-L-arabinose biosynthetic genes in *Yersinia pestis*. *J Biol Chem* 2005; **280**: 14765–72.

**39.** Mathur J, Waldor MK. The *Vibrio cholerae* ToxR-regulated porin OmpU confers resistance to antimicrobial peptides. *Infect Immun* 2004; **72**: 3577–83.

**40.** Lee H, Hsu FF, Turk J *et al*. The PmrA-regulated *pmrC* gene mediates phosphoethanolamine modification of lipid A and polymyxin resistance in *Salmonella enterica*. *J Bacteriol* 2004; **186**: 4124–33.

**41.** Helander IM, Kilpelainen I, Vaara M. Increased substitution of phosphate groups in lipopolysaccharides and lipid A of the polymyxin-resistant *pmrA* mutants of *Salmonella typhimurium*: a 31P-NMR study. *Mol Microbiol* 1994; **11**: 481–7.

**42.** Perez JC, Groisman EA. Acid pH activation of the PmrA/PmrB two-component regulatory system of *Salmonella enterica*. *Mol Microbiol* 2007; **63**: 283–93.

**43.** Delgado MA, Mouslim C, Groisman EA. The PmrA/PmrB and RcsC/YojN/RcsB systems control expression of the *Salmonella* O-antigen chain length determinant. *Mol Microbiol* 2006; **60**: 39–50.

**44.** Reis AO, Luz DA, Tognim MC *et al*. Polymyxin-resistant *Acinetobacter* spp. isolates: what is next? *Emerg Infect Dis* 2003; **9**: 1025–7.

**45.** Urban C, Mariano N, Rahal JJ *et al*. Polymyxin B-resistant *Acinetobacter baumannii* clinical isolate susceptible to recombinant BPI and cecropin P1. *Antimicrob Agents Chemother* 2001; **45**: 994–5.

**46.** Antoniadou A, Kontopidou F, Poulakou G *et al*. Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. *J Antimicrob Chemother* 2007; **59**: 786–90.

**47.** Falagas ME, Bliziotis IA. Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era? *Int J Antimicrob Agents* 2007; **29**: 630–6.

**48.** del Arroyo LA, Garcia-Curiel A, Pachon-Ibanez ME *et al*. Reliability of the E-test method for detection of colistin resistance in clinical isolates of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; **43**: 903–5.

**49.** Pulaski EJ, Baker HJ, Rosenberg ML *et al*. Laboratory and clinical studies of polymyxin B and E. *J Clin Invest* 1949; **28**: 1028–31.

**50.** Sarria JC, Angulo-Pernett F, Kimbrough RC *et al*. Use of intravenous polymyxin B during continuous venovenous hemodialysis. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 340–1.

**51.** Li J, Turnidge J, Milne R *et al*. In vitro pharmacodynamic properties of colistin and colistin methanesulfonate against *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. *Antimicrob Agents Chemother* 2001; **45**: 781–5.

**52.** Li J, Rayner CR, Nation RL *et al*. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. *Antimicrob Agents Chemother* 2005; **49**: 4814–5.

**53.** Kroeger LA, Hovde LB, Mitropoulos IF *et al*. Colistin methanesulfonate against multidrug resistant *Acinetobacter baumannii* in an in vitro pharmacodynamic model. *Antimicrob Agents Chemother* 2007; **51**: 3431–3.

**54.** Tan CH, Li J, Nation RL. Colistin against hetero-resistant *Acinetobacter baumannii* in an *in vitro* Pharmacokinetic/Pharmacodynamic model: antibacterial activity and emergence of resistance. *Antimicrob Agents Chemother* 2007; **51**: 3413–5.

**55.** Tascini C, Menichetti F, Bozza S *et al*. Evaluation of the activities of two-drug combinations of rifampicin, polymyxin B and ampicillin/sulbactam against *Acinetobacter baumannii*. *J Antimicrob Chemother* 1998; **42**: 270–1.

**56.** Yoon J, Urban C, Terzian C *et al*. *In vitro* double and triple synergistic activities of polymyxin B, imipenem, and rifampin against multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2004; **48**: 753–7.

**57.** Landman D, Bratu S, Alam M *et al*. Citywide emergence of *Pseudomonas aeruginosa* strains with reduced susceptibility to polymyxin B. *J Antimicrob Chemother* 2005; **55**: 954–7.

**58.** Bratu S, Quale J, Cebular S *et al*. Multidrug-resistant *Pseudomonas aeruginosa* in Brooklyn, New York: molecular epidemiology and *in vitro* activity of polymyxin B. *Eur J Clin Microbiol Infect Dis* 2005; **24**: 196–201.

**59.** Bratu S, Tolaney P, Karumudi U *et al*. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and *in vitro* activity of polymyxin B and other agents. *J Antimicrob Chemother* 2005; **56**: 128–32.

**60.** Manikal VM, Landman D, Saurina G *et al*. Endemic carbapenem-resistant *Acinetobacter* species in Brooklyn, New York: citywide prevalence, interinstitutional spread, and relation to antibiotic usage. *Clin Infect Dis* 2000; **31**: 101–6.

**61.** Wareham DW, Bean DC. *In-vitro* activity of polymyxin B in combination with imipenem, rifampicin and azithromycin versus multidrug resistant strains of *Acinetobacter baumannii* producing OXA-23 carbapenemases. *Ann Clin Microbiol Antimicrob* 2006; **5**: 10.

**62.** Ouderkirk JP, Nord JA, Turett GS *et al*. Polymyxin B nephrotoxicity and efficacy against nosocomial infections caused by multiresistant gram-negative bacteria. *Antimicrob Agents Chemother* 2003; **47**: 2659–62.

**63.** Sobieszczyk ME, Furuya EY, Hay CM *et al*. Combination therapy with polymyxin B for the treatment of multidrug-resistant Gram-negative respiratory tract infections. *J Antimicrob Chemother* 2004; **54**: 566–9.

**64.** Holloway KP, Rouphael NG, Wells JB *et al*. Polymyxin B and doxycycline use in patients with multidrug-resistant *Acinetobacter baumannii* infections in the intensive care unit. *Ann Pharmacother* 2006; **40**: 1939–45.

**65.** Zavascki AP, Barth AL, Goncalves AL *et al*. The influence of metallo-β-lactamase production on mortality in nosocomial *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother* 2006; **58**: 387–92.

**66.** Zavascki AP, Barth AL, Fernandes JF *et al*. Reappraisal of *Pseudomonas aeruginosa* hospital-acquired pneumonia mortality in the era of metallo-β-lactamase-mediated multidrug resistance: a prospective observational study. *Crit Care* 2006; **10**: R114.

**67.** Pereira GH, Muller PR, Levin AS. Salvage treatment of pneumonia and initial treatment of tracheobronchitis caused by multidrug-resistant Gram-negative bacilli with inhaled polymyxin B. *Diagn Microbiol Infect Dis* 2007; **58**: 235–40.

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

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

**70.** Evagelopoulou P, Katsaros A, Myrianthefs P *et al*. Colistin and rhabdomyolysis: a causative agent or an innocent bystander? *Intensive Care Med* 2007; **33**: 556–7.

**71.** Kazy Z, Puho E, Czeizel AE. Parenteral polymyxin B treatment during pregnancy. *Reprod Toxicol* 2005; **20**: 181–2.

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