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Polymyxins Revisited

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INTRODUCTION

The emergence of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and carbapenemase-producing *Enterobacteriaceae* strains that are resistant to all β-lactams, fluoroquinolones, and aminoglycosides has led to renewed interest in polymyxin antibiotics as therapeutic agents. Once discarded out of concern for their toxicity, polymyxins have now become important therapeutic agents in many medical centers.

Origin and Chemical Structure

The antibiotic property of polymyxins was first recognized in the 1940s (1, 7, 161); colistin was recognized in 1950 (87) and was later identified to be the same as polymyxin E. These are cyclic, positively charged peptide antibiotics derived from various species of *Paenibacillus* (*Bacillus*) *polymyxa*. Of the five polymyxins (polymyxins A to E) originally described, two have been used in the clinical setting. Polymyxin B differs from polymyxin E (colistin) by a single amino acid change (D-phenylalanine replaces D-leucine). The great majority of recent reports involved the study of colistin-derived preparations.

Commercial Formulations

Commercial preparations of colistin sulfate consist of colistins A and B, which differ by their fatty acid residues. Because of toxicity, colistin sulfate is used only for topical therapy. Colistimethate sodium (also referred to as colistin methanesulfonate) is a less toxic preparation for parenteral use and is generated by treating colistin with formaldehyde and sodium bisulfate. Available preparations consist of colistimethates A and B, differing by their fatty acid residues (103).

There are several commercial preparations of colistimethate, and their differences have undoubtedly contributed to confusion when evaluating dosing guidelines. Coly-Mycin M Parenteral is produced by Parkedale Pharmaceuticals in the United States. The package insert states that each vial contains 150 mg of colistin base, and the recommended dose is 2.5 to 5 mg/kg/day in divided doses for patients with normal renal function (Coly-Mycin M Parenteral package insert; Monarch Pharmaceuticals, Inc., Bristol, TN), not to exceed 300 mg per day. Because there is 360 mg of colistimethate per 150 mg of colistin base, this translates into a recommended dose of 6 to 12 mg/kg/day in divided doses of colistimethate (not to exceed 720 mg per day).

Another preparation of colistimethate is Colomycin Injection, manufactured by Alpharma ApS (Denmark). The package insert states that there is 80 mg of colistimethate per 1 million units (12,500 units per mg), and the recommended dosage is 4 to 6 mg/kg per day for ≤60 kg body weight and 240 to 480 mg per day divided into three doses for >60 kg body weight (Colomycin Injection package insert; Forest Laboratories, UK Limited, Bexley, United Kingdom). The preparation of colistin distributed by Norma Pharmaceuticals (Greece) has been reported to have ~12,500 units per mg (48) to 13,333 units per mg (81, 121, 122).

Polymyxin B is manufactured as a sulfate compound consist-

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TABLE 1. Studies examining pharmacokinetic data for polymyxin B and colistimethate[a]

Test group	Agent	Assay	Dose for normal renal function	Peak serum concn (μg/ml)	Description	Reference
10 patients with normal renal function	Colistimethate	Bioassay	150-mg single dose	18	Serum at 4 h, 2 μg/ml; urine, ~250 μg/ml	52
20 patients with variable renal function	Colistimethate	Bioassay	1.25–2.5 mg/kg and then 4.8–6.9 mg/h	5–6	Urine, 90–125 μg/ml	35
39 patients with variable renal function	Colistimethate	Bioassay	75-mg single dose	2.4 (CrCl, >75) 2.2 (CrCl, 20–75) 2.3 (CrCl, 5–20) 5.1 (CrCl, <5)	$t_{1/2}$ = 4.5 h $t_{1/2}$ = 4.75 h $t_{1/2}$ = 12.75 h $t_{1/2}$ = 10.25 h	64
6 patients	Colistimethate	Bioassay	2-mg/kg single dose 4-mg/kg single dose	11–14 17–25	Urine levels reached ~200 μg/ml	119
31 patients with cystic fibrosis and normal renal function	Colistimethate	HPLC	5–7 mg/kg/day colistin base	13–32	$t_{1/2}$ = 3.5 h V = 0.09 liters/kg 62% of dose found in urine	144
22 patients with variable renal function	Colistimethate	Bioassay	1–3.7-mg/kg single dose	~6–12	Prolonged levels in patients with declining renal function; no change with dialysis	112
6 patients with normal renal function	Colistimethate	Bioassay	1 million units in a single dose	5–16		13
12 patients with cystic fibrosis and normal renal function	Colistimethate	HPLC	80–160 mg every 8 h (~7.3 mg/kg/day)	1–3	$t_{1/2}$ of colistin, 4.2 h $t_{1/2}$ of colistimethate, 2.1 h	97
1 patient on continuous venovenous hemodiafiltration	Colistimethate	HPLC	150 mg (2.5 mg/kg) every 48 h	1.8 (colistin) 2.3 (colistimethate)	$t_{1/2}$ of colistin, 7.5 h $t_{1/2}$ of colistimethate, 6.8 h	105
1 patient on continuous venovenous hemodialysis	Polymyxin B	Bioassay	0.8 mg/kg/day	6.25–50		150

[a] CrCl, creatinine clearance (ml/min); $t_{1/2}$, half-life; V, volume of distribution.

ing of polymyxins B_1 and B_2. Each milligram is equivalent to 10,000 units, and the recommended parenteral dose is 15,000 to 25,000 units/kg (1.5 to 2.5 mg/kg) per day in divided doses for normal renal function (Polymyxin B for Injection package insert; Bedford Laboratories, Bedford, OH).

PHARMACOKINETICS

Serum Concentrations

There is a dearth of reliable information concerning the pharmacokinetic data for polymyxins in humans (Table 1). Several older studies using Coly-Mycin noted levels of ~6 to 18 μg/ml of bioactive colistin following a single dose of approximately 75 to 150 mg of colistin base, equivalent to 180 to 360 mg of colistimethate (Coly-Mycin M Parenteral package insert; Monarch Pharmaceuticals, Inc., Bristol, TN) (52, 64, 112). Urinary concentrations typically achieved levels of 250 to 500 μg/ml (Coly-Mycin M Parenteral package insert; Monarch Pharmaceuticals, Inc., Bristol, TN) (52). More prolonged administration of Coly-Mycin at 5 to 7 mg/kg/day of colistin base (equivalent to 12 to 16.8 mg/kg/day of colistimethate) produced higher serum levels (13 to 32 μg/ml) (144). The serum half-life has been estimated to be ~3 to 4.5 h with normal renal function and increases with declining renal function (64, 112, 144). The data regarding Colomycin are similar: single doses of 2 to 4 mg/kg of colistimethate produced serum levels of 11 to 25 μg/ml and urinary concentrations of 200 μg/ml of bioactive colistin (119).

Unfortunately, most of those studies used a microbiological assay to determine colistin levels, and the reliability of these assays has been questioned (102, 103). The parenteral preparation in clinical use, colistimethate, has long been known to possess considerably less microbiological activity than colistin (13, 49, 72). When administered in vivo, colistimethate is hydrolyzed into the active component colistin. This hydrolysis has been recognized to occur, at uneven rates, in vitro as well, particularly in aqueous environments at 37°C (72). Colistimethate is the inactive prodrug of colistin, and it is the colistin formed from colistimethate during incubation that actually provides the antimicrobial activity (10). Therefore, bioassay

results will be misleading, since some of the colistimethate present in the biological fluid will be converted to colistin during the incubation period. In addition, some studies have used colistimethate for the preparation of the control concentrations in the bioassay, adding another source of error (52, 185). Therefore, those studies should be interpreted with caution and serve only as a rough estimate for pharmacokinetic analysis.

Colistin and colistimethate concentrations have also been assayed using high-performance liquid chromatography (HPLC), which has been demonstrated to give more reliable results than the microbiological assays (98, 99). In one report involving 12 patients with cystic fibrosis, the administration of Colomycin at ~7.3 mg/kg/day produced peak concentration levels of colistin of only 1 to 3 μg/ml (97). In that study, colistin had an estimated half-life of 4.2 h, double of that of colistimethate (2.1 h). Since the colistin levels were below the MIC for many pathogens for much of the dosing period, dose-escalating studies were proposed (97). Whether those findings apply to patients without cystic fibrosis and whether increased doses will augment response and/or toxicity rates are unknown.

There is even less information concerning the pharmacokinetic data for polymyxin B. However, since the most widely used preparation for polymyxin B is sulfate, and not methanesulfonate, information derived from microbiological assays should be more reliable for interpretation. Serum levels of 1 to 8 μg/ml following a 50-mg intramuscular dose have been reported (89), and levels of 5 to 6 μg/ml (72) following "usual doses" were also reported. Drug accumulation will occur, and a serum level of 15 μg/ml has been reported following dosing of 2.5 mg/kg/day for 7 days (75, 89). The serum half-life in patients with normal renal function is ~6 h and is increased with renal insufficiency (90). Sixty percent of the administered dose can be recovered in the urine, and urinary concentrations of 10 to 100 μg/ml are attained (72). The remainder of the dose is eliminated by nonrenal mechanisms; there is no excretion via the biliary system (89).

Distribution and Concentrations in Body Fluids

In rabbits, considerable binding of polymyxin B and colistin to kidney, brain, liver, muscle, heart, and lung has been observed using a bioassay to measure drug concentrations (91). In rats, approximately 50% of colistin is protein bound (100). In patients with cystic fibrosis, the volume of distribution of colistimethate has been reported to be 340 ml/kg (97). Penetration into the pleural space has been reported to be poor (89). Colistin was not evident in the cerebrospinal fluid (CSF) following systemic administration in children with hydrocephalus (184), and low concentrations were noted in healthy subjects (13). In patients with meningitis, colistin levels in the CSF following intravenous therapy have been reported to reach 1.25 μg/ml and to have a half-life of 2.7 h (77, 78). Given the fact that these levels are just above the MIC for many nosocomial pathogens, clinicians should have a low threshold for administering intrathecal or intraventricular therapy for patients with meningitis.

Dosing Guidelines

On the basis of the limited pharmacokinetic data obtained decades ago, several dosing guidelines (3, 43, 72, 89, 112) have been proposed (Table 2). It is important to reiterate the different dosing recommendations that are suggested for the two different colistimethate preparations. For Coly-Mycin M Parenteral (generally used in North America), the recommended dose for a 70-kg person with normal renal function would be 300 mg of colistin base per 24 h or 720 mg of colistimethate (Coly-Mycin M Parenteral package insert; Monarch Pharmaceuticals, Inc., Bristol, TN). For Colomycin (generally used in Europe), the recommended dose for the same individual would be 1 to 2 million units thrice daily or 240 to 480 mg of colistimethate per day (Colomycin Injection package insert; Forest Laboratories, UK Limited, Bexley, United Kingdom). Until accurate pharmacokinetic data for each formulation are available to clarify this issue, it would seem prudent to follow the dosing guidelines in the package insert for each preparation.

For polymyxin B, ~2 mg/kg/day divided into two doses is generally recommended for patients with normal renal function (Table 2). However, the package insert does not give recommendations for dosing for patients with renal insufficiency (Polymyxin B for Injection package insert; Bedford Laboratories, Bedford, OH); the advice noted in two reviews (43, 72) offers some guidance, but again, this is based on very limited information.

Dosing guidelines for critically ill patients and those with renal insufficiency need to be reevaluated. One report noted a removal rate of colistimethate during peritoneal dialysis of 0.9 mg/h (64). While older studies did not find any effect of hemodialysis on colistimethate (64, 112), the effect of high-flux dialyzers is unknown. Similarly, there is negligible information concerning the effect of other forms of renal replacement therapy, including venovenous hemofiltration (105, 150). With the increasing use of polymyxins, accurate pharmacokinetic data for these populations are sorely needed (101).

ANTIMICROBIAL PROPERTIES

Spectrum of Activity and Mechanism of Action

Both polymyxin B and colistin possess antibacterial activities against a wide variety of gram-negative pathogens (89). The great majority of isolates of *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp., all important nosocomial pathogens, are usually susceptible to polymyxins. In addition, considerable activity exists against *Salmonella* spp., *Shigella* spp., *Pasteurella* spp., and *Haemophilus* spp. Several pathogens possess intrinsic resistance to the polymyxins: *Proteus* spp., *Providencia* spp., and most isolates of *Serratia* spp. In addition, isolates of *Brucella* spp., *Neisseria* spp., *Chromobacterium* spp., and *Burkholderia* spp. are resistant.

Mechanism of Action and Resistance

Polymyxins are cationic agents that bind to the anionic bacterial outer membrane, leading to a detergent effect that dis-

TABLE 2. Dosage guidelines for colistimethate and polymyxin B parenteral therapy[a]

Formulation	Dosage guidelines		Reference
	Normal renal function	Altered renal function	
Colistimethate (Colomycin)	1–2 million U (80–160 mg colistimethate) every 8 h (>60 kg); 50,000–75,000 U (4–6 mg colistimethate)/kg/day divided into 3 doses (≤60 kg)	CrCl of 20–50 ml/min, 1–2 million U (80 –160 mg colistimethate) every 8 h CrCl of 10–20 ml/min, 1 million U (80 mg colistimethate) every 12–18 h CrCl of <10 ml/min, 1 million U (80 mg colistimethate) every 18–24 h (>60 kg)	51
Colistimethate (Coly-Mycin)	5 mg/kg/day colistin base in 2 doses	Cr level of 1.3–1.5 mg/dl, 2.5–3.8 mg/kg/day colistin base (2 doses) Cr level of 1.6–2.5 mg/dl, 2.5mg/kg/day colistin base (1 or 2 doses) Cr level of 2.6–4.0 mg/dl, 1.5 mg/kg colistin base every 36 h	123
Colistimethate (Coly-Mycin)	5 mg/kg/day colistin base in 2 doses	CrCl of 30–80 ml/min, 3.0 mg/kg and then 2.5–3.8 mg/kg/day colistin base (2 doses) CrCl of <30 ml/min, 3.0 mg/kg and then 2.5 mg/kg/day colistin base (2 doses) Anuric CrCl level, 2.5 mg/kg and then 1.5 mg/kg colistin base every 36 h	43
Colistimethate (Coly-Mycin)	2.5–5.0 mg/kg/day in 2 doses	CrCl of >20 ml/min, 75–100% of daily dose (2 doses) CrCl of 5–20 ml/min, 50% of daily dose (2 doses) CrCl of <5 ml/min, 30% of daily dose (2 doses every 12–18 h)	89
Colistimethate (Coly-Mycin)	5 mg/kg/day in 2 doses	CrCl of 40–70 ml/min, 2.4–5 mg/kg/day (2 doses) CrCl of 10–25 ml/min, 2.5 mg/kg every 36 h BUN level of >100 mg/dl, 2.0 mg/kg every 48–72 h Anuric level, 2.0 mg/kg every "many days"	112
Colistimethate	2.5–5 mg/kg/day in 2–4 doses	CrCl of 50–80 ml/min, 2.5–3.8 mg/kg/day in 2 doses CrCl of 10–50 ml/min, 2.5 mg/kg/day in 1 to 2 doses CrCl of <10 ml/min, 1.5 mg/kg every 36 h	3
Polymyxin B	1.5–2.5 mg/kg/day in 2 doses	CrCl of 30–80 ml/min, 2.5 mg 1× and then 1–1.5 mg/kg/day CrCl of <30 ml/min, 2.5 mg/kg and then 1–1.5 mg/kg/day every 2–3 days Anuric CrCl, 2.5 mg/kg and then 1 mg/kg every 5–7 days	43
Polymyxin B	1.5–2.5 mg/kg/day in 2–4 doses		6
Polymyxin B	1.5–2.5 mg/kg/day in divided doses		89
Polymyxin B	2.5–3.0 mg/kg/day in divided doses	CrCl of 30–80 ml/min, 2.5 mg 1× and then 1–1.5 mg/kg/day CrCl of <25 ml/min, 2.5 mg/kg and then 1–1.5 mg/kg/day every 2–3 days Anuric CrCl, 2.5 mg/kg and then 1 mg/kg every 5–7 days	72
Polymyxin B	1.5–2.5 mg/kg/day in 2 doses	CrCl of 5–20 ml/min, 0.75–1.25 mg/kg/day in 2 doses CrCl of <10 ml/min, 0.225–0.375 mg/kg/day in 2 doses	3

[a] Cr, creatinine; CrCl, creatinine clearance; BUN, blood urea nitrogen.

rupts membrane integrity. In particular, polymyxins show a high affinity for the lipid moiety of lipopolysaccharide and can preferentially displace Mg^{2+} and Ca^{2+} from cationic binding sites (65). Besides leading to cytoplasmic leakage, this binding can have a neutralizing effect on the biological properties of endotoxins (147, 182). Because of the disruptive effect on membrane integrity, gram-negative bacteria may become more susceptible to hydrophobic antimicrobials (e.g., erythromycin) following exposure to the polymyxins (32, 61, 155).

Isolates with intrinsic resistance to polymyxins have alterations in lipid A that account for reduced binding. In *Proteus mirabilis*, polymyxin resistance has been associated with the

4′-phosphate moiety of lipopolysaccharide being linked to 4-amino-4-deoxy-L-arabinopyranose (156). Similar changes in lipid A have been observed for other bacteria that intrinsically resistant to polymyxins, including *Burkholderia cepacia* and *Chromobacterium violaceum* (36, 69).

Acquired resistance to polymyxins has been observed in *Salmonella* spp. and *Escherichia coli*. For these pathogens, the substitution of phosphate groups in lipopolysaccharide led to a reduced susceptibility to polymyxins (14, 132, 140, 186). Esterification of the lipid A moieties 4′-phosphate (with 4-amino-4-deoxy-L-arabinopyranose) and the glycosidic diphosphate (with 2-aminoethanol) results in a decrease in anionic charges. The change in the surface charge has been correlated with decreased binding sites for the cationic polymyxins. For *Klebsiella pneumoniae*, lipopolysaccharide-related phosphate substitution with 4-amino-4-deoxy-L-arabinopyranose has also been linked with resistance to polymyxins (71).

It has been recognized that for some bacteria, polymyxin resistance is affected by the conditions of the culture medium. Reduced polymyxin susceptibility can be found in cells of *Salmonella enterica* serovar Typhimurium starved of carbon, nitrogen, or phosphate and in stationary cells (118). Also, in *Salmonella enterica* serovar Typhimurium, the presence of low levels of Mg^{2+} leads to the induction of PhoP, a transcriptional activator of the *pmrCAB* locus (65). Through PmrA-activated genes, the negative charges in lipopolysaccharide molecules are diminished, thereby reducing the membrane requirement for Mg^{2+} (65). With fewer cationic binding sites, the activity of polymyxin is decreased, and resistance ensues.

The development of resistance in *P. aeruginosa* is also a complex process. Two separate pathways leading to polymyxin resistance have been proposed (126). When subcultured in increasing concentrations of polymyxin, *P. aeruginosa* can acquire resistance attributed to changes involving its outer membrane (61), including the conversion of acidic phospholipids to neutral lipids (25) and alterations in membrane lipids, proteins, and carbohydrates (32, 62). These adaptive changes are quickly lost when cells are grown in the absence of polymyxin. A second, genetically stable mechanism involves the increased production of the outer membrane protein H1 (129). This protein can be induced by the growth of cells with reduced Mg^{2+} levels and also affords protection against chelating agents. It has been purported that this protein exerts its protective effect by functionally replacing divalent cations in the cell membrane (129). Given the propensity for developing resistance, appropriate dosing and achieving therapeutic levels of polymyxins cannot be overemphasized when treating serious infections.

IN VITRO STUDIES

Susceptibility Testing Methodology

Currently, there are conflicting recommendations regarding breakpoints from the Clinical and Laboratory Standards Institute (CLSI), the British Society for Antimicrobial Chemotherapy (BSAC), and the Societe Francaise de Microbiologie (SFM) (21, 31, 159). The CLSI recommends breakpoints of ≤2 μg/ml as being susceptible, 4 μg/ml as being intermediate, and 8 μg/ml as being resistant for *P. aeruginosa* and ≤2 μg/ml as being susceptible and ≥4 μg/ml as being resistant for *Acinetobacter* spp. No recommendation is made for *Enterobacteriaceae*. These recommendations apply for both polymyxin B and colistin. The BSAC recommends breakpoints of ≤4 μg/ml as being susceptible and >4 μg/ml as being resistant for *P. aeruginosa* and *Enterobacteriaceae*. These recommendations are for colistin only. The SFM recommends breakpoints of ≤2 μg/ml as being susceptible and >2 μg/ml as being resistant for all species. The SFM suggests that susceptibility testing results for colistin should apply to polymyxin B as well. The primary difference is whether isolates with a polymyxin MIC of 4 μg/ml should be considered susceptible. There are no clinical data regarding outcomes of patients treated with polymyxins for infections due to organisms with an MIC of 4 μg/ml. Studies suggested that peak serum concentrations of colistin can reach 10 to 30 μg/ml (144). Similarly, peak polymyxin B concentrations can reach 15 μg/ml after repeated dosing (89). More clinical data will be needed to define the optimal susceptibility breakpoints.

Cation concentrations have been known to affect the activity of polymyxins. A recent study demonstrated that increasing the Ca^{2+} concentration from 25 to 75 μg/ml resulted in slightly higher polymyxin B MICs against strains of *P. aeruginosa* and *Acinetobacter* spp. (157). Previously, lowering the Mg^{2+} concentration was shown to significantly decrease the in vitro killing of *P. aeruginosa* by polymyxin B (129). This effect was countered by the addition of Ca^{2+} to the medium. Clearly, more information is needed to assess the effects of cation concentrations on MIC tests. Currently, standard cations should be supplemented for broth dilution testing and not for agar dilution testing, as recommended by the CLSI and BSAC (21, 31).

Colistimethate has been demonstrated to be an inactive prodrug of colistin (10). Both during in vitro broth studies and in vivo, the activity of colistimethate was demonstrated to be due to hydrolysis to the active colistin sulfate. Moreover, colistimethate MICs are generally three- to eightfold higher than colistin sulfate MICs. Therefore, all susceptibility testing of colistin should be done with colistin sulfate (10). Several studies have shown nearly complete agreement in the susceptibility results for polymyxin B and those for colistin, particularly when colistin sulfate was used (41, 49, 55, 130, 153, 179). In one report, the MICs of colistin were slightly higher than those of polymyxin B using agar dilution, although the categorical agreement was very good when the 4-μg/ml breakpoint was used (73). Taken together, the data suggest that susceptibility to one agent should nearly always imply susceptibility to the other.

Heteroresistance, a small subpopulation with markedly reduced susceptibility to colistin, in *A. baumannii* (106) and *Enterobacter cloacae* (111) has recently been described. This phenomenon cannot be detected by standard dilution MIC tests and usually requires population analysis studies (106). However, the existence of heteroresistance may be indicated by the presence of colonies within the zone of inhibition of the Etest and disk diffusion test (111). The clinical significance of this laboratory phenomenon is presently unknown.

Differing recommendations have been made regarding disk susceptibility methodology for polymyxins. The CLSI recommends a 10-μg colistin disk or a 300-μg polymyxin B disk,

Mueller-Hinton agar, a solution with a 0.5 McFarland standard for the inoculum, and a 16- to 18-h incubation time. Recommended breakpoints are ≤10 mm and ≥11 mm for colistin and ≤11 mm and ≥12 mm for polymyxin B (30). The CLSI recommendation is only for *P. aeruginosa*. The BSAC recommends a 25-μg colistin disk, Isosensitest agar, a 1:100 dilution of a solution with a 0.5 McFarland standard for the inoculum, and an 18- to 20-h incubation time. This recommendation is for colistin only; no recommendations for polymyxin B are given. Recommended breakpoints are ≤13 mm and ≥14 mm (21). The SFM recommends a 50-μg colistin disk, Mueller-Hinton agar, a 1:100 dilution of a solution with a 0.5 McFarland standard for the inoculum, and an 18- to 24-h incubation time. Recommended breakpoints are ≤14 mm and ≥15 mm for colistin. Results for colistin apply for polymyxin B as well (159).

The accuracy of disk diffusion testing of polymyxins has been questioned for decades due to the poor diffusion of these agents in agar. Considerable variations exist regarding the species and selection criteria used, zone diameter breakpoints, and MIC susceptibility breakpoints. Several recent and older studies have found very major error rates of ~5% to 20% (isolates falsely identified as being susceptible) and have concluded that disk diffusion testing is unreliable (4, 55, 83, 86, 111, 116, 130, 146, 167, 179). Therefore, despite the inclusion of disk diffusion testing guidelines by several groups, the accumulated data suggest that alternative methods should be used for susceptibility testing of polymyxins.

Broth microdilution and agar dilution MIC testing are the standard methods for susceptibility testing of polymyxins. Several recent studies compared these two methods (15, 55, 73, 111). Although MICs tended to be slightly higher using agar dilution, in general, a very good correlation between the two methods was seen using CLSI methodology (15, 55, 111). One study demonstrated a poor correlation, although results were improved when broth MICs were read after 48 h and a susceptibility breakpoint of 4 μg/ml was used (73). Those findings may be related to the large number of *P. aeruginosa* isolates from cystic fibrosis patients included, which frequently have MICs near the susceptibility breakpoints of 2 to 4 μg/ml. Taken together, the data suggest that either method is acceptable for testing either colistin or polymyxin B.

Automated microdilution susceptibility testing of polymyxins has been assessed for colistin. Three published studies compared Vitek-2 (bioMerieux, France) with MIC testing of colistin. One study found 100% agreement between Vitek-2 and agar dilution against 44 *Acinetobacter* sp. strains, but all of the isolates were colistin susceptible (169). A second study comparing Vitek-2 with broth microdilution against 102 mixed gram-negative bacterial strains (half were colistin resistant) found excellent correlation, with 93% and 7% within one and two twofold dilutions, respectively (111). Heteroresistance in six isolates of *Enterobacter cloacae* was not detected by the Vitek system (111). In contrast, a third study comparing Vitek-2 with agar dilution for 172 mixed gram-negative bacterial strains (31% colistin resistant) revealed a false-susceptible rate of 18% (168). The very major error rate was significantly higher for *P. aeruginosa*. Those authors concluded that Vitek-2 was unreliable for the detection of colistin resistance. The latter studies both used a susceptibility breakpoint of 2 μg/ml. The discrepancy between the studies may be due to the preponderance of *Enterobacteriaceae* (rather than *P. aeruginosa*) and the absence of isolates with an MIC of 4 μg/ml in the study by Lo-Ten-Foe et al. (111). It is unclear if use of a breakpoint of 4 μg/ml would result in greater agreement between methods. More data are needed before automated microdilution testing can be routinely recommended.

In view of the inaccuracy of disk diffusion and the difficulty in performing MIC tests routinely in the clinical laboratory, the Etest method might present an attractive alternative. Several studies (5, 15, 63, 111, 130, 168, 179) comparing Etest with broth or agar dilution MICs are listed in Table 3. The reported accuracy of the Etest varies markedly among the studies. In general, the Etest for polymyxins tends to produce sharp endpoints with *Enterobacteriaceae* but not with nonfermenters. The manufacturer suggests reading the MIC by extrapolating the colonies from above to the strip when testing nonfermenters. Only one of the studies in Table 3 indicated how the endpoint was determined (5). The accuracy of the Etest appears to be greater for *Enterobacteriaceae* and less for *P. aeruginosa* and *Stenotrophomonas maltophilia* due to the indistinct endpoints and frequent MICs near the susceptibility breakpoint with the latter species. These factors may partially explain the discrepant findings of the studies. More data are needed before susceptibility testing of polymyxins by Etest can be routinely recommended.

Susceptibility Reports

The emergence of multidrug resistance has renewed interest in the use of polymyxins against strains of *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *Enterobacter*, and *S. maltophilia*. For *P. aeruginosa* (24, 27, 54, 55, 73, 93, 94, 127, 152, 167, 174, 179), the activity of polymyxins has remained excellent, with ~90% to 100% of isolates being susceptible at the 4-μg/ml breakpoint and in most studies at the 2-μg/ml breakpoint as well. The susceptibility rate was somewhat lower in two studies involving fewer isolates (167, 174). Whether the clonal spread of a single strain with reduced susceptibility occurred in those areas is unknown. Of note, virtually no resistance to *P. aeruginosa* was detected in a surveillance study in Brooklyn, NY, in 2006, an area with considerable usage of polymyxins due to endemic multidrug-resistant *A. baumannii* and *K. pneumoniae* strains (94). Similarly, in most studies, ~95% to 100% of *A. baumannii* isolates were susceptible (24, 38, 54, 55, 94, 146, 167, 174); however, in one report from Spain, only 83% of isolates were susceptible (5).

Most studies examining *K. pneumoniae* reported very high polymyxin susceptibility rates, approaching ~90% to 95% (18, 23, 24, 54, 94, 127, 138, 167). Susceptibility has been noted to be lower in multidrug-resistant isolates, including carbapenemase-producing strains (4, 18, 23). Substantial variation was seen in reports concerning *S. maltophilia* (54, 55, 73, 127, 130) and *Enterobacter* spp. (23, 24, 54, 94, 138, 167), with susceptibility rates ranging from 28% to 77% and 79% to 97%, respectively. The reasons for this variability are unclear; however, the variable susceptibility underscores the need for accurate susceptibility testing to guide treatment decisions.

TABLE 3. Studies evaluating the Etest susceptibility testing method for polymyxins[a]

Drug(s)	Comparator method (breakpoint [μg/ml])	Species tested (no. of isolates tested)	VME rate (%)	% Agreement within 1 $\log_2$ dilution	% Categorical agreement	Description	Reference
Colistin, polymyxin B	Agar dilution (2)	*S. maltophilia* (70)	Colistin, 9; polymyxin B, 12	Colistin, 97; polymyxin B, 89	Not stated	Correlation good, especially for colistin; VME rate due to many with MIC of 4	130
Colistin, polymyxin B	Broth microdilution (2)	*A. baumannii* (327), *P. aeruginosa* (46)	Not stated	Colistin, >98; polymyxin B, >99	Colistin, 99; polymyxin B, 99	Etest is equivalent	15
Colistin	Broth microdilution (2)	*A. baumannii* (115)	1.7	16.5	98	Correlation is good, although Etest MICs were often 4–8-fold higher; confirm MICs if 1–2 μg/ml	5
Colistin	Agar dilution (4)	Mixed (170)	*P. aeruginosa*, 11; others, 0	91	*P. aeruginosa*, 89; others, 100	Correlation is good, although Etest MICs were often 2–8-fold lower	63
Colistin	Broth microdilution (2)	Mixed (102)	NA	73	Not stated	Correlation is fairly good, although Etest MICs were often 4–8-fold higher	111
Colistin, Polymyxin B	Broth microdilution (2)	Multidrug-resistant *P. aeruginosa* (78)	Colistin, NA; polymyxin B, 1.2	Colistin, 80; polymyxin B, 33	Not stated	Correlation poor; Etest MICs higher; many minor errors	179
Colistin	Agar dilution (2)	Mixed (172)	4.7	75	87	May need confirmatory test; most errors with *P. aeruginosa*	168

[a] NA, not applicable; VME, very major error.

Bactericidal Activity and Synergy Studies

Numerous studies have been performed in recent years using a pharmacodynamic model or standard time-kill studies of the effect of polymyxin B or colistin against strains of *P. aeruginosa* (19, 59, 67, 93, 107, 166), *A. baumannii* (60, 88, 106, 136, 160, 170), and *K. pneumoniae* (20). Nearly all studies demonstrated that the polymyxins produce concentration-dependent killing, with an initial kill followed by regrowth. The initial killing is very rapid (107, 136, 166), with a large decrease in CFU/ml occurring as soon as 5 min after antibiotic exposure. After regrowth occurs, the surviving bacteria often have significantly higher MICs that appear to be stable (19, 88, 106). It is not clear if exposure to polymyxins induces resistance or selects out a small (heteroresistant) subpopulation (106). In reports using an in vitro pharmacodynamic model with *P. aeruginosa*, compared to a dosing schedule of every 8 h, dosing regimens mimicking extended-interval dosing (dosing of every 12 and 24 h) were more likely to be associated with the emergence of resistant subpopulations (9, 166). For colistin, the extended-interval regimens also had a greater time period in which the concentrations fell below the MIC of the *P. aeruginosa* isolate (9). It is also noteworthy that extended-interval dosing of colistimethate has been associated with increased rates of neurotoxicity and nephrotoxicity in rats (181). Although clinical data are lacking, it would appear that dosing regimens using shorter time intervals may be favored. The routine occurrence of regrowth also raises the question of whether combination therapy might prove to be more efficacious and/or prevent the emergence of resistance to polymyxins.

The in vitro activities of polymyxins combined with other agents against *A. baumannii* (16, 26, 74, 104, 113, 172, 174), *P. aeruginosa* (26, 93, 165, 171, 174), *K. pneumoniae* (26), and *Serratia marcescens* (134, 173, 177) have been studied by the checkerboard methodology (Table 4). Against *A. baumannii*, the combination of polymyxin B or colistin with rifampin or azithromycin produced the synergistic inhibition (generally defined as a fractional inhibitory concentration index of ≤0.5) of most isolates. Synergistic inhibition was more variable when polymyxin was combined with a carbapenem. Against *P. aeruginosa*, the combination of polymyxins with other agents yielded conflicting results and was only synergistic against a minority of isolates. Interestingly, the combination of polymyxins with rifampin was routinely synergistic against *S. marcescens*, an organism that is intrinsically resistant to polymyxins.

The findings of time-kill studies involving polymyxins against *A. baumannii* (26, 60, 160, 170, 187), *P. aeruginosa* (19, 59, 67, 93, 139, 171), *Stenotrophomonas maltophilia* (58), *K. pneumoniae* (20), and *S. marcescens* (134, 173) are summarized in Table 5. Against *A. baumannii*, the combination of polymyxin B or colistin with rifampin produced synergistic activity (generally defined as a ≥100-fold increase in killing) against most isolates. Similar results were seen in two studies that combined polymyxin B with imipenem and in a recent study using minocycline. Against *P. aeruginosa*, combinations with rifampin produced synergistic killing in 90% to 100% of isolates in all but one study; the difference in the latter study may have been the use of colistimethate rather than colistin sulfate or polymyxin B. Synergy was also demonstrated in a few studies using azithromycin or imipenem, but antagonism was also rarely observed. The combination of polymyxins with rifampin also showed bactericidal synergy against isolates of *K. pneumoniae*, *S. maltophilia*, and *S. marcescens* (20, 58, 134, 173).

The in vitro drug combination studies provide several arguments to favor the use of combination therapy. First, exposure to polymyxin B or colistin alone routinely results in regrowth. Many

TABLE 4. Synergy studies of polymyxins by checkerboard methods

Organism (no. of isolates)	Polymyxin studied	Combined-drug synergy (% of isolates with synergy)	Reference
A. baumannii (13)	Colistin	Rifampin (85)	74
A. baumannii (5)	Polymyxin B	Rifampin (60); ampicillin-sulbactam (0)	172
A. baumannii (55)	Polymyxin B	Rifampin (76); imipenem (58)	26
A. baumannii (24)	Polymyxin B	Azithromycin (83); rifampin (54); meropenem (38); cotrimazole (25)	113
A. baumannii (5)	Colistin	Rifampin (80), meropenem (60), azithromycin (60)	174
A. baumannii (8)	Colistin	Rifampin (100)	104
A. baumannii (6)	Colistin	Rifampin (100)	16
P. aeruginosa (55)	Polymyxin B	Rifampin (0); imipenem (0)	26
P. aeruginosa (5)	Colistin	Rifampin (40); meropenem (0), azithromycin (0)	174
P. aeruginosa (7)	Colistin	Rifampin (14)	171
P. aeruginosa (10)	Polymyxin B	Azithromycin (60); imipenem (20); rifampin (10)	93
P. aeruginosa (40)	Polymyxin B	Rifampin (not stated)	165
K. pneumoniae (55)	Polymyxin B	Rifampin (46); imipenem (15)	26
S. marcescens (12)	Polymyxin B	Rifampin (100)	177
S. marcescens (12)	Polymyxin B	Rifampin (100)	134
S. marcescens (13)	Colistimethate	Cotrimazole (not stated); rifampin (not stated); chloramphenicol (not stated)	173

of the studies listed in Tables 4 and 5 demonstrated that antibiotic combinations could reduce or eliminate regrowth and possibly prevent the development of polymyxin resistance. Second, antibiotic combinations demonstrated bactericidal activity even against polymyxin-resistant strains of *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, *S. maltophilia*, and *S. marcescens*. Third, some combination studies demonstrated bactericidal activity against polymyxin-susceptible strains even when sub-MIC concentrations were used. This might allow the use of lower doses of polymyxins, thereby reducing drug toxicity. Clearly, clinical studies are needed to determine whether combining polymyxins with rifampin or other agents improves the outcome of therapy and/or prevents the emergence of polymyxin resistance.

A few studies have looked at the in vivo efficacies of polymyxins against multidrug-resistant *P. aeruginosa* and *A. baumannii* strains in animal models of infection (28, 29, 124, 125, 137, 149). In general, there was a tendency for colistin to reduce mortality in sepsis models using single-dose therapy (29, 137). However, colistin monotherapy appeared to be less effective against *A. baumannii* in pneumonia and endocarditis models (124, 125, 149). The addition of rifampin or imipenem enhanced bacterial clearance in several studies (28, 29, 137). Definitive conclusions cannot be drawn from the studies because of the varied experimental models, varied drug preparation and dosage, small number of strains tested, and brief duration of therapy used. In particular, the potential advantage of combination therapy in preventing the emergence of resistance may not be evident after very brief (or single-dose) treatment. Additional studies will be needed to assess the efficacy of monotherapy and combination therapy, particularly in the pneumonia model.

CLINICAL STUDIES

Clinical and Microbiological Outcomes

The clinical experience with colistin and polymyxin B comprises case reports and series. Virtually all patients received other antimicrobial agents, and their impact on outcomes at-

TABLE 5. Synergy studies of polymyxins by time-kill methods

Organism (no. of isolates)[a]	Polymyxin studied	Combined-drug synergy (% of isolates with synergy/% with bactericidal activity)	Reference
A. baumannii (NA)	Polymyxin B	Rifampin (100/100); imipenem (100/100)	26
A. baumannii (6)	Colistimethate	Rifampin (100/100)	60
A. baumannii (8)	Polymyxin B	Rifampin (88/88); imipenem (88/88); rifampin + imipenem (100/100)	186
A. baumannii (8)	Colistimethate	Rifampin (100/100)	160
A. baumannii (13)	Colistin	Minocycline (92/69)	170
P. aeruginosa (5)	Polymyxin B	Rifampin (100/100)	139
P. aeruginosa (17)	Colistimethate	Rifampin (12/12)	59
P. aeruginosa (2)	Colistin	Ceftazidime (100/100); ciprofloxacin (0/0)	67
P. aeruginosa (2)	Colistin	Rifampin (100/100)	171
P. aeruginosa (13)	Polymyxin B	Azithromycin (70/70)	19
P. aeruginosa (10)	Polymyxin B	Azithromycin (40/40); imipenem (80/80); rifampin (90/90); rifampin + imipenem (100/100)	93
S. maltophilia (24)	Colistimethate	Rifampin (63/not stated); cotrimazole (42/not stated)	58
K. pneumoniae (16)	Polymyxin B	Rifampin (89/89); imipenem (44/44)	20
S. marcescens (4)	Polymyxin B	Rifampin (100/100)	134
S. marcescens (13)	Colistimethate	Cotrimazole (85/85), rifampin (not stated/not stated); chloramphenicol (not stated/not stated)	173

[a] NA, not available.

TABLE 6. Clinical and microbiological outcomes of patients infected with multidrug-resistant gram-negative pathogens treated with a polymyxin antibiotic

No. of cases	Pathogen(s) (no. of isolates)	Polymyxin used	Estimated colistimethate dose for patients with normal renal function (mg/kg/day)	Avg duration of therapy (days)	% of cases with respiratory tract infection	% of cases with clinical improvement	Microbiological eradication rate (%)	Overall mortality rate (%)	Reference
60	*P. aeruginosa* (21), *A. baumannii* (39)	Colistimethate	6–12[a]	12.6	33	58	93	37	96
21	*A. baumannii* (21)	Colistimethate	2.5–5	14.7	100	57	67	61.9	56
26	*P. aeruginosa* (20), *A. baumannii* (6)	Colistimethate	~10.3[b]	13.5	57.7	73		43.3	114
23	*P. aeruginosa* (23)	Colistimethate	12[a]	17	78	60.9			108
7	*P. aeruginosa* (5), *K. pneumoniae* (2)	Colistimethate	3.4–10.3[b]	28	42.9	71.4		28.6	45
19	*P. aeruginosa* (12), *A. baumannii* (5), *K. pneumoniae* (2)	Colistimethate	5 (320 mg/day)	43.4	68	73.7		41.2	48
55	*A. baumannii* (36), *P aeruginosa* (19)	Colistimethate	12[a]	13	53	15		29	145
43	*P. aeruginosa* (35), *A. baumannii* (8)	Colistimethate	9.6–10.3	18.6	72	74.5	67	27.8	122
45	Not stated	Colistimethate	Not stated	Not stated	100	66.7		75.6	121
54	*A. baumannii* (28), *P. aeruginosa* (23)	Colistimethate	~4.6[b]	21.3	33	66.7		24	81
12	*A. baumannii* (12)	Colistimethate	2.3–6.8[b]	11	0		90.9	18	11
78	*P. aeruginosa* (35), *A. baumannii* (43)	Colistimethate	6–12	9.3	78.2	76.9			79
31	*A.* baumannii (26), *P. aeruginosa* (11)	Colistimethate	Not stated	12.2	100			51.6	148
4	*P. aeruginosa* (4)	Colistimethate + rifampin	2.3[a]	16.5	75	100	100		171
14	*A. baumannii* (14)	Colistimethate	~6.8[a]	12	100		64	50	141
60	*A. baumannii* (48), *P. aeruginosa* (4)	Polymyxin B		13	65		88		135
29	*A. baumannii* (16), *P. aeruginosa* (12)	Polymyxin B		19	100	76	41	48	158

[a] A 150-mg colistin base equals 360 mg of colistimethate.
[b] Approximate dose for a 70-kg person.

tributed to the polymyxins cannot be determined. Most of the recent experience has involved colistimethate. Reports from the 1960s clearly demonstrated the utility of colistimethate to treat a variety of infections (35, 40, 119), with particularly high success rates (~85% to 95%) in treating urinary tract infections. In the late 1990s, colistimethate used in the treatment of respiratory exacerbations in patients with cystic fibrosis was reported (33, 34, 95). In those reports, patients with chronic bronchopulmonary infection with *P. aeruginosa* were given a ~2-week trial, with modest clinical and spirometric improvement.

The emergence of multidrug-resistant *P. aeruginosa* and *A. baumannii* strains in hospitalized patients spurred renewed interest in polymyxins (11, 45–48, 56, 78, 79, 81, 96, 114, 121, 122, 141, 145, 148, 156). Most series used colistimethate at doses ranging from ~5 to 10 mg/kg/day for patients with normal renal function (Table 6). Overall clinical improvement was seen in approximately 60% to 70% of cases. Mortality rates were high in most studies involving patients with multidrug-resistant *P. aeruginosa* and *A. baumannii* infection (Table 6). This undoubtedly reflects the severity of underlying illness; most patients are located in intensive care areas and have received prolonged courses of antibiotics. Mortality rates, including ventilator-associated mortality, and lengths of hospital stay of patients treated with colistimethate have been comparable to those of patients treated with other antibiotics (56, 145, 148). Given the fact that polymyxins are typically not considered for empirical therapy, most patients with multidrug-resistant bacterial infections do not receive adequate initial therapy. Although the delay in appropriate therapy was associated with adverse outcome in one report (148), this finding was not noted in other studies (56, 145).

Therapy of nosocomial respiratory tract infections, the most common site of infection for multidrug-resistant *P. aeruginosa* and *A. baumannii* strains, has been more difficult. One study documented clinical improvement in only 25% (5 of 20) of patients with pneumonia due to *P. aeruginosa* or *A. baumannii* infection (96), although in subsequent studies, 56 to 74% of patients were noted to have clinical improvement (56, 79, 81, 108, 114). In one report, the outcome of patients treated with imipenem (for carbapenem-susceptible *A. baumannii* strains) was identical to that of patients treated with colistimethate (57% clinical cure). Microbiological eradication from the respiratory tract has proven even more difficult, with only 33% to 67% of cases being cleared of the pathogen (56, 108, 114). Given the lower response rates for respiratory tract infections and the in vitro synergy between colistin and rifampin, combination therapy has been used in a few patients (141, 171). The combination of colistimethate and rifampin resulted in a microbiological clearance rate of 64% in patients with pneumonia due to multidrug-resistant *A. baumannii* infection (141). Whether this combination therapy results in improved clinical outcome remains uncertain.

Aerosolized therapy with colistimethate has also been used to treat bronchopulmonary infections. A prolonged course in patients with cystic fibrosis resulted in improved clinical scores

TABLE 7. Case studies involving the intrathecal or intraventricular administration of colistimethate or polymyxin B for gram-negative bacterial meningitis[a]

Study subjects and no. of cases	Pathogen	Intrathecal or -ventricular agent	Intraventricular or -thecal dose	Parenteral therapy	No. of cases of microbiological eradication/ total no. of cases	Reference
Children						
1	*P. aeruginosa*	Polymyxin B	2 mg q12h × 3 courses	Polymyxin B	1/1	70
1	*E. coli*	Polymyxin B	2 mg/day × 5 days		1/1	39
6	*Haemophilus influenzae*	Polymyxin B or polymyxin E	Variable	Polymyxin B or polymyxin E	8/8	164
1	*P. aeruginosa*	Polymyxin B	40,000 U q24h × 37 days	Polymyxin B	1/1	176
1	*P. aeruginosa*	Polymyxin B	1–2 mg × 25 days	Polymyxin B	1/1	12
1	*P. aeruginosa*	Polymyxin B	5 mg × 7 days	Streptomycin	1/1	12
1	*P. aeruginosa*	Polymyxin B	3 mg/day × 5 doses	Polymyxin B	1/1	133
8	*P. pyocyanea*	Polymyxin B methanesulfonic acid	5,000 U	i.m. 50,000 U every 6 h	6/8	30
4	*P. aeruginosa*	Polymyxin B	Not stated; 3–31 days		3/4	183
1	*K. pneumoniae*	Polymyxin B	2 mg/day × 38 days	Cephalothin	1/1	143
1	*A. baumannii*	Colistimethate	5 mg b.i.d. × 19 days	Tobramycin	1/1	50
1	*A. baumannii*	Colistimethate	1–4 mg/day × 24 days	Colistimethate + amikacin	1/1	128
Adults						
1	*P. aeruginosa*	Polymyxin B	5 mg × 3 doses	Polymyxin B	1/1	175
1	*Alcaligenes faecalis*	Polymyxin B	100,000 U b.i.d. × 3 doses	Polymyxin B	1/1	117
4	*P. aeruginosa*	Polymyxin B	5–10 mg/day for 7–10 doses	Polymyxin B	4/4	183
2	*P. aeruginosa*	Polymyxin B	Not stated; ~10–14 doses	Carbenicillin + polymyxin B; colistimethate	2/2	57
1	*K. pneumoniae*	Polymyxin B	50,000 U q24h × 7 days	Meropenem	1/1	154
1	*A. baumannii*	Colistimethate	5–10 mg b.i.d. × 17 days	Tobramycin	1/1	50
1	*A. baumannii*	Colistimethate	5–10 mg q.d. × 22 days	None stated	1/1	180
1	*P. aeruginosa*	Colistimethate	20–60,000 U × 26 days	Colistimethate, amikacin, ceftazidime	1/1	151
1	*A. baumannii*	Colistimethate	40,000 IU/day × 17 days	Ampicillin-sulbactam	1/1	8
2	*A. baumannii*	Colistimethate	40,000 U × 42 days	Colistin, amikacin	2/2	82
1	*A. baumannii*	Colistimethate	5 mg b.i.d. for 21 days	Colistin	1/1	22
2	*P. aeruginosa*	Colistimethate	10 mg/day × 14 days	Colistin	2/2	142
2	*A. baumannii*	Colistimethate	10 mg b.i.d.–20 mg q.d. × 8–10 days	Colistimethate	2/2	11
1	*P. aeruginosa*	Colistimethate	10 mg/day × 10 days	Colistin	1/1	66
1	*A. baumannii*	Colistimethate	10–20 mg/day × 10 days	Colistin	1/1	163
4	*A. baumannii*	Colistimethate	5–10 mg/day × 3–19 days	Amikacin	4/4	128
1	*A. baumannii*	Colistimethate	10 mg/day × 21 days	Not stated	0/1	120

[a] q12h, every 12 h; i.m., intramuscular; b.i.d., twice a day; q.d., once a day.

and spirometric measurements, but rates of eradication of *P. aeruginosa* from the respiratory tract have been more variable (76, 110, 178). Several studies have examined the efficacy of aerosolized therapy to treat multidrug-resistant *P. aeruginosa* and *A. baumannii* infections (11, 68, 92, 121). Using doses of 120 to 720 mg per day for 10 to 14 days, clinical improvement was noted in 57% to 87% of cases (68, 92, 121). Compared to parenteral colistimethate therapy, microbiological eradication has generally been more successful with aerosolized therapy, occurring in 80% to 92% of cases (11, 92, 121). These improved rates may have infection control implications, since multidrug-resistant *P. aeruginosa* and *A. baumannii* strains may chronically colonize the respiratory tract.

Meningitis due to multidrug-resistant *A. baumannii* or *P. aeruginosa* infection is a feared complication for the neurosurgical patient, and polymyxins have assumed an important role in treatment. While cure with intravenous colistimethate therapy alone has been documented (53, 77, 78, 96, 115), several reports noted failures with systemic therapy (8, 22, 50, 133, 183). Numerous case reports demonstrated successful outcomes with intraventricular or intrathecal instillation of colistimethate or polymyxin B in children (12, 30, 39, 50, 70, 128, 133, 143, 164, 176, 183) and adults (8, 11, 22, 50, 57, 66, 82, 117, 120, 128, 141, 142, 154, 163, 175, 180, 183) with or without corresponding intravenous therapy (Table 7). Given the fact that peak CSF levels of colistin approximate the MIC for most multidrug-resistant *P. aeruginosa* and *A. baumannii* strains (77, 78), clinicians should have a low threshold for the administration of intrathecal or intraventricular therapy, especially if prompt improvement does not occur with intravenous therapy.

In the great majority of reports prior to 1999, polymyxin B was the agent used intrathecally or intraventricularly, whereas the use of preparations of colistimethate predominated in more recent studies (188). Doses used in adults for intrathecal or intraventricular administration have generally been 10 to 20 mg of colistimethate every 24 h (11, 50, 128, 142) and 5 mg of

polymyxin B every 24 h (44, 154). Chemical arachnoiditis following intrathecal or intraventricular administration has been reported for both polymyxin B and colistimethate (57, 70, 117, 128, 175, 176). Microbiological cures have been noted in the great majority of studies; however, this outcome is likely skewed, since successful therapies are more likely to be reported.

Similar to early reports, patients with urinary tract infections had the highest response rates, ranging from 83% to 100% (11, 79, 81, 96). Patients with primary bloodstream infections also generally had good outcomes, with response rates of 60% to 100% (11, 79, 81, 96). Only a few cases of patients with surgical wound infections have been reported, with response rates of ~50% (81, 96, 108).

Most studies involve a mixture of infections caused by multidrug-resistant *P. aeruginosa* and *A. baumannii* strains, and it appears that the response rates to colistimethate therapy are similar for these two pathogens. In one report, overall clinical improvement was noted in 16 of 23 (70%) patients with *P. aeruginosa* infection, compared to 19 of 28 (68%) patients with *A. baumannii* infection (81). In independent studies, 56% of patients with pneumonia due to *P. aeruginosa* experienced clinical improvement (108), compared to 57% of patients with infection due to *A. baumannii* (56). Experience treating other pathogens is extremely limited. Colistimethate and polymyxin B have been used to treat infections due to *K. pneumoniae*, including bacteremia, pneumonia, and urinary tract infections, but the numbers are too low for meaningful conclusions (17, 35, 48, 80, 81). Similarly, sporadic cases of infections due to *E. coli* (35) and *Enterobacter* spp. (48) have been treated with colistimethate.

There is only limited recently reported experience with polymyxin B therapy. An overall microbiological cure rate, from a variety of sites of infection, of 88% has been reported (135). However, as with colistimethate, the outcome of polymyxin B therapy for respiratory tract infection appears to be lower, with clinical and microbiological response rates of 76% and 41% being reported (158).

Initial Toxicity Reports

Studies from several decades ago identified several adverse events attributed to polymyxins. Approximately 2% of patients will develop allergic manifestations including fever, eosinophilia, and macular and urticarial rashes (85, 95). However, the more prominent side effects involve neurotoxicity and nephrotoxicity, perhaps attributed to high binding to brain and renal tissue (91). Neurological side effects, occurring in 7% to 27% of patients, typically manifested as paresthesias (often perioral) and ataxia (35, 40, 49, 75, 85, 119, 184). These symptoms usually quickly resolved once the dose was reduced or discontinued. Other reported neurotoxic side effects have included diplopia, ptosis, and nystagmus (109, 184). A more serious adverse reaction is respiratory paralysis requiring ventilatory support, often lasting 10 to 48 h (85, 109).

Polymyxins were also associated with high rates of nephrotoxicity. Overall, as many as 20% of patients experienced a decline in renal function while on polymyxin therapy (85). In most instances, azotemia developed within the first 4 days and tended to resolve by 2 weeks following discontinuation of the agent (85). However, irreversible acute oliguric renal failure, with death from uremia, has been attributed to polymyxins (42, 183). Patients with abnormal renal function at the start of therapy have consistently been identified as being at high risk for nephrotoxic side effects (75, 85, 184). With these reported adverse effects, and the emergence of other antimicrobial agents, polymyxins quickly fell out of favor in the 1970s.

Two other adverse reactions warrant note. While inhalational therapy of colistimethate or polymyxin B appears to be well tolerated (11, 68, 92, 121, 158, 178), reports have noted episodes of bronchospasm following administration (2, 37, 110), particularly in patients with underlying cystic fibrosis. Decreases of 10% to 15% in FEV_1 (forced expiratory volume in 1 second) have been reported, especially in subjects at high risk for bronchospasm, and the effects can last for ~30 min (2, 37). If continued aerosolized polymyxin therapy is warranted, prophylactic bronchodilator therapy should be considered for patients experiencing bronchospasm. The second adverse reaction includes aseptic meningitis following intraventricular or intrathecal administration of colistimethate or polymyxin B. Chemical meningitis following intrathecal or intraventricular administration has been reported for both polymyxins, warranting a reduction in the dose or discontinuation of therapy (57, 70, 116, 128, 175, 176).

When administered to give serum concentrations with comparable antibacterial activities, it appears that colistimethate and polymyxin B share similar toxicity rates and profiles (131). While the sulfated forms (such as polymyxin B) are more toxic, they possess considerably more antibacterial activity than the sulfomethyl forms (such as colistimethate) (13, 41, 49, 72), and the lower toxicity rates are lost when higher doses are administered. Despite the unresolved issues regarding the dosing of colistimethate, there does not appear to be a clear advantage of one preparation over another.

Toxicity in Contemporary Studies

Polymyxins fell out of favor due to reports of nephrotoxicity and neurotoxicity (35, 40, 85, 119). Case series examining the efficacy of polymyxins against multidrug-resistant pathogens have provided some reassurance regarding the toxicity rates of these agents (Table 8), and even prolonged courses have been well tolerated (48, 162). Using a variety of definitions for renal toxicity, approximately 10% to 37% of patients receiving colistimethate (generally in doses of 5 to 12 mg/kg per day) experience an increase in serum creatinine levels (48, 56, 79, 81, 96, 114, 122). Fortunately, few patients will require renal replacement therapy. As most of these patients are acutely ill and receive other nephrotoxic agents, additional factors undoubtedly contribute to the development of renal insufficiency. Compared to a cohort receiving carbapenems and other agents for infections due to *P. aeruginosa* and *A. baumannii* strains, patients receiving colistimethate experience similar rates of nephrotoxicity (56, 145, 148). Compared to patients with normal baseline renal function, patients with preexisting renal insufficiency had a greater likelihood of developing nephrotoxicity during colistin therapy (5% to 27% versus 18% to 58%) (56, 96, 122). Neurotoxicity rates have remained low in most studies, ranging from 0% to 5% of patients (11, 48, 56, 79, 81, 96,

TABLE 8. Contemporary studies examining toxicity of polymyxins when they are used as therapy against multidrug-resistant pathogens

Therapy and no. of cases	Published dose for patients with normal renal function	Estimated colistimethate dose (mg/kg/day)	Duration of therapy (days)	Definition of renal toxicity[c]	Renal toxicity rate (%)	Neurotoxicity rate (%)	Reference
Colistimethate							
60	2.5–5.0 mg/kg/day colistin base (presumed)	6–12[a]	12.6	Worsening of renal function	37	0	96
21	2.5–5 mg/kg/day colistimethate	2.5–5	14.7	Creatinine level of >2 or increase by 50%, reduction in CrCl by 50%, need for renal replacement	24	0	56
26	720 mg/day colistimethate	10.3[b]	13.5	Increase of creatinine level by >1 mg/dl	14.3	0	114
23	5 mg/kg/day colistin base	12[a]	17	Need for hemodialysis or venovenous hemofiltration	Not stated	4.3	108
19	5 mg/kg/day colistimethate (presumed)	5	43.4	>50% increase of baseline creatinine level to >1.3 mg/dl	5.6	5.3	48
55	5 mg/kg/day colistin base	12[a]	13	Creatinine level of ≥2, decrease of CrCl by 50%, or renal replacement	0	Not stated	145
43	675–720 mg/day colistimethate	9.6–10.3	18.6	Increase of creatinine level by 2 or doubling of baseline creatinine level	18.6	0	122
54	~320 mg/day colistimethate	~4.6[b]	21.3	Increase of >50% to >1.3 or need for renal replacement therapy	8	2	81
14	6 million U/day colistimethate + rifampin	~6.8[b]	12	Not stated	7.1	0	141
12	160–480 mg/day colistimethate	2.3–6.8[b]	11	Not stated	0	0	11
78	6–12 mg/kg/day colistimethate	6–12	9.3	Creatinine level of >150 μmol/liter or BUN level of >10 mmol/liter without preexisting renal disease	9.0	1.3	79
31	5 mg/kg/day colistin base (presumed)	12[a]	12.2	Not stated	0	Not stated	148
Polymyxin B							
60	1.5–2.5 mg/kg/day		13	Doubling of creatinine level to >2	14	Not stated	135
23	1.5–2.5 mg/kg/day		19	Doubling of creatinine	6	7	158

[a] A 150-mg colistin base equals 360 mg of colistimethate.
[b] Approximate dose for a 70-kg person.
[c] CrCl, creatinine clearance; BUN, blood urea nitrogen.

114, 122), often manifesting as muscular weakness or polyneuropathy (81, 108, 158). Given that most of the recent experience with polymyxins involved acutely ill patients (often sedated and on respiratory support therapy), it is likely that the other neurotoxic effects (oral paresthesias and dizziness) noted in the older studies are missed.

Although there has been less recent clinical experience with polymyxin B, similar rates of nephrotoxicity (6 to 14%) and neurotoxicity (7%) have been reported for patients receiving 1.5 to 2.5 mg/kg per day (135, 158). Nephrotoxicity rates have also been noted to be higher in patients with preexisting renal disease treated with polymyxin B (75).

Emergence of Resistant Pathogens

As might be expected, the increased use of polymyxins has resulted in the emergence of pathogens that are resistant to these agents. The appearance of gram-negative pathogens with intrinsic resistance to polymyxins, including species of *Proteus*, *Serratia*, and *Stenotrophomonas*, has been documented in patients receiving these agents (35, 49, 108, 119). Compared to *P. aeruginosa* and *A. baumannii*, these pathogens tend to have greater susceptibility to commonly used antimicrobial agents. A more disconcerting finding is the emergence of polymyxin resistance in multidrug-resistant strains of *Acinetobacter*,

Pseudomonas, and *Klebsiella* from throughout the world. Polymyxin resistance in 5% to 28% of isolates of *A. baumannii*, including multidrug-resistant strains, from Brazil, the United States, and South Korea has been documented (84, 94, 146). Polymyxin resistance in *P. aeruginosa* has remained low, occurring in 5% of isolates in one report (93). Perhaps the greatest concern is the emergence of polymyxin resistance in nosocomial strains of *K. pneumoniae*. Approximately 10% to 25% of isolates of multidrug-resistant (including carbapenem-resistant) strains of *K. pneumoniae* in New York City were resistant to polymyxins (18, 20). An outbreak of several polymyxin-resistant clones of *K. pneumoniae* in Greece has also been documented; many of these isolates also carried metallo-β-lactamase, conferring resistance to most β-lactams (4).

CONCLUDING REMARKS

The rapid spread of multidrug-resistant strains of *A. baumannii*, *P. aeruginosa*, and *Enterobacteriaceae* has prompted a renewed interest in polymyxins. These agents have certainly been invaluable for the therapy of serious nosocomial pathogens. However, concerns regarding nephrotoxicity and neurotoxicity continue to surround polymyxins. Given the limited knowledge regarding the proper dosing of polymyxins in critically ill patients and the burgeoning reports of polymyxin-resistant isolates, the clinical utility of these agents has been predicted to be short-lived (101). Effective and appropriate use of these agents will likely prolong their therapeutic value. Polymyxins will hopefully serve as a bridge until novel and effective therapeutic agents are developed.

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