In Vitro Assessment and Multicenter Cohort Study of Comparative Nephrotoxicity Rates Associated with Colistimethate versus Polymyxin B Therapy

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Despite concerns of nephrotoxicity, polymyxin antibiotics often remain the only susceptible agents for multidrug-resistant (MDR) Gram-negative bacteria. Colistin has been more commonly used clinically due to a perceived safety benefit. We compared the nephrotoxicity of colistin to polymyxin B. The *in vitro* cytotoxicity of colistin was compared to polymyxin B in two mammalian renal cell lines. To validate the clinical relevance of the findings, we evaluated adult patients with normal renal function who received a minimum of 72 h of polymyxin therapy in a multicenter study. The primary outcome was the prevalence of nephrotoxicity, as defined by the RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria. Colistin exhibited an *in vitro* cytotoxicity profile similar to polymyxin B. A total of 225 patients (121 receiving colistimethate, 104 receiving polymyxin B) were evaluated. Independent risk factors for colistimethate-associated nephrotoxicity included age (odds ratio [OR], 1.04; 95% confidence interval [CI], 1.00 to 1.07; $P = 0.03$), duration of therapy (OR 1.08; 95% CI, 1.02 to 1.15; $P = 0.02$), and daily dose by ideal body weight (OR 1.40; 95% CI, 1.05 to 1.88; $P = 0.02$). In contrast, cystic fibrosis was found to be a protective factor in patients who received colistimethate (OR, 0.03; 95% CI, 0.001 to 0.79; $P = 0.04$). In a matched analysis based on the risk factors identified ($n = 76$), the prevalence of nephrotoxicity was higher with colistimethate than with polymyxin B (55.3% versus 21.1%; $P = 0.004$). Polymyxin B was not found to be more nephrotoxic than colistin and may be the preferred polymyxin for MDR infections. A prospective study comparing the two polymyxins directly is warranted.

Multidrug-resistant (MDR) Gram-negative infections pose a significant threat worldwide; they are associated with poor clinical outcomes and high mortality rates (1–3). With no first-line agents effective for these drug-resistant infections and a lack of new agents in development, there is an immediate need to find viable treatment options.

There has been renewed interest in the polymyxin antibiotics. These antibiotics have been clinically available since the 1960s and have *in vitro* activity against many MDR Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (4). There are two agents commercially available for clinical use, colistin (polymyxin E) and polymyxin B, which differ in structure by only one amino acid. They share the same spectrum of activity and mechanism of action (4). Shortly after the polymyxins were used clinically, reports of nephrotoxicity led to a significant decline in their utilization. Recently, however, the increasing rate of MDR infections has led to a revival of the polymyxins, but nephrotoxicity continues to be of major concern to clinicians. Although the relative safety of these agents is not well established, colistin is more commonly used clinically, presumably due to a perceived benefit of a better safety profile (5).

The reported rates of nephrotoxicity associated with colistin and polymyxin B vary widely in the literature. In a systematic review of literature from 1950 through 2005, rates of colistin-associated nephrotoxicity ranged from 0% to 50.0% (6). Recent literature has shown similar rates of nephrotoxicity, ranging from 10.0% to 45.0% (7–9). In comparison, there is limited clinical evidence demonstrating a higher rate of nephrotoxicity with poly-

myxin B than with colistimethate sodium (CMS)/colistin (5, 10). Literature reports involving polymyxin B found nephrotoxicity rates around 10.0 to 14.0% (11, 12). Of note, a small study comparing the safety and efficacy of colistin versus polymyxin B found no significant differences in the rates of nephrotoxicity (13). Possible explanations for the discrepancy in the reported rates of nephrotoxicity between colistin and polymyxin B include varying definitions of nephrotoxicity, differences in the dosing regimens used, and a lack of control for risk factors such as underlying renal insufficiency.

Colistin is commercially available as colistimethate sodium (CMS), a prodrug that must be converted to its pharmacologically active form to be effective. In patients with moderate to good renal function, most of the colistimethate dose will be renally excreted, with only a small fraction of the dose converted to its active form

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(14). Thus, a toxicity comparison based on the dosing of the pro-drug could be potentially misleading as the pharmacologically active form must also be considered. The objectives of this study were (i) to compare the nephrotoxicity of CMS/colistin to polymyxin B and (ii) to determine independent risk factor(s) associated with nephrotoxicity in patients on polymyxin therapy. The agent with a better benefit-to-toxicity ratio would be preferred for treating MDR Gram-negative infections.

MATERIALS AND METHODS

In vitro cytotoxicity. The relative toxicity of polymyxins was assessed using two mammalian cell lines, HEK 293 (human embryonic renal cells) and NRK-52E (rat renal proximal tubular epithelial cells), as detailed previously (15). Briefly, the cells were grown in Dulbecco's modified Eagle medium (DMEM) and exposed to different constant concentrations of colistin sulfate or polymyxin B sulfate (USP) (Sigma-Aldrich, St. Louis, MO). After incubation for 48 h, cell viability was assessed in triplicate by absorbance at 595 nm using a cell proliferation kit (Roche, Indianapolis, IN). A placebo (negative) control was used for each experiment, and gentamicin sulfate (USP) was used as a positive control. The experiments were repeated at least once on a different day. A sigmoid inhibitory maximum effect ($E_{\max}$) model was used to fit the mean data, and the relative toxicity was compared using the drug concentration resulting in 50% of maximal reduction in cell viability (50% inhibitory concentration [IC_{50}]). The weighted least squares method in ADAPT II (University of Southern California, Los Angeles, CA) was used.

Clinical study design and sites. To validate the clinical relevance of the *in vitro* findings, a multicenter, retrospective, cohort study was conducted from 2006 to 2011 in 4 U.S. teaching hospitals: St. Luke's Episcopal Hospital (a 900-bed hospital in Houston, TX), Houston Methodist (a 900-bed hospital in Houston, TX), New York Hospital Queens (a 520-bed hospital in Flushing, NY), and Maimonides Medical Center (a 700-bed tertiary teaching hospital in Brooklyn, NY). Institutional Review Board (IRB) approval at each study site and the University of Houston was obtained prior to the initiation of this study. In view of the retrospective nature of the study, the need for informed consent was not mandated.

Patient selection criteria. Patients 18 years or older who received at least 72 h of intravenous polymyxin (colistimethate or polymyxin B) daily for suspected or documented infections were included in this study. Patients with underlying baseline renal insufficiency (baseline serum creatinine of >1.5 mg/dl or requiring any form of renal replacement therapy) or fluctuating renal function (increase or decrease in serum creatinine of more than 50% in the 72 h immediately prior to polymyxin initiation) were excluded. Baseline renal function was defined as the serum creatinine on the day of polymyxin initiation.

Study variables/outcome definition. Data collected included demographics (e.g., age, ethnicity, and gender), comorbidities (e.g., hypertension, cystic fibrosis, and diabetes mellitus), pertinent laboratory findings (e.g., serum creatinine, site of infection, and organisms isolated), polymyxin therapy regimens (daily dose and duration), and concomitant nephrotoxins (e.g., aminoglycosides, loop diuretics, vasopressors, calcineurin-inhibitors, vancomycin, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers). The primary outcome of the study was the prevalence of nephrotoxicity, as defined according to the RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria (16). Secondary outcomes included independent risk factors associated with nephrotoxicity, onset of nephrotoxicity, and hospital mortality. Patients were followed for up to 30 days or until hospital discharge.

Statistical analysis. Continuous variables were compared using a Student *t* test or Kruskal-Wallis one-way analysis of variance. Alpha adjustment was not performed. Categorical variables were compared using Fisher's exact test. To identify independent risk factors associated with nephrotoxicity, univariate logistic regression was used to derive odds ratios (ORs) and 95% confidence intervals (CIs) of different variables. Variables with *P* values of <0.2 in the univariate analysis were included in a

TABLE 1 *In vitro* toxicity of various drugs in different cell lines^a

Cell line	IC_{50} (mM) ^b		
	Polymyxin B	Colistin	Gentamicin
HEK 293	0.383 $\pm$ 0.182	0.456 $\pm$ 0.0656	16.3 $\pm$ 14.4
NRK-52E	0.713 $\pm$ 0.102	1.48 $\pm$ 0.441	21.6 $\pm$ 7.76

^a All drugs tested were a USP mixture of sulfate salts; working molecular weights for the mixtures were 1302 (polymyxin B), 1155 (colistin), and 576 (gentamicin).

^b Data shown are means $\pm$ SDs. IC_{50} (drug concentration resulting in 50% of maximal reduction in cell viability) estimates of polymyxin B and colistin were not statistically different.

multivariate analysis, using both forward- and backward-stepwise selection processes. *P* values of ≤ 0.05 were considered significant. To account for underlying differences in risk factors, a (1:1) matching process was used to compare the prevalence of nephrotoxicity in the two treatment cohorts. Based on the results of the preliminary analyses, patients with cystic fibrosis and those given lower than the standard daily doses (colistimethate [colistin base activity], <2.5 mg/kg by ideal body weight [IBW]; polymyxin B, <1.5 mg/kg) were excluded. Each patient given colistimethate was matched to a patient given polymyxin B based on duration of therapy (within 3 days) and age (within 5 years). The time to nephrotoxicity in the matched cohorts was compared using the Kaplan-Meier survival analysis and log rank test. All statistical analyses were performed using SYSTAT, version 12 (SYSTAT Software, Inc., Chicago, IL).

Sample size (power) analysis. This study was a multicenter study in which subjects were recruited from multiple study sites using the same set of inclusion/exclusion criteria. Based on the RIFLE criteria, the rate of nephrotoxicity associated with colistimethate therapy was reported to be 43.0% (8). Using an alpha of 0.05, beta of 0.20, and delta of 20.0%, 97 subjects in each patient cohort were needed for the comparison.

RESULTS

In vitro cytotoxicity. All model fits were satisfactory ($r^2 \geq 0.95$). Using the pharmacologically active forms, both polymyxins were found to be more nephrotoxic than gentamicin (Table 1). The difference in the IC_{50} s between colistin and polymyxin B was within the expected interday experimental variation. There were no considerable differences in most drug concentrations investigated; the toxicity profiles of colistin sulfate and polymyxin B sulfate were deemed similar overall.

Baseline characteristics of patients. A total of 225 patients were evaluated; 121 patients received colistimethate (49.6% male, 61.5% Caucasian), and 104 patients received polymyxin B (46.2% male, 61.5% Caucasian). The characteristics of all of the patients are summarized in Table 2. Patients in the Texas hospitals were given colistimethate sodium as the primary polymyxin while patients in the New York hospitals were predominantly given polymyxin B.

Comparison in intention-to-treat patients. In the intention-to-treat analysis, nephrotoxicity (overall) was observed in 24 patients (23.1%) who received polymyxin B and 41 patients (33.9%) who received colistimethate ($P = 0.08$) (Fig. 1). In patients without cystic fibrosis (83 receiving colistimethate and 104 receiving polymyxin B), nephrotoxicity was observed in 40 patients (48.2%) who received colistimethate and 24 patients (23.1%) who received polymyxin B ($P < 0.001$) (see Table S1 in the supplemental material). In a multivariate analysis, the duration of therapy was the only variable identified as an independent risk factor for polymyxin B-associated nephrotoxicity (OR, 1.08; 95% CI, 1.02 to 1.16; $P = 0.02$). In contrast, independent risk factors for nephrotoxicity in the colistimethate cohort included age (OR, 1.04; 95%

TABLE 2 Characteristics of all patients

Parameter ^a	Value for the cohort		P value
	Colistin (n = 121)	Polymyxin B (n = 104)	
Age (yr [mean $\pm$ SD])	46.4 $\pm$ 18.9	71.4 $\pm$ 17.0	<0.001
No. of male patients (%)	60 (49.6)	48 (46.2)	0.69
No. of Caucasians (%)	67 (61.5) ^b	64 (61.5)	1.00
ABW (kg)	68.8 $\pm$ 22.0	71.6 $\pm$ 20.4	0.33
IBW (kg)	61.5 $\pm$ 12.1 ^b	57.7 $\pm$ 10.6 ^c	0.02
Length of hospital stay (days)	29.0 $\pm$ 31.2	39.2 $\pm$ 35.6	0.03
Duration of therapy (days)	10.5 $\pm$ 9.7	11.7 $\pm$ 7.2	0.33
Total daily dose (mg/day) ^d	275.2 $\pm$ 106.8 ^e	103.9 $\pm$ 40.1	
Daily dose by ABW (mg/kg/day) ^d	4.1 $\pm$ 1.0 ^e	1.5 $\pm$ 0.5	
Daily dose by IBW (mg/kg/day) ^d	4.6 $\pm$ 1.8 ^{b,c}	1.8 $\pm$ 0.6 ^c	
Baseline creatinine (mg/dl)	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3	0.23
No. of concurrent nephrotoxins (mean $\pm$ SD)	1.3 $\pm$ 1.2	1.9 $\pm$ 1.0	<0.001
Comorbidities (no. of patients [%])			
Hypertension	51 (42.1)	16 (15.4)	<0.001
Cystic fibrosis	38 (31.4)	0 (0.0)	<0.001
Diabetes mellitus	45 (37.2)	32 (30.8)	0.33
Source of infection (no. of patients [%]) ^{b,f}			
Blood	8 (6.8)	14 (13.5)	0.12
Lungs	64 (54.7)	64 (61.5)	0.34
Urinary tract	13 (11.1)	25 (24.0)	0.01
Wound	21 (18.0)	8 (7.7)	0.03
Abdomen	6 (5.1)	1 (1.0)	0.12
Other	5 (4.3)	2 (1.9)	0.45
Infecting organism (no. of patients [%]) ^f			
<i>Pseudomonas aeruginosa</i>	66 (55.9)	37 (35.6)	0.003
<i>Acinetobacter baumannii</i>	33 (28.0)	41 (39.4)	0.09
<i>Escherichia coli</i>	3 (2.5)	8 (7.7)	0.12
<i>Klebsiella pneumoniae</i>	21 (17.8)	31 (29.8)	0.04
Other	7 (5.9)	10 (9.6)	0.32
Hospital mortality (no. of patients [%])	10 (8.3)	32 (30.8)	<0.001
Nephrotoxicity within 30 days (no. of patients [%])			
Overall	41 (33.9)	24 (23.1)	0.08
Risk	17 (14.0)	5 (4.8)	0.02
Injury	14 (11.6)	7 (6.7)	0.25
Failure	10 (8.3)	12 (11.5)	0.50

^a ABW, actual body weight; IBW, ideal body weight.^b n < 121 due to missing patient data.^c n < 104 due to missing patient data.^d Direct comparison of doses not appropriate due to different drug moiety used in each group.^e Dose of colistimethate based on colistin base activity.^f May not add up to 100%.

FIG 1 Comparison of nephrotoxicity rates. *, P = 0.003.

CI, 1.00 to 1.07; $P = 0.03$), duration of therapy (OR, 1.08; 95% CI, 1.02 to 1.15; $P = 0.02$), and daily dose by ideal body weight (OR, 1.40; 95% CI, 1.05 to 1.88; $P = 0.02$). Of note, cystic fibrosis (average age $\pm$ standard deviation [SD], 28.6 $\pm$ 7.5 years) was found to be protective against the development of nephrotoxicity in patients who received colistimethate (OR, 0.03; 95% CI, 0.001 to 0.79; $P = 0.04$) (Table 3).

Comparison in matched patients. To account for underlying differences in risk factors, a total of 76 patients (38 pairs) were matched and analyzed. The characteristics of all matched patients are described in Table 4. In the matched cohorts, the prevalence of nephrotoxicity (overall) was found to be significantly higher in patients who received colistimethate than in those receiving poly-

TABLE 3 Risk factors of nephrotoxicity associated with colistin therapy^a

Variable ^b	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.06 (1.03–1.09)	<0.001	1.04 (1.00–1.07)	0.03
Male gender	1.28 (0.60–2.72)	0.52		
Ethnicity				
Caucasian	0.98 (0.43–2.22)	0.96		
African American	0.80 (0.30–2.14)	0.65		
Hispanic	1.26 (0.42–3.79)	0.68		
Duration of therapy	1.03 (0.99–1.08)	0.12	1.08 (1.02–1.15)	0.02
Total daily dose	1.01 (1.00–1.01)	0.002		
Daily dose by ABW	1.36 (0.94–1.98)	0.11		
Daily dose by IBW	1.50 (1.15–1.95)	0.003	1.40 (1.05–1.88)	0.02
No. of concurrent nephrotoxins	1.54 (1.10–2.16)	0.01		
Comorbidities				
Hypertension	3.81 (1.73–8.42)	0.001		
Cystic fibrosis	0.03 (0.004–0.22)	0.001	0.03 (0.001–0.79)	0.04
Diabetes mellitus	0.96 (0.44–2.10)	0.92		

^a Receiver operating characteristic area under the curve of 0.857.^b ABW, actual body weight; IBW, ideal body weight.

myxin B (55.3% versus 21.1%; $P = 0.003$) (Fig. 1). Furthermore, the onset of nephrotoxicity was earlier in patients given colistimethate than in those receiving polymyxin B ($P = 0.003$) (Fig. 2). Fourteen pairs of patients had the same positive culture sites (8 lungs, 3 urine, 2 blood, and 1 wound); there was no difference in overall hospital mortality between the colistimethate (21.4%) and polymyxin B (21.4%) cohorts.

DISCUSSION

Polymyxins are increasingly used to treat MDR infections. In view of colistin and polymyxin B sharing a similar mechanism of action and cross-resistance pattern (17), there does not appear to be an advantage with one agent versus another. An older study by Nord and Hoeprich compared the relative potency/acute toxicity between the agents and found polymyxin B sulfate to be more toxic (5). Despite a lack of substantial clinical evidence to support this claim, colistimethate is more commonly used worldwide (18). Published literature has revealed significant variability in nephrotoxicity rates associated with the polymyxins. Older studies demonstrated rates of nephrotoxicity ranging from 0% to 50.0% with intravenous or intramuscular colistimethate sodium (6). However, these reports have been limited to small case series. Lower rates of nephrotoxicity were observed in the early 2000s, contributing to the perception that colistimethate was safer than previously reported (19, 20). More recent studies have demonstrated colistimethate-associated nephrotoxicity rates to be as high as 45.0% (8, 9). Until recently, only a limited number of studies evaluated nephrotoxicity in patients who received polymyxin B. Among these reports, similar discrepancies in nephrotoxicity rates exist, ranging from 4.0% to 60.0% (21–23).

Reasons for the discrepancies encountered in the literature may be multifactorial. Many of the inconsistencies regarding nephrotoxicity may be attributed to nonstandardized definitions. The majority of older studies did not provide a formal definition

TABLE 4 Characteristics of matched patients

Parameter ^a	Value for the cohort		
	Colistin (n = 38)	Polymyxin B (n = 38)	P value
Age (yr [mean $\pm$ SD])	63.0 $\pm$ 15.7	63.3 $\pm$ 15.5	0.92
No. of male patients (%)	16 (42.1)	17 (44.7)	1.00
No. of Caucasians (%)	21 (61.8) ^b	20 (52.6)	0.48
ABW (kg)	70.5 $\pm$ 19.2	79.1 $\pm$ 20.6	0.06
IBW (kg)	59.1 $\pm$ 12.9	59.9 $\pm$ 10.4	0.76
Length of hospital stay (days)	35.5 $\pm$ 35.2	27.2 $\pm$ 27.2	0.25
Duration of therapy (days)	9.2 $\pm$ 5.1	9.2 $\pm$ 5.2	0.98
Total daily dose (mg/day) ^c	291.7 $\pm$ 99.3 ^d	125.9 $\pm$ 35.3	
Daily dose by ABW (mg/kg/day) ^c	4.2 $\pm$ 1.1 ^d	1.7 $\pm$ 0.5	
Daily dose by IBW (mg/kg/day) ^c	5.0 $\pm$ 1.5 ^d	2.1 $\pm$ 0.6	
Baseline creatinine (mg/dl)	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3	0.07
Concurrent vasopressor (no. of patients [%])	11 (28.9)	8 (21.1)	0.60
Comorbidities (no. of patients [%])			
Hypertension	22 (57.9)	10 (26.3)	0.01
Diabetes mellitus	12 (31.6)	14 (36.8)	0.81
Source of infection (no. of patients [%]) ^e			
Blood	3 (7.9)	6 (15.8)	0.48
Lungs	15 (39.5)	20 (52.6)	0.26
Urinary tract	7 (18.4)	12 (31.6)	0.29
Wound	7 (18.4)	3 (7.9)	0.31
Abdomen	4 (10.5)	0 (0.0)	0.12
Other	2 (5.3)	0 (0.0)	0.49
Infecting organism (no. of patients [%]) ^e			
<i>Pseudomonas aeruginosa</i>	17 (44.7)	14 (36.8)	0.50
<i>Acinetobacter baumannii</i>	15 (39.5)	9 (23.7)	0.15
<i>Escherichia coli</i>	0 (0.0)	4 (10.5)	0.06
<i>Klebsiella pneumoniae</i>	12 (31.6)	12 (31.6)	1.00
Other	1 (2.6)	2 (5.3)	1.00
Hospital mortality (no. of patients [%]) ^f	3 (21.4)	3 (21.4)	1.00
Nephrotoxicity within 30 days (no. of patients [%])			
Overall	21 (55.3)	8 (21.1)	0.003
Risk	7 (18.4)	1 (2.6)	0.06
Injury	7 (18.4)	3 (7.9)	0.31
Failure	7 (18.4)	4 (10.5)	0.52

^a ABW, actual body weight; IBW, ideal body weight.^b $n < 38$ due to missing patient data.^c Direct comparison of doses not appropriate due to different drug moiety used in each group.^d Dose of colistimethate based on colistin base activity.^e May not add up to 100%.^f Among 14 pairs of matched patients with the same site of positive culture.

of nephrotoxicity. In 2004, a consensus definition for acute renal failure, the RIFLE criteria, was developed (18). Studies conducted prior to the development of the RIFLE criteria reported rates of colistimethate-associated nephrotoxicity around 14.0 to 18.0% (19, 20). In two recent studies evaluating 126 and 66 patients utilizing the RIFLE criteria, colistimethate-associated nephrotoxicity was observed in 45.0% and 43.0% of patients, respectively (8, 9).

FIG 2 Comparison between the matched cohorts of the onset of nephrotoxicity. Patients who fell into any category of the RIFLE definition for nephrotoxicity were included. Solid line, polymyxin B cohort; dashed line, colistin cohort ($P = 0.003$).

The definition of nephrotoxicity used in earlier studies may not have been stringent enough to capture subtle changes in renal function.

The lack of control for underlying risk factors may be another source of discrepancy. Several studies identified underlying renal impairment as an independent risk factor for nephrotoxicity (24, 25). Evaluating nephrotoxicity in patients who already have an underlying renal impairment may introduce bias. It is often difficult to distinguish whether the acute renal injury is due to a drug or to the progression of the underlying disease. Inconsistency in dosing was also commonly seen and was typically at the discretion of the attending physician. The daily dose of colistimethate or polymyxin B has been identified as an independent risk factor for nephrotoxicity (8, 9, 25). Thus, doses outside the normal range may also account for some of the variability seen in the nephrotoxicity rates reported. Finally, since intravenous colistimethate sodium is an inactive prodrug, it is difficult to make a fair comparison of nephrotoxicity to polymyxin B. Patients with moderate to good renal function and those in a hypermetabolic state (e.g., cystic fibrosis or burn patients) can excrete the majority of a colistimethate sodium dose, leaving only a small fraction of the dose to be converted to its active form (14, 26, 27).

To advance medical care based on the best available scientific evidence, we conducted *in vitro* experiments exposing mammalian kidney cell lines to colistin or polymyxin B sulfate. In view of the unpredictable conversion of colistimethate sodium to the active form, we focused on the active moiety for a direct comparison of nephrotoxicity. In contrast to the study by Nord and Hoeprich (5), we examined a range of drug concentrations on different kidney cell lines, which provided a more specific assessment of nephrotoxicity. We found that the relative nephrotoxicity was much less dramatic than previously reported; the toxicity profiles between colistin and polymyxin B were similar. Although this study is often cited, only acute toxicity of the polymyxins was previously examined in animals, not specifically nephrotoxicity. Moreover, consistent evidence of renal parenchymal damage was not detected in the experimental animals.

To validate the clinical relevance of our *in vitro* results, we

assessed nephrotoxicity in more than 200 patients on polymyxin therapy in a multicenter study. To our knowledge, this is the largest study to date that is adequately powered for a head-to-head comparison between colistimethate and polymyxin B. Using the RIFLE criteria, the observed nephrotoxicity rate in our patients who received colistimethate (33.9%) was comparable to rates observed previously (8, 9). Of note, there has been one study that evaluated polymyxin B-associated nephrotoxicity using the RIFLE criteria (22). This study evaluated 73 patients and found a 60.0% rate of nephrotoxicity, which is higher than previous reports. However, patients with underlying renal insufficiency were not excluded from this study. We specifically excluded patients with underlying baseline insufficiency or fluctuating renal function prior to the initiation of polymyxin therapy to eliminate the association of nephrotoxicity with this potential confounder. Similar to our findings, a recent study by Akajagbor et al. evaluating 173 critically ill patients found significantly higher rates of nephrotoxicity associated with colistin than with polymyxin B (60.4% versus 41.8%, $P = 0.02$) (28). Patients in this study received a lower average daily dose of colistin (3.9 mg/kg/day by IBW) than in our study (4.6 mg/kg/day). However, those who received >5 mg/kg/day by IBW of colistin had a numerically higher rate of nephrotoxicity than those receiving lower doses. Interestingly, a higher APACHE II score and a baseline serum creatinine of ≥ 1.5 mg/dl were found to be associated with a lower risk of nephrotoxicity while older age was an independent predictor of nephrotoxicity. The authors hypothesized that patients with higher APACHE II scores had worsening renal function and might have been dosed more conservatively, leading to lower drug exposures and a decreased toxicity risk.

Daily dose of colistimethate by ideal body weight was identified as an independent risk factor for nephrotoxicity. Patients received an average of 4.6 mg/kg/day and 1.8 mg/kg/day by ideal body weight in the colistimethate and polymyxin B cohorts, respectively. Studies conducted in the United States found that nephrotoxicity was greater than 40% when the average daily dose of colistimethate exceeded 300 mg (8, 9). In contrast, studies outside the United States used lower doses of colistimethate and observed nephrotoxicity rates of less than 20%. Based on the correlation seen between dose and nephrotoxicity, the higher colistimethate dose used in our study might account for the higher rates of nephrotoxicity observed. However, it was difficult to determine how much and the exact anatomical site where colistimethate sodium was actually converted to its active form. Thus, we could not make a fair dosing comparison to polymyxin B in terms of a dose equivalent. As a prodrug, colistimethate sodium was administered at a higher dose (on a mg/kg basis) than polymyxin B. The greater nephrotoxicity rate observed could be due to a higher than expected extent of colistin conversion systemically. Other independent risk factors identified include age and duration of therapy for colistin and duration of therapy only for polymyxin B. Our findings are consistent with the risk factors identified in previous studies (8, 22, 29). Colistin-associated nephrotoxicity was well explained by the independent risk factors (as reflected in a receiver operating characteristic area under the curve of >0.85), resulting in a high degree of concordance using the identified risk factors. Also, we found that cystic fibrosis was a protective factor against the development of nephrotoxicity in patients who received colistimethate. However, some other risk factors, such as concomitant nephrotoxins and concomitant rifampin administration, were not

found in our study. Investigations are ongoing to evaluate the predictability of different published models.

To adjust for underlying differences in each treatment group, the identified risk factors were matched. Higher rates of colistimethate-associated nephrotoxicity were observed in both the intention-to-treat and matched analyses, attesting to the robustness of our assessment. The mortality rates in the intention-to-treat analysis were 8.3% and 30.8% in the colistimethate and polymyxin B cohorts, respectively, although there were differences in the sites of infection, length of hospital stay, duration of therapy, and baseline severity of illness. In addition, specific concurrent treatment was not controlled for with respect to appropriateness of therapy as this was not our study focus. When we matched the patients by age, duration of therapy, and site(s) of infection, no differences in mortality were observed.

There are several limitations in our study. Due to the retrospective nature of study design, we were unable to stratify for specific factors such as dose, duration of therapy, and site of infection. Baseline severity of illness was not systematically assessed as we reasoned that commonly used indices (e.g., APACHE II) were not designed to predict the likelihood of nephrotoxicity. However, based on the proportion of (hemodynamically unstable) patients on concurrent vasopressor therapy or with bacteremia, patients given colistimethate in the matched analysis did not appear to be more acutely ill. Thus, the difference in the prevalence of nephrotoxicity observed was unlikely explained solely by the underlying severity of illness. Because this is a multicenter study, there could also be interinstitution variability in patient population, the standards of care, and the polymyxin available on formulary at each study site. These differences may have an impact on patient outcomes. Where readily available, therapeutic drug monitoring of polymyxins should be employed for safety and efficacy evaluation. Our study excluded patients with underlying renal insufficiency; therefore the results cannot be extrapolated to this patient population. Finally, we defined nephrotoxicity according to the RIFLE criteria, which were mostly based on serum creatinine measurements. While elevated serum creatinine is indicative of significant renal damage, it may not be as reliable for detecting early, subtle injuries. This may potentially have led to an underestimation of the rates of nephrotoxicity seen in our patients.

In conclusion, polymyxin B was not found to be more nephrotoxic than colistin, contrary to common belief. In view of the more predictable systemic drug exposure and similar *in vitro* potency, polymyxin B may be the preferred polymyxin for MDR Gram-negative infections. A prospective study comparing the two polymyxins directly is warranted.

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