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SUSCEPTIBILITY

In Vitro Potency and Spectrum of the Novel Polymyxin MRX-8 Tested against Clinical Isolates of Gram-Negative Bacteria

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ABSTRACT The polymyxins display excellent *in vitro* antimicrobial activity against most *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolates, but their clinical utility has been limited because of class-specific toxicity problems. Therefore, new polymyxin analogs with improved safety properties are needed to combat serious infections caused by resistant Gram-negative pathogens. MRX-8 is a novel polymyxin B analog that displays reduced toxicity in *in vitro* and animal assays and is currently being evaluated in a phase 1 clinical trial. In this nonclinical study, the *in vitro* potency and spectrum of MRX-8 and comparators were evaluated against a large set of Gram-negative clinical isolates collected in the United States in 2017 to 2020. MRX-8, colistin, and polymyxin B exhibited nearly identical antimicrobial activities against the *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolate sets. MRX-8 MIC_{50} and MIC_{90} values were 0.12 and 0.25 mg/L, respectively, for the set of *Enterobacterales* isolates not intrinsically resistant to colistin and 0.5 and 1 mg/L, respectively, against both the *A. baumannii* and *P. aeruginosa* isolate sets. All three polymyxin-class compounds retained activity against meropenem-resistant and multidrug-resistant isolate subsets but were inactive against isolates displaying acquired or intrinsic resistance to polymyxins. These results support the continued development of MRX-8 to treat serious Gram-negative infections.

KEYWORDS polymyxin, colistin, lipopeptide, Gram-negative, resistance, MRX-8

Many recent reports and reviews have emphasized the need for the development of novel or improved antimicrobial agents to treat serious infections caused by Gram-negative species or groups like the *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, which often display resistance to frontline drugs (1–4). Of particular concern are Gram-negative isolates that are multidrug resistant (MDR) or carbapenem resistant due to multifactorial mechanisms or the production of class B metallo-β-lactamases (3, 5).

The clinically relevant polymyxin antimicrobial class is composed of colistin (polymyxin E) and polymyxin B, which are structurally related polycationic molecules with a cyclic lipodecapeptide structure composed of a heptapeptide core, a tripeptide linear linker, and an N-terminal fatty acyl group. The structures of colistin and polymyxin B differ only at the R6 peptide position, which is D-phenylalanine in polymyxin B and D-leucine in colistin (6). Commercial preparations of polymyxin B and colistin also typically contain mixtures of components with related but distinct fatty acid groups (7). Three free amino groups are present within the heptapeptide core, and two free amino groups are present within the linker region. Both colistin and polymyxin B exhibit rapid *in vitro* bactericidal activity against many important Gram-negative pathogens. The mode of action is principally mediated by disruption of the bacterial outer membrane through binding of the lipopeptide cationic groups to lipopolysaccharide, followed by disruption of the inner membrane, which likely involves interaction with the fatty acid polymyxin tail (6, 8).

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The authors declare no conflict of interest.

Received 28 January 2022
Returned for modification 20 February 2022
Accepted 5 April 2022
Published 27 April 2022

Gram-positive species and several *Enterobacterales* species (e.g., *Providencia* spp. and *Proteus* spp.) are intrinsically resistant to the polymyxins (9, 10). In contrast, *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *P. aeruginosa*, and *A. baumannii* are intrinsically susceptible to the polymyxins but resistance can be acquired by various methods, including mutations in chromosomal genes or the acquisition of mobile genetic elements that contain *mcr-1* or related genes encoding enzymes that modify the bacterial lipopolysaccharide structure (9, 11, 12).

Although the discovery of polymyxins was reported in 1947 (for a review, see reference 7), their clinical use was short-lived due to concerns about nephrotoxicity and neurotoxicity and the discovery of safer alternative antimicrobials (7, 13). Because of the recent rise in highly drug-resistant Gram-negative pathogens, however, there has been a resurgence in the clinical use of the polymyxins as drugs of last resort (14).

Several groups have launched research efforts to improve the clinical utility of the polymyxins with the goal of maintaining their excellent antimicrobial features while abrogating their toxicity (6, 14). Other groups have instead focused on designing truncated polymyxins, with decreased antimicrobial activity and decreased toxicity, that still maintain the ability to potentiate the entry of other antimicrobials through the Gram-negative outer membrane (8).

MRX-8 is a polymyxin-class antimicrobial under development that was designed using soft drug principles, in which the goal is to produce molecules that are "deactivated in a predictable and controllable way after achieving their therapeutic goal" (15). To that end, MRX-8 is an analog of polymyxin B in which the fatty acid tail is linked to the rest of the molecule by a polar ester group. MRX-8 maintains *in vitro* antimicrobial activity (reference 16 and see below). After exerting its antimicrobial effect *in vivo*, however, MRX-8 is intended to be converted by endogenous esterases into a nontoxic metabolite that displays minimized cell culture toxicity and animal nephrotoxicity properties, compared to polymyxin B (16).

Lepak et al. (17) recently investigated the pharmacodynamic (PD) properties of MRX-8, compared to polymyxin B, in mouse thigh and lung infection models involving *Enterobacterales*, *P. aeruginosa*, and *A. baumannii* strains. They found that MRX-8 exhibited efficacy in those animal models and that AUC/MIC and maximum drug concentration (C_{max})/MIC were the pharmacokinetic (PK)/PD indices that best correlated with the observed antimicrobial therapeutic effects.

The safety, tolerability, and human PK features of MRX-8 following intravenous dosing are currently being evaluated in a phase 1 clinical trial (ClinicalTrials registration no. NCT04649541), with results expected in 2022. In this study, we investigated the *in vitro* antimicrobial potency and spectrum of MRX-8, compared to colistin and polymyxin B, when tested against a large set of Gram-negative pathogens collected in the United States from 2017 to 2020.

RESULTS

Cumulative distributions of MIC values for each isolate set tested against MRX-8, colistin, and polymyxin B are shown in Table 1, Table S1, and Table S2, respectively. Summary tables that display MIC_{50} and MIC_{90} values, MIC ranges, and percentages of susceptible, intermediate, or resistant isolates for various species are displayed in Table 2 and in Tables S3 to S6 in the supplemental material.

Activity of MRX-8 and comparators against *Enterobacterales* species. The activity of MRX-8 and comparator lipopeptides was first measured against *Enterobacterales* species that are not intrinsically resistant to colistin, including *E. coli* (Table 2), *Klebsiella pneumoniae* (Table 2), *Citrobacter* spp. (see Table S3), *Enterobacter cloacae* species complex (see Table S4), *Klebsiella aerogenes* (see Table S5), and *Klebsiella oxytoca* (see Table S6). Within each species or group of isolates, the MIC_{50} and MIC_{90} values for MRX-8, colistin, and polymyxin B agreed within 2-fold. For example, against the *E. coli* subset (Table 2), the MIC_{50} and MIC_{90} values for MRX-8, colistin, and polymyxin B were 0.12 and 0.25 mg/L, 0.25 and 0.25 mg/L, and 0.25 and 0.5 mg/L, respectively. There was also

TABLE 1 Cumulative MIC distributions for MRX-8 tested against the main species and organism groups

Species/organism group (no. of isolates)	No. (cumulative %) of isolates inhibited with MIC of:													MIC_{50} (mg/L)	MIC_{90} (mg/L)
	≤0.015 mg/L	0.03 mg/L	0.06 mg/L	0.12 mg/L	0.25 mg/L	0.5 mg/L	1 mg/L	2 mg/L	4 mg/L	8 mg/L	16 mg/L	32 mg/L	>[a]		
Enterobacterales (787)															
Enterobacterales isolates not intrinsically resistant to colistin (685)[b]	0 (0.0)	2 (0.3)	86 (12.8)	450 (78.5)	109 (94.5)	20 (97.4)	0 (97.4)	1 (97.5)	0 (97.5)	3 (98.0)	2 (98.2)	1 (98.4)	11 (100.0)	0.12	0.25
Citrobacter spp. (47)		0 (0.0)	10 (21.3)	30 (85.1)	6 (97.9)	1 (100.0)								0.12	0.25
Enterobacter cloacae species complex (52)		0 (0.0)	8 (15.4)	27 (67.3)	6 (78.8)	1 (80.8)	0 (80.8)	0 (80.8)	0 (80.8)	1 (82.7)	0 (82.7)	0 (82.7)	9 (100.0)	0.12	>32
Escherichia coli (261)	0 (0.0)	2 (0.8)	46 (18.4)	164 (81.2)	40 (96.6)	7 (99.2)	0 (99.2)	0 (99.2)	0 (99.2)	1 (99.6)	1 (100.0)			0.12	0.25
Klebsiella aerogenes (23)		0 (0.0)	1 (4.3)	15 (69.6)	5 (91.3)	2 (100.0)								0.12	0.25
Klebsiella oxytoca (37)		0 (0.0)	6 (16.2)	30 (97.3)	1 (100.0)									0.12	0.12
Klebsiella pneumoniae (265)		0 (0.0)	15 (5.7)	184 (75.1)	51 (94.3)	9 (97.7)	0 (97.7)	1 (98.1)	0 (98.1)	1 (98.5)	1 (98.9)	1 (99.2)	2 (100.0)	0.12	0.25
Colistin-resistant (17)									0 (0.0)	3 (17.6)	2 (29.4)	1 (35.3)	11 (100.0)	>32	>32
Meropenem-resistant (10)		0 (0.0)	1 (10.0)	3 (40.0)	3 (70.0)	0 (70.0)	0 (70.0)	0 (70.0)	0 (70.0)	1 (80.0)	0 (80.0)	0 (80.0)	2 (100.0)	0.25	>32
MDR (54)		0 (0.0)	3 (5.6)	34 (68.5)	11 (88.9)	3 (94.4)	0 (94.4)	0 (94.4)	0 (94.4)	1 (96.3)	0 (96.3)	0 (96.3)	2 (100.0)	0.12	0.5
Enterobacterales isolates intrinsically resistant to colistin (102)[c]											0 (0.0)	2 (2.0)	100 (100.0)	>32	>32
Acinetobacter baumannii (264)	0 (0.0)	1 (0.4)	3 (1.5)	33 (14.0)	58 (36.0)	116 (79.9)	35 (93.2)	12 (97.7)	1 (98.1)	0 (98.1)	2 (98.9)	1 (99.2)	2 (100.0)	0.5	1
Meropenem-resistant (74)			0 (0.0)	9 (12.2)	20 (39.2)	32 (82.4)	9 (94.6)	1 (95.9)	0 (95.9)	0 (95.9)	1 (97.3)	1 (98.6)	1 (100.0)	0.5	1
Colistin-nonresistant (258)	0 (0.0)	1 (0.4)	3 (1.6)	33 (14.3)	58 (36.8)	116 (81.8)	35 (95.3)	12 (100.0)						0.5	1
Colistin-resistant (6)								0 (0.0)	1 (16.7)	0 (16.7)	2 (50.0)	1 (66.7)	2 (100.0)	16	
MDR (104)			0 (0.0)	15 (14.4)	23 (36.5)	44 (78.8)	15 (93.3)	4 (97.1)	0 (97.1)	0 (97.1)	1 (98.1)	1 (99.0)	1 (100.0)	0.5	1
Pseudomonas aeruginosa (263)		0 (0.0)	3 (1.1)	9 (4.6)	19 (11.8)	166 (74.9)	64 (99.2)	2 (100.0)						0.5	1
Meropenem-resistant (31)			0 (0.0)	3 (9.7)	3 (19.4)	20 (83.9)	5 (100.0)							0.5	1
MDR (46)		0 (0.0)	1 (2.2)	4 (10.9)	6 (23.9)	22 (71.7)	12 (97.8)	1 (100.0)						0.5	1

[a]Greater than the highest concentration tested.
[b]Species included *Citrobacter amalonaticus/farmeri* (1 isolate), *Citrobacter freundii* (1 isolate), *C. freundii* species complex (26 isolates), *Citrobacter koseri* (19 isolates), *Enterobacter asburiae* (1 isolate), *Enterobacter cloacae* (14 isolates), *E. cloacae* species complex (36 isolates), *Enterobacter hormaechei* (1 isolate), *Escherichia coli* (261 isolates), *Klebsiella aerogenes* (23 isolates), *Klebsiella oxytoca* (37 isolates), and *Klebsiella pneumoniae* (265 isolates).
[c]Species included *Morganella morganii* (10 isolates), *Proteus mirabilis* (46 isolates), *Proteus penneri* (1 isolate), *Proteus vulgaris* group (5 isolates), *Providencia rettgeri* (7 isolates), *Providencia stuartii* (8 isolates), *Serratia liquefaciens* complex (3 isolates), and *Serratia marcescens* (22 isolates).

10.1128/aac.00139-22

TABLE 2 Antimicrobial activity of MRX-8 and comparator agents tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* isolates

Species and antimicrobial agent	No. of isolates	MIC findings (mg/L)			Susceptibility results (%) using:					
					CLSI criteria[a]			EUCAST criteria[a]		
		MIC_{50}	MIC_{90}	MIC range	S	I	R	S	I	R
Escherichia coli										
MRX-8	261	0.12	0.25	0.03 to 16						
Colistin	261	0.25	0.25	0.06 to 8		99.2	0.8	99.2		0.8
Polymyxin B	261	0.25	0.5	0.12 to 8		99.6	0.4			
Amikacin	261	2	8	0.5 to 16	100.0	0.0	0.0	98.9[b]		1.1
Ceftazidime	261	0.25	8	0.03 to >32	88.1	2.7	9.2	82.8	5.4	11.9
Ceftazidime-avibactam (fixed at 4 mg/L)	77	0.06	0.25	≤0.015 to 0.25	100.0		0.0	100.0		0.0
Ceftriaxone	261	≤0.06	>8	≤0.06 to >8	83.5	0.0	16.5	83.5,[c] 83.5[d]	0.0[d]	16.5,[c] 16.5[d]
Gentamicin	261	1	>16	≤0.12 to >16	87.0	0.4	12.6	86.6[b]		13.4
Levofloxacin	260	≤0.03	16	≤0.03 to >16	68.8	0.8	30.4	68.8	0.8	30.4
Meropenem	261	0.015	0.03	0.008 to 8	99.6	0.0	0.4	99.6,[c] 99.6[d]	0.4[d]	0.4,[c] 0.0[d]
Piperacillin-tazobactam (fixed at 4 mg/L)	261	2	8	1 to >128	96.6	1.5	1.9	94.3		5.7
Tigecycline	261	0.12	0.25	≤0.06 to 1	100.0[e]	0.0	0.0	98.9		1.1
Klebsiella pneumoniae										
MRX-8	265	0.12	0.25	0.06 to >32						
Colistin	265	0.12	0.25	0.12 to >32		98.1	1.9	98.1		1.9
Polymyxin B	265	0.25	0.5	0.12 to >32		98.1	1.9			
Amikacin	265	1	2	0.5 to >32	98.9	0.0	1.1	98.5[b]		1.5
Ceftazidime	265	0.25	16	0.03 to >32	88.3	1.1	10.6	87.5	0.8	11.7
Ceftazidime-avibactam (fixed at 4 mg/L)	102	0.12	0.25	0.03 to 1	100.0		0.0	100.0		0.0
Ceftriaxone	265	≤0.06	>8	≤0.06 to >8	87.9	0.0	12.1	87.9,[c] 87.9[d]	0.0[d]	12.1,[c] 12.1[d]
Gentamicin	265	0.25	0.5	≤0.12 to >16	94.0	0.4	5.7	94.0[b]		6.0
Levofloxacin	265	0.06	1	≤0.03 to >16	86.0	4.2	9.8	86.0	4.2	9.8
Meropenem	265	0.03	0.03	0.015 to >16	97.4	0.4	2.3	97.7,[c] 97.7[d]	0.4[d]	2.3,[c] 1.9[d]
Piperacillin-tazobactam (fixed at 4 mg/L)	265	4	16	0.12 to >128	93.6	2.3	4.2	89.4		10.6
Tigecycline	265	0.5	1	≤0.06 to 8	98.1[e]	1.5	0.4			
Acinetobacter baumannii										
MRX-8	264	0.5	1	0.03 to >32						
Colistin	264	0.25	1	0.06 to >32		97.7	2.3	97.7		2.3
Polymyxin B	264	0.25	0.5	0.12 to 16		98.5	1.5			
Amikacin	264	4	>32	0.5 to >32	83.3	1.9	14.8	79.2[b]		20.8
Ceftazidime	264	8	>32	1 to >32	65.2	7.2	27.7			
Ceftazidime-avibactam (fixed at 4 mg/L)	91	8	32	1 to >32						
Ceftriaxone	224	>8	>8	4 to >8	21.4	0.0	0.0			
Gentamicin	264	1	>16	≤0.12 to >16	75.4	5.7	18.9	75.4[b]		24.6
Levofloxacin	264	0.25	>16	≤0.015 to >16	65.9	1.1	33.0	62.9	2.3	34.8
Meropenem	264	0.5	>16	0.06 to >16	71.2	0.8	28.0	71.2,[c] 71.2[d]	1.1[d]	28.8,[c] 27.7[d]
Piperacillin-tazobactam (fixed at 4 mg/L)	258	8	>128	≤0.06 to >128	58.5	7.0	34.5			
Tigecycline	264	0.5	4	≤0.06 to 8						
Pseudomonas aeruginosa										
MRX-8	263	0.5	1	0.06 to 2						
Colistin	263	0.5	1	0.12 to 2		100.0	0.0	100.0		0.0
Polymyxin B	263	0.5	1	0.12 to 2		100.0	0.0			
Amikacin	263	4	8	≤0.25 to >32	96.2	1.1	2.7	96.2[b]		3.8
Ceftazidime	263	2	32	0.25 to >32	82.1	4.2	13.7	[f]	82.1	17.9
Ceftazidime-avibactam (fixed at 4 mg/L)	92	2	4	0.25 to >32	98.9		1.1	98.9		1.1
Ceftriaxone	211	>8	>8	0.5 to >8						
Gentamicin	263	2	8	≤0.12 to >16	87.8	6.5	5.7			
Levofloxacin	263	0.5	8	≤0.03 to >16	68.4	11.8	19.8	[f]	68.4	31.6
Meropenem	263	0.5	8	0.015 to >16	82.5	5.7	11.8	82.5,[c] 82.5[d]	8.7[d]	17.5,[c] 8.7[d]
Piperacillin-tazobactam (fixed at 4 mg/L)	263	4	128	≤0.06 to >128	79.8	7.6	12.5	[f]	79.8	20.2
Tigecycline	263	8	>8	0.25 to >8						

[a]Criteria as published by CLSI (10) and EUCAST (23). I, intermediate; R, resistant; S, susceptible.
[b]For infections originating from the urinary tract. For systemic infections, aminoglycosides must be used in combination with other active therapy.
[c]Using meningitis breakpoints.
[d]Using nonmeningitis breakpoints.
[e]Using FDA breakpoints.
[f]An arbitrary susceptible breakpoint of ≤0.001 mg/L and/or >50 mm has been published by EUCAST, indicating that susceptible should not be reported for this organism-agent combination and intermediate should be interpreted as susceptible-increased exposure.

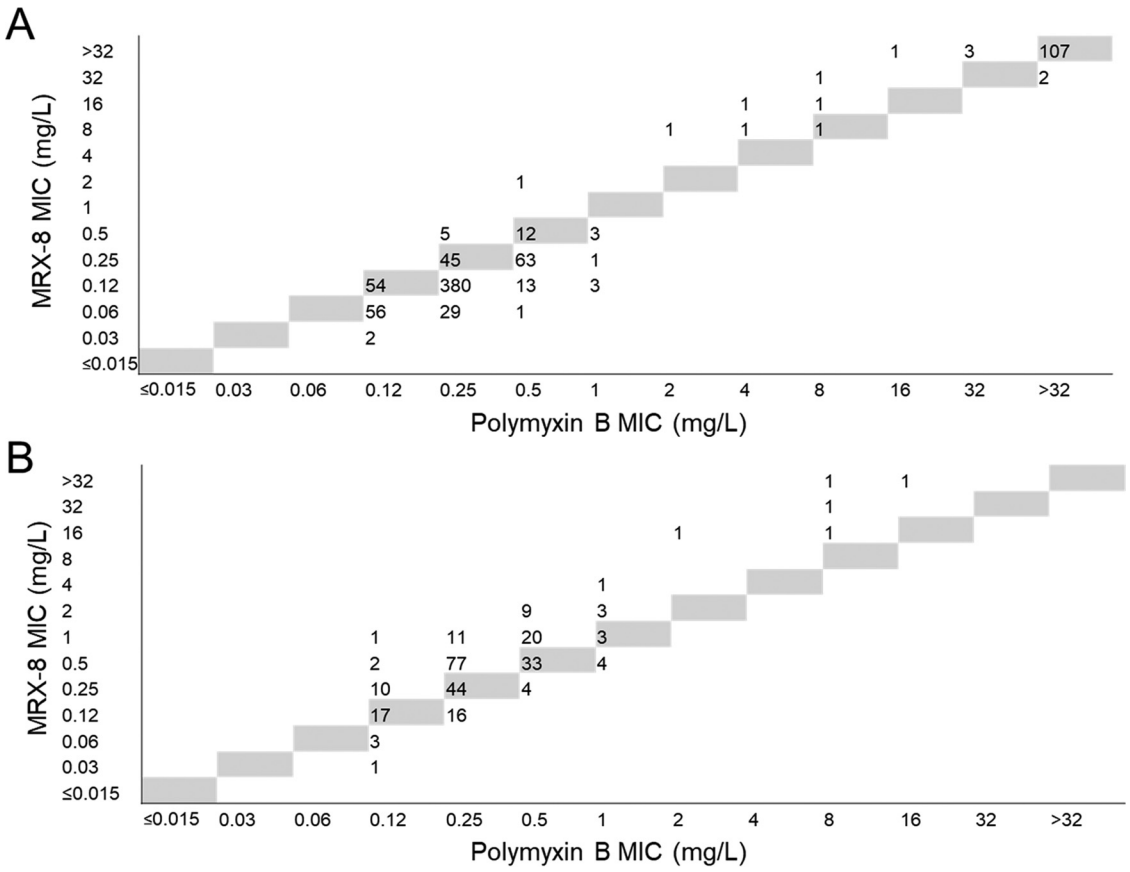

FIG 1 Scatterplot of MRX-8 and polymyxin B MIC values when tested against *Enterobacterales* and *Acinetobacter baumannii* isolates. The figure displays a scatterplot of MIC values for MRX-8 and polymyxin B tested against *Enterobacterales* isolates (787 isolates) (A) and *Acinetobacter baumannii* isolates (264 isolates) (B). The gray cells represent identical MIC values for the two antimicrobials.

little variation in the different polymyxin MIC_{50} and MIC_{90} values among the various *Enterobacterales* species and groups. Except for the *E. cloacae* species complex isolate subset, the MIC_{50} and MIC_{90} values were 0.12 and 0.12 to 0.25 mg/L, respectively, for MRX-8 (Table 1), 0.12 to 0.25 and 0.25 mg/L, respectively, for colistin (see Table S1), and 0.25 and 0.25 to 0.5 mg/L, respectively, for polymyxin B (see Table S2). In contrast, the *E. cloacae* species complex isolate subset MIC_{90} values for MRX-8 (Table 1), colistin (see Table S1), and polymyxin B (see Table S2) were much higher (>32 mg/L for each lipopeptide) than those for the other species groups of *Enterobacterales* that are not intrinsically resistant to colistin. This difference is due to the larger percentage of *Enterobacter* species isolates that displayed acquired colistin resistance (19.2%) (see Table S4), compared to the other species.

MRX-8 (Table 1) and colistin (see Table S1) were 2-fold more potent (MIC_{50} and MIC_{90} values for both antimicrobials of 0.12 and 0.25 mg/L, respectively) than polymyxin B (MIC_{50} and MIC_{90} of 0.25 and 0.5 mg/L, respectively) (see Table S2) against the combined set of *Enterobacterales* isolates not intrinsically resistant to colistin. This potency difference can be observed in the scatterplot of polymyxin B MIC values versus MRX-8 MIC values shown in Fig. 1A.

Against the meropenem-resistant subset of 10 *Enterobacterales* isolates from species that are not intrinsically resistant to colistin, the MIC_{50} and MIC_{90} values for MRX-8, colistin, and polymyxin B were 0.25 and >32 mg/L (Table 1), 0.12 and >32 mg/L (see Table S1), and 0.25 and 16 mg/L (see Table S2), respectively. Against the MDR subset of 54 *Enterobacterales* isolates from species that are not intrinsically resistant to colistin, the MIC_{50} and MIC_{90} values for MRX-8, colistin, and polymyxin B were 0.12

and 0.5 mg/L (Table 1), 0.12 and 0.5 mg/L (see Table S1), and 0.25 and 0.5 mg/L (see Table S2), respectively. MRX-8, colistin, and polymyxin B were all inactive (MIC_{50} values of ≥32 and MIC_{90} values of >32 mg/L) against the subset of 17 *Enterobacterales* isolates from species that are not intrinsically resistant to colistin but displayed acquired colistin resistance (Table 1; also see Tables S1 and S2 in the supplemental material).

Finally, we investigated the activity of MRX-8 and comparator lipopeptides against a subset of *Enterobacterales* species that are intrinsically resistant to colistin, such as *Morganella morganii* (10) (Table 1; also see Tables S1 and S2). As expected, MRX-8, colistin, and polymyxin B were all inactive against this isolate set (MIC_{90} values of >32 mg/L for all three antimicrobials).

Activity of MRX-8 and comparators against *Acinetobacter baumannii*. The *in vitro* antimicrobial activities of MRX-8 and comparators against the *A. baumannii* isolate subset are displayed in Table 2. The MIC_{50} and MIC_{90} values for the three lipopeptides agreed within 2-fold (MIC_{50} and MIC_{90} ranges of 0.25 to 0.5 and 0.5 to 1 mg/L, respectively). Colistin and polymyxin B were 2-fold more potent than MRX-8 according to MIC_{50} values. This effect can be observed in the scatterplot of polymyxin B MIC values and MRX-8 MIC values shown in Fig. 1B. The three lipopeptides maintained their nearly equivalent potencies against the meropenem-resistant *A. baumannii* isolate subset. *In vitro* activity was lost for each antimicrobial, however, against the subset of colistin-resistant *A. baumannii* isolates, although 2 of the 6 colistin-resistant isolates remained intermediate to polymyxin B (Table 1; also see Tables S1 and S2). MDR status did not significantly affect the MIC_{50} and MIC_{90} values for any of the three polymyxins (Table 1; also see Tables S1 and S2).

Activity of MRX-8 and comparators against *Pseudomonas aeruginosa*. The *in vitro* antimicrobial activities of MRX-8 and comparators against the *P. aeruginosa* isolate subset are displayed in Table 2. The MIC_{50} and MIC_{90} values for the three polymyxins were identical (MIC_{50} and MIC_{90} values of 0.5 and 1 mg/L, respectively), and the antimicrobial activities were unchanged against the subsets of meropenem-resistant and MDR *P. aeruginosa* isolates (Table 1; also see Tables S1 and S2).

No colistin-resistant *P. aeruginosa* isolates were present within the randomly selected set tested in this study (Table 2). However, we measured MIC values for the three polymyxins against a JMI Laboratories colistin-resistant *P. aeruginosa* isolate (collection no. 991784). All three polymyxins were inactive against this isolate, which displayed modal MIC values for MRX-8, colistin, and polymyxin B of >32 mg/L, 32 mg/L, and 8 mg/L, respectively (data not shown).

DISCUSSION

In this study, the *in vitro* antimicrobial activity of MRX-8 and polymyxin comparators was measured against a large set of clinically relevant Gram-negative pathogens, including various *Enterobacterales* species, *A. baumannii*, and *P. aeruginosa*. MRX-8, colistin, and polymyxin B exhibited identical or nearly identical *in vitro* antimicrobial activities against almost all of the Gram-negative species and groups tested, including MDR and meropenem-resistant subsets. Against the *Enterobacterales* isolates not intrinsically resistant to colistin, MRX-8 was 2-fold more potent than polymyxin B according to MIC_{50} and MIC_{90} values. In contrast, MRX-8 was about 2-fold less potent than polymyxin B against the *A. baumannii* set and equipotent against the *P. aeruginosa* isolate set according to MIC_{50} and MIC_{90} values. In general, all three polymyxins exhibited potent *in vitro* activity against the pathogen groups, e.g., the MRX-8 MIC_{50} and MIC_{90} values were 0.12 and 0.25 mg/L, respectively, against the set of *Enterobacterales* isolates not intrinsically resistant to colistin and 0.5 and 1 mg/L, respectively, against the *A. baumannii* and *P. aeruginosa* isolate sets. MRX-8, colistin, and polymyxin B were all inactive against isolate subsets that displayed acquired or intrinsic resistance to colistin.

MRX-8 merits additional study for the potential treatment of serious infections caused by Gram-negative pathogens. This conclusion is based on the following considerations. First, the *in vitro* potency and spectrum of MRX-8 are similar to those of colistin and

polymyxin B. Second, the unique soft drug structure of MRX-8, featuring an ester bond that is cleaved by enzymes *in vivo*, is designed to decrease the potential for nephrotoxicity, which is a significant treatment-limiting side effect of colistin and polymyxin B use. Although further work is needed, *in vitro* and *in vivo* toxicology studies have generated favorable data in support of this hypothesis (16). Third, recent animal data suggest that MRX-8 exhibits PK/PD properties equivalent or superior to those of colistin and polymyxin B (17).

Finally, when evaluating the potential clinical utility of MRX-8, it is important to consider the existing and potential future colistin resistance rates within target pathogen groups, because MRX-8, like other polymyxins, is inactive against such resistant isolates. Global longitudinal rates of colistin resistance have recently been reviewed for the *Enterobacterales* (18), *A. baumannii* (19), and *P. aeruginosa* (20). In general, European Committee on Antimicrobial Susceptibility Testing (EUCAST) colistin susceptibility rates remain high for these target pathogens, but significant global variation exists. Against the *Enterobacterales*, colistin susceptibility rates were generally >90%, but increased resistance has been noted for *Klebsiella* spp. and *Enterobacter* spp. (9, 18). Against *A. baumannii* isolates, colistin susceptibility was >93% overall in each global region examined from 2005 to 2016, although resistance rates did increase over this period, particularly in Europe (9, 19). Importantly, colistin resistance rates are also typically higher against carbapenem-resistant *A. baumannii* isolate subsets than against randomly selected isolate sets. In contrast to the *Enterobacterales* and *A. baumannii* findings, *P. aeruginosa* susceptibility rates for colistin have remained >99% from 2005 to the present (9, 20).

In summary, we have shown that MRX-8, which is a lipopeptide designed to abrogate the cytotoxic properties common to other polymyxins, displayed *in vitro* antimicrobial activity against *Enterobacterales*, *A. baumannii*, and *P. aeruginosa* clinical isolates that was identical or nearly identical to the activities of colistin and polymyxin B.

MATERIALS AND METHODS

Bacterial isolates. A total of 1,314 nonduplicate, Gram-negative clinical isolates from the SENTRY Antimicrobial Surveillance Program (21) were randomly selected from 77 medical centers located in all 9 U.S. Census Bureau divisions in 2017 to 2020 (see Table S7 in the supplemental material). The isolate sets were composed of various *Enterobacterales* species (including some species intrinsically resistant to polymyxins), *Acinetobacter baumannii-calcoaceticus* species complex (referred to as *A. baumannii*), and *Pseudomonas aeruginosa*. All organisms were isolated from various documented infection types (see Table S8), and only 1 isolate per patient infection episode was included in the surveillance collection. The number of isolates tested per species was arbitrary and should not be interpreted as representing the clinical prevalence of the species for these infection types. Species identification was performed at the participating medical centers and confirmed at the monitoring laboratory (JMI Laboratories, North Liberty, IA, USA) using standard microbiological methods and matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker, Billerica, MA, USA).

Isolates were categorized as MDR if they were nonsusceptible to at least one antimicrobial from ≥3 drug classes using Clinical and Laboratory Standards Institute (CLSI) breakpoints (10). For the *A. baumannii* isolate set, the antimicrobial classes were extended-spectrum cephalosporins (ceftriaxone, ceftazidime, or cefepime), carbapenems (meropenem, doripenem, or imipenem), piperacillin-tazobactam (fixed at 4 mg/L), quinolones (ciprofloxacin or levofloxacin), aminoglycosides (gentamicin, tobramycin, or amikacin), polymyxins (colistin or polymyxin B), tetracyclines (tetracycline, doxycycline, or minocycline), and ampicillin-sulbactam (2:1). For the *P. aeruginosa* isolate set, the antimicrobial classes were cephalosporins (ceftazidime or cefepime), carbapenems (meropenem, doripenem, or imipenem), piperacillin-tazobactam (fixed at 4 mg/L), quinolones (ciprofloxacin or levofloxacin), aminoglycosides (gentamicin, tobramycin, or amikacin), and polymyxins (colistin or polymyxin B). For the set of *Enterobacterales* isolates that are not intrinsically resistant to colistin (*E. coli*, *Enterobacter* spp., *Citrobacter* spp., and *Klebsiella* spp.), the antimicrobial classes were extended-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime, or cefepime), carbapenems (meropenem, doripenem, or imipenem), piperacillin-tazobactam (fixed at 4 mg/L), quinolones (ciprofloxacin or levofloxacin), aminoglycosides (gentamicin, tobramycin, or amikacin), polymyxins (colistin or polymyxin B), and tigecycline. The MIC data for MDR categorizations were obtained from the SENTRY Antimicrobial Surveillance Program (21). A subset of MIC data for comparator antimicrobials is displayed in Table 2 and Tables S3 to S6. We also tested the activity of MRX-8 and comparators against a rare colistin-resistant *P. aeruginosa* isolate (JMI 991784) collected in 2017.

Susceptibility testing methods. MRX-8 powder was supplied by MicuRx Pharmaceuticals. Colistin (catalog no. C4461) and polymyxin B (catalog no. 1547007) were obtained from Sigma-Aldrich and United States Pharmacopeia, respectively. MRX-8, colistin, and polymyxin stocks were made in water.

Susceptibility to MRX-8 and comparator agents was measured using current CLSI methods (10, 22). The test medium was cation-adjusted Mueller-Hinton broth. Non-tissue-culture-treated polystyrene

plates were used. CLSI and EUCAST interpretive criteria were applied according to current guidelines (10, 23). U.S. FDA product package insert interpretive criteria were used for tigecycline (24).

The current EUCAST interpretive criteria for colistin against *Enterobacterales*, *P. aeruginosa*, and *A. baumannii* isolates are ≤2 mg/L for susceptible and ≥4 mg/L for resistant (23). In contrast, CLSI categorizes an *Enterobacterales*, *P. aeruginosa*, or *A. baumannii* isolate as resistant if it exhibits a colistin or polymyxin MIC value of ≥4 mg/L (10). CLSI does not currently recognize a susceptible category for colistin or polymyxin B against any of these organism groups or species. Rather, isolates from these groups and species that display a colistin or polymyxin B MIC value of ≤2 mg/L are categorized as intermediate (10).

JMI Laboratories followed current CLSI quality assurance practices when performing the susceptibility tests. MIC values were validated by concurrently testing CLSI- and EUCAST-recommended (10, 25) ATCC or National Collection of Type Cultures (NCTC) quality control (QC) reference strains. The QC strains included *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC BAA 1705, *K. pneumoniae* ATCC 700603, and *E. coli* NCTC 13846. QC ranges for the tested reference strains were the criteria published by CLSI or EUCAST (10, 25). The inoculum density during susceptibility testing was monitored by bacterial colony counts.

Data availability. Data will be made available upon reasonable request.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

We thank Amy Chen and Judy Oberholser for their help during the preparation of the manuscript.

This study was performed by JMI Laboratories and supported by MicuRx Pharmaceuticals Inc., which included funding for services related to preparing the manuscript. This study was partially funded by CARB-X.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Health and Human Services Office of the Assistant Secretary for Preparedness and Response.

JMI Laboratories contracted to perform services in 2021 for AbbVie Inc., Affinity Biosensors, AimMax Therapeutics, Inc., Alterity Therapeutics, Amicrobe, Inc., Arietis Pharma, Armata Pharmaceuticals, Inc., Astrellas Pharma Inc., Basilea Pharmaceutica AG, Becton, Dickinson and Company, bioMérieux, Inc., Boost Biomes, Brass Dome Ventures Ltd., Bravos Biosciences, Bugworks Research Inc., Centers for Disease Control and Prevention, Cerba Research, Cidara Therapeutics, Cipla Ltd., ContraFect Corp., CXC7, Diamond V, Enveda Biosciences, Fedora Pharmaceuticals, Inc., Fimbrion Therapeutics, First Light Diagnostics, Forge Therapeutics, Inc., Fox Chase Cancer Center, GlaxoSmithKline plc, Harvard University, Institute for Clinical Pharmacodynamics, International Health Management Associates, Inc., Iterum Therapeutics plc, Janssen Research & Development, Johnson & Johnson, Kaleido Biosciences, Inc., Laboratory Specialists, Inc., Meiji Seika Pharma Co., Ltd., Melinta Therapeutics, Menarini Group, Merck & Co., Inc., MicuRx Pharmaceuticals Inc., Mutabilis, Nabriva Therapeutics, National Institutes of Health, Novome Biotechnologies, Omnix Medical Ltd., Paratek Pharma, Pattern Bioscience, Pfizer Inc., Prokaryotics Inc., Pulmocide Ltd., QPEX Biopharma, Inc., Roche Holding AG, Roivant Sciences, SeLux Diagnostics, Inc., Shionogi Inc., Sinovent Pharmaceuticals, Inc., SNIPR Biome ApS, Spero Therapeutics, Summit Therapeutics, Inc., T2 Biosystems, TenNor Therapeutics, Thermo Fisher Scientific, University of Southern California, University of Wisconsin, USCAST, U.S. Food and Drug Administration, Venatorx Pharmaceutics, Inc., Weill Cornell Medicine, and Wockhardt Ltd.

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