

The novel polymyxin analogue SPR206 exhibits higher activity than colistin against both colistin-susceptible and colistin-resistant strains of *Acinetobacter baumannii*

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ABSTRACT Colistin resistance is increasing globally and complicates treatments of *A. baumannii* infections. The next-generation polymyxin SPR206 shows potent activity against multidrug-resistant Gram-negative pathogens with low toxicity. SPR206 exhibited higher activity against colistin-susceptible and colistin-resistant strains (MIC₅₀/MIC₉₀ = 0.12/0.25 mg/L), and resistance was only detected in 1/118 strains (MIC ≥4 mg/L). Mutations in *pmrCAB*, linked to colistin resistance, do not seem to confer resistance to the novel polymyxin, which retains a high level of activity.

KEYWORDS antimicrobial resistance, colistin, SPR206, *Acinetobacter baumannii*

Acinetobacter baumannii is a nosocomial pathogen associated with a wide range of infections, particularly ventilator-associated pneumonia and bacteremia. Carbapenem-resistant *A. baumannii* is renowned for its ability to acquire antimicrobial resistance (1), and it was therefore classified by the World Health Organization (WHO) as a critical priority pathogen for new drug development in 2024 (2). Colistin (polymyxin E) is considered a “last-resort” drug for treating *A. baumannii* infections; however, despite its potent antimicrobial activity, its use is associated with significant drawbacks, including high nephrotoxicity, neurotoxicity, and challenging PK/PD properties (3). Furthermore, the increasing incidence of colistin resistance in recent years highlights the urgent need for new antimicrobials to combat *A. baumannii* infections (4).

In response to this situation, several new polymyxin analogs with promising activity have been developed. Among these, SPR206 (also known as EVER206) exhibits potent activity against *Enterobacteriales*, *Pseudomonas aeruginosa*, and *A. baumannii*, as well as significantly lower toxicity (5–7). SPR206, which shows structural similarities to colistin, has a diaminopropionate residue adjacent to the cyclic core (Fig. 1) and is designed to maintain potent polymyxin activity by destabilizing the bacterial outer membrane, ultimately resulting in cell lysis. The optimized structure enhances the compound's selectivity for the bacterial membrane, reducing interactions with human cell membranes (5). The safety and pharmacokinetics of this next-generation polymyxin in humans have been evaluated in several Phase 1 clinical trials, with promising results. FDA approval has recently been given for Phase 2 trials to be undertaken to evaluate the efficacy of the compound against carbapenem-resistant *A. baumannii* and *P. aeruginosa* infections (8). Although SPR206 has demonstrated high activity against *A. baumannii*, including carbapenem-resistant strains (7), its activity against colistin-resistant strains has scarcely been studied. The activity of SPR206 is not well understood, and the extent to which it is affected by resistance mechanisms that limit other classical polymyxins, such as colistin, is not clear. Further evaluation of the activity of SPR206 by using large, fully

Editor Laurent Poirer, University of Fribourg, Fribourg, Switzerland

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The authors declare no conflict of interest.

See the funding table on p. 7.

Received 26 December 2024

Accepted 16 April 2025

Published 20 May 2025

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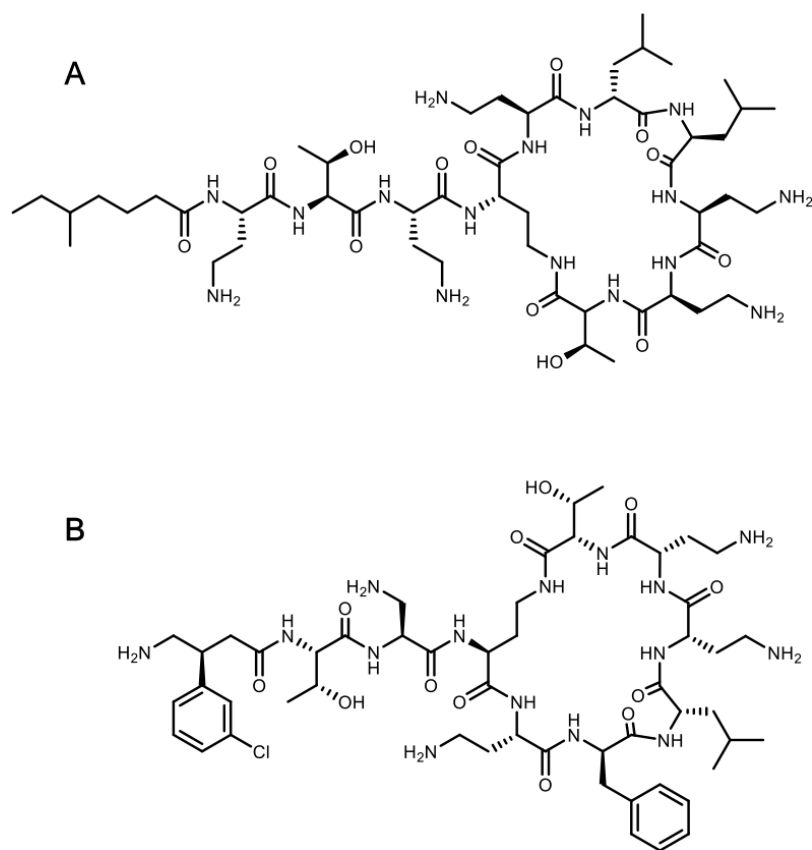


FIG 1 Chemical structures of colistin (A) and SPR206 (B).

sequenced multicenter collections is essential to comprehensively define its therapeutic range and limitations. Such studies will also provide valuable insights to inform strategies aimed at mitigating the emergence and spread of antimicrobial resistance and guiding the clinical use of the compound for difficult-to-treat pathogens.

In this study, the activity of SPR206 and colistin was tested against *A. baumannii* strains with different and well-characterized colistin resistance mechanisms, including lipid A modifications (e.g., *pmrB*) and the less frequent deficits in LPS biosynthesis. Three isogenic pairs of colistin-susceptible and colistin-resistant clinical isolates (ABRIM/ABRIM*pmrB*, GR.AB248/GR.AB249*pmrB*, and GR.AB299/GR.AB347*pmrB*) were included, with the last two pairs collected consecutively from two intensive care unit patients, pre- and post- colistin treatment (9). The *A. baumannii* type strain ATCC 19606 was also included, along with its isogenic colistin-resistant mutants: ATCC 19606*pmrB*, ATCC 19606Δ*pxA*, ATCC 19606Δ*pxC*, and ATCC 19606Δ*pxD* (10). The molecular mechanisms of colistin resistance of these strains have been extensively characterized in previous studies (Table 1). A total of 118 sequenced clinical strains of *A. baumannii*, isolated in a Spanish national multicenter study, conducted in 2020 and involving more than 20 hospitals (4), were also included. In addition, 12 colistin-resistant *A. baumannii* isolates, 4 from the Institute for Global Health (Barcelona, Spain), and 8 from the previous Spanish National *A. baumannii* 2010 Study were tested against both polymyxins.

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of colistin and SPR206 were determined using broth microdilution, following CLSI criteria (14), and MIC₅₀/MIC₉₀ values and resistance rates were calculated. Time–kill curve analyses were conducted using colistin and SPR206 at concentrations of 0.5×, 1×, and 2× MIC with two pairs of isogenic clinical isolates: GR.AB299/GR.AB347*pmrB* and GR.AB248/GR.AB249*pmrB*. *A. baumannii* cultures were grown overnight, and 3 × 10⁵ CFUs/mL were used to inoculate 150 μL of Mueller-Hinton II broth in 96-well

plates. Growth was monitored for 48 h using an Epoch 2 Microplate Spectrophotometer (BioTek), and bacterial counts (CFU/mL) were determined after 0, 2, 4, 8, 24, and 48 h, by plating the strains onto Mueller-Hinton agar plates.

Comparative WGS analysis was performed for colistin-resistant and phylogenetically related susceptible clinical isolates using Panaroo (15) for presence/absence analysis, Snippy (16) for SNP detection, and manual curation for IS*Aba* elements identification. Previously described resistance mechanisms and membrane-related elements were systematically analyzed as detailed in Table S1. Core genome multilocus sequence typing (cgMLST) was performed using ChewBBACA with the public *A. baumannii* schema at a 95% presence threshold. The allelic profiles were used to construct a Neighbor-Joining tree with GrapeTree based on allelic differences. The resulting Newick tree was visualized using the *ggtree* package in R.

SPR206 exhibited higher *in vitro* activity than colistin against the full panel of colistin-susceptible strains, with lower MICs (8- to 16-fold lower). Notably, SPR206 also demonstrated significantly higher activity against colistin-resistant strains due to *pmrB* mutations, yielding lower MICs (16- to 32-fold lower). However, SPR206 did not improve colistin activity against the strains resistant to this antibiotic due to alterations in the *lpx* genes (MICs ≥ 256 mg/L for both antibiotics against all strains), likely reflecting the absence of lipid A in these mutants, which is the target structure of polymyxins (Table 1).

The MIC₅₀/MIC₉₀ values were significantly lower for the novel polymyxin than for colistin (0.12/0.25 mg/L and 1/4 mg/L, respectively) against the entire set of 118 isolates (Table 2). Applying the colistin resistance breakpoints recommended by CLSI and EUCAST (MIC ≥ 4 mg/L) to both antibiotics, the *A. baumannii* collection exhibited 11.9% resistance to colistin (14 strains with MICs ≥ 4 mg/L), but only 0.8% resistance to SPR206 (1 strain, MIC = 8 mg/L). The phylogenetic tree based on cgMLST analysis of the 14 colistin-resistant strains showed that they belonged to diverse sequence types and genetic lineages, supporting their epidemiological unrelatedness (Fig. S1).

The robust activity of SPR206 against colistin-susceptible isolates of *A. baumannii* has previously been described, with SPR206 and colistin MIC₅₀/MIC₉₀ values of 0.06/0.12 mg/L and 0.25/0.25 mg/L, respectively, in a small collection of 20 isolates (7). Focusing on the comparative analysis of the activity of SPR206 and colistin against the colistin-susceptible strains here (104/118) (Table 2), we obtained MIC₅₀/MIC₉₀ values of 0.12/0.25 mg/L and 1/1 mg/L for SPR206 and colistin, respectively. Therefore, both

TABLE 1 Description of *A. baumannii* strains and MICs of colistin and SPR206 (mg/L)

<i>A. baumannii</i> strain	Colistin	SPR206	Description	Source or reference
ATCC 17978	0.5	0.03	<i>A. baumannii</i> reference strain	ATCC
ABRIM	0.5	0.06	<i>A. baumannii</i> clinical strain	(11)
ABRIM <i>pmrB</i>	32	1	Isogenic derivative mutant of ABRIM; single amino acid substitution (Asn353Tyr) in PmrB	(12)
GR.AB299	0.5	0.03	<i>A. baumannii</i> clinical strain	(9)
GR.AB347 <i>pmrB</i>	64	2	Isogenic clinical strain derivative of GR.AB299; single amino acid substitution (Pro170Leu) in PmrB	(9)
GR.AB248	0.5	0.03	<i>A. baumannii</i> clinical strain	(9)
GR.AB249 <i>pmrB</i>	256	4	Isogenic clinical strain derivative of GR.AB248; single amino acid substitution (Pro233Ser) in PmrB	(9)
ATCC 19606	0.5	0.06	<i>A. baumannii</i> type strain	ATCC
ATCC 19606 <i>pmrB</i>	32	2	Isogenic derivative mutant of ATCC 19606; single amino acid substitution (Ala227Val) in PmrB	(12)
ATCC 19606 Δ <i>lpxA</i>	256	≥ 256	Isogenic derivative mutant of ATCC 19606; 445 bp deletion at nucleotide 364 within the <i>lpxA</i> gene and frameshift after His121	(13)
ATCC 19606 Δ <i>lpxC</i>	1024	≥ 256	Isogenic derivative mutant of ATCC 19606; 84 bp deletion at nucleotide 858 within the <i>lpxC</i> gene and frameshift after Thr285	(13)
ATCC 19606 Δ <i>lpxD</i>	1024	≥ 256	Isogenic derivative mutant of ATCC 19606; single-base deletion at nucleotide 952 of the <i>lpxD</i> gene and frameshift after Lys317	(13)

TABLE 2 Cumulative distribution of the minimum inhibitory concentration (MIC) and percentage of resistance (MIC ≥ 4 mg/L) to colistin and SPR206 relative to the set of *A. baumannii* strains isolated in a national multicenter study^b

Antimicrobial	Cumulative % of strains at MIC in mg/L (num. of total strains) ^c											% of resistance	
	≤ 0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32		≥ 64
All sets of strains (N = 118)													
Colistin			0.9 (1) ^d	5.9 (7)	33.9 (40)	83.9 (99)	88.1 (104)	94.1 (111)	99.2 (117)	99.2 (117)	99.2 (117)	100 (118)	11.9
SPR206	9.3 (11) ^d	32.2 (38)	<u>72.8</u> (86)	97.5 (115)	99.2 (117)	99.2 (117)	99.2 (117)	99.2 (117)	100 (118)				0.8
Colistin-susceptible strains (N = 104)													
Colistin			1.0 (1)	6.7 (7)	38.5 (40)	95.2 (99)	100 (104)						0
SPR206	9.6 (10) ^d	34.6 (36)	<u>76.0</u> (79)	98.1 (102)	100 (104)								0
Colistin-resistant strains (N = 14)													
Colistin								50.0 (7)	92.9 (13)	92.9 (13)	92.9 (13)	100 (14)	100
SPR206	7.1 (1) ^d	14.3 (2)	<u>50</u> (7)	92.9 (13)	92.9 (13)	92.9 (13)	92.9 (13)	92.9 (13)	100 (14)				7.1

^aActual MIC value is equal to or inferior to that indicated in the table.

^bMIC₅₀ underlined, MIC₉₀ in bold.

^cEmpty cells indicate that the MIC was not evaluated at that specific concentration for that group of strains.

studies revealed that SPR206 was more active than colistin against colistin-susceptible strains, with MIC₅₀/MIC₉₀ values 4- to 8-fold lower. Higher activity of SPR206 was also observed in another recent study using a collection of 30 clinical strains of *A. baumannii*, including 17 colistin-resistant strains (17). Considering only the 17 colistin-resistant strains included in that study, MIC₅₀/MIC₉₀ values of 1/16 mg/L for SPR206 and 16/256 mg/L for colistin were obtained. In the present study, analysis of the 14 colistin-resistant isolates yielded MIC₅₀/MIC₉₀ values of 0.12/0.25 mg/L for SPR206 and 4/8 mg/L for colistin (Table 2). Thus, both studies demonstrated the excellent activity of SPR206 against colistin-resistant strains (MIC values 16- to 32-fold lower). The differences in the MIC values obtained in both studies are probably due to the genetic diversity and the underlying mechanisms of colistin resistance of the strains included in the collections. Interestingly, the isolate with the highest resistance to colistin in our study (MIC \geq 64 mg/L) was also the isolate with the highest SPR206 MIC (8 mg/L).

In order to confirm the potent activity of SPR206 against colistin-resistant *A. baumannii* strains, the MIC of 12 additional isolates from two other different collections was also evaluated. This pool of 12 colistin-resistant *A. baumannii* strains showed MIC₅₀/MIC₉₀ values for colistin of 16/32 mg/L and for SPR206 of 0.12/1 mg/L (Table S3), concordant with the MICs obtained for the strains above. Thus, when combined with the 14 strains from the Spanish National 2020 Study collection (total 26 strains), the resulting MIC₅₀/MIC₉₀ values were 8/32 mg/L for colistin and 0.12/1 mg/L for SPR206, confirming the higher potency of SPR206 against resistant isolates (Table S4).

Later, the MBC, defined as the lowest drug concentration that reduces the initial bacterial inoculum by \geq 99.9% (18), was assessed for colistin-resistant *A. baumannii* isolates carrying *pmrAB*-dependent resistance mechanisms, as well as their colistin-susceptible parental strains. MBC determinations matched MIC values for all strains (displayed in Table 1), confirming a potent bactericidal activity of SPR206, similar to that previously described for colistin (19).

In order to evaluate the stability of this new polymyxin, time-kill assays were performed using two isogenic clinical pairs of colistin-susceptible and colistin-resistant *A. baumannii*. As shown in Fig. S2, SPR206 exhibits sustained antimicrobial activity over a 48-hour period when used at concentrations close to the MIC (0.5 \times , 1 \times , and 2 \times MIC), similar to that shown by colistin. Of note, however, SPR206 was administered at absolute concentrations significantly lower than those used for colistin (16- to 64-fold lower).

WGS analysis of the colistin resistance mechanisms in the 14 colistin-resistant strains included in the 2020 collection revealed that 4 strains had mutations in the *pmrABC* genes (Table S2). Mutations in *pmrABC* genes have previously been associated with the addition of phosphoethanolamine residues to lipid A, which reduces the LPS charge and increases the resistance to colistin (12, 20). Analysis of the isolate that yielded the highest colistin and SPR206 MICs (isolate AB171) revealed a nonfunctional Tim44 lipid-binding transport protein (frameshift Lys146fs), along with a \sim 10 kb deletion encompassing transcriptional regulators (*irp* and *acrR*), efflux transporters (*rhtA* and *araJ*), and an acyltransferase (annotated as lauroyl acyltransferase/LpxL by homology with *Pseudomonas* sp.). Given the size of the deletion and the diverse functions of the affected genes, multiple factors in this region may be contributing to the observed high-level colistin resistance (MIC \geq 64 mg/L). Notably, even this highly resistant isolate with significant membrane-related alterations had moderately elevated SPR206 MIC to 8 mg/L, thus highlighting the increased stability of SPR206 against these colistin resistance mechanisms.

The underlying mechanism of resistance in the remaining nine colistin-resistant strains remains unclear. Other genes, such as *mcr* (PEtN transferase), *basRS* (two-component signal transduction system), outer membrane proteins, and others involved in membrane modifications, have been implicated in colistin resistance in Gram-negative pathogens (20, 21) (Table S1); however, no *mcr* genes or changes in the coding sequences of these proteins or in the promoter regions were identified.

These results, together with those obtained with the isogenic colistin-resistant mutants, suggest that SPR206 retains substantial activity against strains harboring *pmrB* mutations, despite the associated high-level colistin resistance. However, further detailed studies are required to elucidate the underlying causes of reduced susceptibility to SPR206 in *A. baumannii*. Similarly, we observed, for the first time, that the complete loss of LPS caused by mutations in the *lpx* genes confers high resistance to both colistin and SPR206. The total loss of LPS due to alterations in *lpx* genes is rarely identified in clinical isolates, probably due to the high fitness cost, thus suggesting that the emergence of *lpx*-mediated resistance is expected to scarcely occur (22).

In conclusion, our study findings demonstrate the improved activity of SPR206 against both colistin-susceptible and colistin-resistant strains of *A. baumannii*. In addition, high activity against both clinical strains and isogenic strains with specific mutations in *pmrCAB* that confer colistin resistance was observed. However, mutations in *lpx* genes, which are infrequent among colistin-resistant strains of clinical origin, compromised the activity of SPR206. Due to its limited toxicity and its ability to overcome colistin resistance, SPR206 could potentially be considered a therapeutic option for treating infections caused by *A. baumannii*, particularly in cases where current treatments are limited due to resistance or toxicity.

ACKNOWLEDGMENTS

This research is part of the Spanish National Study *Acinetobacter* spp. 2020 and was made possible by the helpful collaboration of the following researchers: Javier Colomina, David Navarro, Mercè Garcia, Felipe Fernández-Cuenca, Álvaro Pascual, Matxalen Vidal, Helena Gil, Ana Madueño, Ángel Rodríguez-Villodres, Jerónimo Pachón, Javier Aznar, Irene García-Ahufinger, Luis Martínez-Martínez, Frederic Gómez, Genoveva Yagüe, Javier Fernández, Andrés Canut, Marina Fernández, Dolores Quesada, Lourdes Matas, Jorge Calvo, Fátima Galán-Sánchez, Manuel Rodríguez-Iglesias, David Velasco, Begoña Fernández, Xavier Mulet, Antonio Oliver, and Ana Fernández.

We thank J.D. Boyce for the kind gift of the *A. baumannii* ATCC 19606Δ*lpxA* (AL1851), ATCC 19606Δ*lpxC* (AL1842), and ATCC 19606Δ*lpxD* (AL1852) strains, and S. Pournaras for providing the *A. baumannii* GR.AB248, GR.AB249*pmrB*, GR.AB299, and GR.AB347*pmrB* strains. We are also grateful to J. Vila for the kind gift of the eight colistin-resistant *A. baumannii* clinical isolates from the Institute for Global Health (Barcelona, Spain). We would also like to thank the Fundación Pública Galega de Investigación Biomédica INIBIC for their support.

This research was supported by Projects PI20/01212 and PI23/00851 awarded to A.B., Project PI21/00704 awarded to G.B., and Project PI22/01212 awarded to J.A.-S., all funded by Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union. The research was also supported by CIBER-Consortio Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III (CB21/13/00055), Ministerio de Ciencia e Innovación, and Unión Europea—NextGenerationEU, and by project IN607D 2021/12 awarded to A.B., IN607A 2020/05 to G.B., and IN607D 2024/008 to J.A.-S. (GAIN-Agencia Gallega de Innovación). Consellería de Economía, Emprego e Industria). J.A.-S. was financially supported by the Juan Rodés program (ISCIII, JR21/00026). J.C.V.-U. was financially supported by IN606B-2022/009 (Xunta de Galicia). P.G.-S. was financially supported by IN606A 2021/021 Grant (Xunta de Galicia). M.O.-G. was financially supported by IN606A 2023/023. I.A.-G. was financially supported by the Juan Rodés (ISCIII, JR23/00036). A.B.-B. was financially supported by IN606A-2024/033.

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FUNDING

Funder	Grant(s)	Author(s)
MEC Instituto de Salud Carlos III (ISCIII)	PI20/01212, PI23/00851	Alejandro Beceiro
MEC Instituto de Salud Carlos III (ISCIII)	JR23/00036	Isaac Alonso-García
Axencia Galega de Innovación	IN606A 2024/033	Gabriela Alejandra Báez-Barroso
MEC Instituto de Salud Carlos III (ISCIII)	PI21/00704,CB21/13/00055	German Bou
MEC Instituto de Salud Carlos III (ISCIII)	PI22/01212, JR21/00026	Jorge Arca-Suárez
Axencia Galega de Innovación	IN606A 2021/021	Paula Guijarro-Sánchez
Axencia Galega de Innovación	IN606A 20223/023	Michelle Outeda-García
Axencia Galega de Innovación	IN607D 2021/12	Alejandro Beceiro
Axencia Galega de Innovación	IN607A 2020/05	German Bou
Axencia Galega de Innovación	IN607D 2024/008	Jorge Arca-Suárez
Axencia Galega de Innovación	IN606B 2022/009	Juan C. Vázquez-Ucha

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DATA AVAILABILITY

The data supporting this work are available under NCBI bioproject [PRJNA991768](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA991768).

ADDITIONAL FILES

The following material is available [online](#).

Supplemental Material

Supplemental material (AAC01940-24-s0001.docx). Tables S1 to S4; Fig. S1 and S2.

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