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SUSCEPTIBILITY

In Vitro Susceptibility Testing of a Novel Benzimidazole, SPR719, against Nontuberculous Mycobacteria

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ABSTRACT Nontuberculous mycobacterium (NTM) infections are increasing globally. The *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* are the most frequently encountered NTM among clinical laboratories, and treatment options are extremely limited. In this study, the *in vitro* potency of a novel benzimidazole, SPR719, the microbiologically active form of the orally available prodrug SPR720, was tested against several species of NTM. MICs were determined for 161 isolates of NTM of 13 taxa (seven species, three subspecies, and three groups/complexes) in cation-adjusted Mueller-Hinton Broth, as described and recommended by the Clinical and Laboratory Standards Institute (CLSI M24-A2). Comparator antimicrobials included amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, minocycline, moxifloxacin, tigecycline, and trimethoprim-sulfamethoxazole (TMP-SMX) for the rapidly growing mycobacteria (RGM), amikacin and clarithromycin for the MAC, and amikacin, ciprofloxacin, clarithromycin, doxycycline, linezolid, moxifloxacin, rifabutin, rifampin, and TMP-SMX for the other slowly growing NTM. SPR719 was found to be potent against multiple clinical strains of NTM with an MIC_{50} range of 0.25 to 4 μg/ml for several species of NTM. These findings support the further advancement of SPR720 for the treatment of NTM disease.

KEYWORDS benzimidazole, nontuberculous mycobacteria, SPR719

Nontuberculous mycobacterium (NTM) infections are increasing globally. These organisms, once thought to be merely environmental species, are responsible for a wide range of infections, including respiratory, cutaneous, and disseminated disease, highlighting the importance for expanding the antimicrobial armamentarium (1).

SPR719, formerly VXc-486, is a novel aminobenzimidazole which has been shown to inhibit the ATPase activity of gyrase in *Mycobacterium tuberculosis* (2). Initial studies have shown that this antimicrobial inhibits both drug-susceptible *Mycobacterium tuberculosis* and multidrug-resistant *M. tuberculosis* with *in vitro* MICs of 0.03 to 0.3 μg/ml and 0.08 to 5.48 μg/ml, respectively (2). Additional *in vitro* studies revealed that multiple isolates of *Mycobacterium abscessus*, *Mycobacterium avium* complex (MAC), and *Mycobacterium kansasii* were also inhibited, with MICs of 0.1 to 2 μg/ml (2).

(A portion of this study was presented at ASM Microbe 2018, Atlanta, GA, 6 to 11 June, 2018 [3].)

RESULTS

Of the total 161 NTM isolates tested, SPR719 MICs were 0.02 to 8 μg/ml. For all rapidly growing mycobacteria (RGM) ($n = 93$), the range of MIC_{50} values was 0.06 to 4 μg/ml, with the lowest MICs seen with the *M. mucogenicum* group (MIC_{50}, 0.06 μg/ml) and the *M. fortuitum* group (MIC_{50}, 0.25 μg/ml). The highest MIC_{50} values were seen with the isolates of *M. chelonae* and *M. immunogenum* (4 μg/ml). The largest group of RGM was the *M. abscessus* complex ($n = 53$). The MIC_{50} value for all three subspecies, including subspecies *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* and the hybrid subspecies, was 2 μg/ml (Table 1).

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Among the slowly growing mycobacteria (SGM) ($n = 68$), the range of MIC_{50} values was 0.002 to 4 μg/ml. The lowest MICs were seen with the isolates of *M. kansasii* (0.002 to 0.015 μg/ml); the highest MIC_{50} values were seen with isolates of *M. simiae* and the *Mycobacterium avium* complex X (MAC-X) (each species, 1 μg/ml). Among the 41 isolates of the MAC, SPR719 MIC_{90} values were $\leq$2 μg/ml, and the MIC_{50} values were $\leq$1 μg/ml (Table 1).

Interestingly, isolates intrinsically resistant to ciprofloxacin and moxifloxacin (e.g., those of *M. abscessus* complex) had an MIC_{50} value of 2 μg/ml for SPR719, in contrast to the MIC_{50} value of $\geq$4 μg/ml for both ciprofloxacin and moxifloxacin.

Most SPR719 MIC_{50} values were equivalent to or at least 1 dilution less than the MIC_{50} values for ciprofloxacin and moxifloxacin. All other MICs for the comparator agent were within expected ranges for the species/subspecies tested (Table 1).

Quality control. The manufacturer's acceptable range of MICs for *Staphylococcus aureus* ATCC 29213 with SPR719 was $\leq$0.015 to 0.12 μg/ml, but it was based on a limited number of runs ($n = 5$). All 43 replicates of *S. aureus* ATCC 29213 tested had an SPR719 MIC within the acceptable range (Table 2). Each quality control for the comparator agents was within the CLSI acceptable range for *M. peregrinum* ATCC 700686, *M. marinum* ATCC 927, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 2912.

Seventeen replicates of *M. peregrinum* ATCC 700686 had an MIC range for SPR719 of 0.12 to 0.5 μg/ml (mode, 0.25 μg/ml), while 23 replicates of *M. marinum* ATCC 927 had an MIC range of 0.06 to 0.5 μg/ml (mode, 0.06 μg/ml) (experiments were performed weekly). The additional bacterial reference strains *E. coli* ATCC 25922 (16 replicates) and *E. faecalis* ATCC 29212 (11 replicates) had an SPR719 MIC range of 0.5 to 1 μg/ml (modes, 0.5 μg/ml and 0.015 μg/ml, respectively) (Table 2).

DISCUSSION

SPR719 (previously VXc-486) is a novel aminobenzimidazole, which is a class of gyrase inhibitors that target the ATPase subunits, resulting in growth inhibition of *M. tuberculosis* (2). This compound was previously reported to be active against *M. kansasii* and *M. tuberculosis* in a murine model (2).

Importantly, no cross-resistance to the fluoroquinolone class of gyrase inhibitors, including moxifloxacin, has been reported, even in fluoroquinolone-resistant isolates (e.g., from extremely drug-resistant [XDR] *M. tuberculosis*, which is resistant to fluoroquinolones). The compound is slowly bactericidal for *M. tuberculosis*, achieving a $\geq$3-fold reduction in CFU (MIC, 0.06 μg/ml) after 14 days, and slow killing of *M. tuberculosis* against actively growing cells of *M. tuberculosis* strain Erdman (2). Additionally, SPR719 has shown activity against drug-susceptible strains of *M. tuberculosis* in an *in vitro* model of quiescent mycobacterial survival and was more active than moxifloxacin and gatifloxacin (2).

SPR719 also inhibits the growth of strain H37Ra cultured in human and mouse macrophage-like cell lines. Docking studies using the published crystal structure of the *M. tuberculosis* gyrase B were used to produce a three-dimensional model of SPR719 interaction or binding. The predicted binding was consistent with experimental studies reported for closely related compounds in complex with the gyrase B enzyme from *Staphylococcus aureus*, suggesting that the compound binds to gyrase B by altering the hydrogen bonding network and destabilizing the catalytic water present in the active sites (2).

Locher and colleagues also showed the potency of the compound against a small number of *M. abscessus*, *M. avium*, *M. kansasii*, and *Nocardia* spp. (2). The investigators reported the compound's bactericidal activity against *M. kansasii*.

The SPR719 MIC_{50} values of our clinical isolates of NTM in the current study were comparable to those from the preliminary studies by Locher et al. (2). However, the SPR719 MIC_{50} for all *M. abscessus* complex isolates in this study ($n = 53$) was 2 μg/ml compared with 1 μg/ml in the Locher et al. study ($n = 22$; the Locher et al. study did not differentiate subspecies of the *M. abscessus* complex). In this study, 33 isolates were *M. abscessus* subsp. *abscessus* and 20 isolates were *M. abscessus* subsp. *massiliense* or *M. abscessus* subsp. *abscessus*/*M. abscessus* subsp. *massiliense* hybrid [i.e., *M. abscessus*

TABLE 1 MIC ranges, MIC_{50} values, and MIC_{90} values of 161 isolates of 13 taxa of the nontuberculous mycobacteria against antimicrobials[a]

Antimicrobial[b]	*M. abscessus* subsp. *abscessus*	*M. abscessus* subsp. *massiliense*	*M. abscessus/massiliense* hybrid	*M. chelonae*	*M. immunogenum*	*M. fortuitum* group	*M. mucogenicum* group	*M. kansasii*	*M. marinum*	*M. simiae*	*M. avium*	*M. intracellulare*	MAC-X
No. of isolates	33	10	10	10	10	10	10	8	9	10	12	19	10
SPR719													
MIC range	0.25 to 8	0.12 to 4	0.06 to 2	2 to 4	4 to 8	0.06 to 1	0.015 to 0.25	0.002 to 0.03	0.12 to 1	0.5 to 4	0.5 to 2	0.12 to 2	0.12 to 1
MIC_{50}	2	2	2	4	4	0.25	0.06	0.015	0.5	1	0.5	0.5	1
MIC_{90}	4	2	2	4	8	1	0.25	0.03	0.5	2	2	2	1
AMK													
MIC range	2 to 64	4 to 16	8 to 16	16 to 32	8 to 16	≤1	≤1	2 to 8	≤1 to 2	8 to 32	8 to >64	8 to >64	8 to 32
MIC_{50}	8	16	16	16	8	≤1	≤1	4	≤1	16	16	16	8
MIC_{90}	16	16	16	32	16	≤1	≤1	8	2	16	64	>64	32
FOX													
MIC range	16 to 64	32 to 64	32 to 64	>128	>128	32 to 64	≤4 to 16						
MIC_{50}	64	64	32	>128	>128	64	8						
MIC_{90}	64	64	64	>128	>128	64	16						
SXT													
MIC range	4/76 to 8/152	4/76 to 8/152	4/76 to 8/152	≤2/38 to >4/76	2/38 to 8/152	≤0.25/4.75 to 1/19	≤0.25/4.75 to 0.5/9.5	≤0.12 to 0.25/4.75	≤0.25/4.75 to 1/19	1/19 to 4/76			
MIC_{50}	4/76	4/76	4/76	≥4/76	4/76	0.5/9.5	≤0.25/4.75	0.25/4.75	0.5/9.5	2/38			
MIC_{90}	8/152	8/152	8/152	≥4/76	8/152	1/19	0.5/9.5	0.25/4.75	1/19	4/76			
LZD													
MIC range	2 to 32	2 to 16	2 to 16	2 to 16	4 to 32	≤1 to 8	≤1 to 4	≤1 to 4	≤1 to 2	16 to >64	2 to 64	8 to >64	8 to 64
MIC_{50}	8	8	8	8	16	2	≤1	≤1	2	32	32	32	32
MIC_{90}	16	16	16	16	32	4	4	4	2	64	64	64	64
CIP													
MIC range	2 to >4	≥4	4 to >4	2 to >4	2 to 8	≤0.12	0.25 to 4	2 to 4	1 to 16	4 to >16			
MIC_{50}	>4	4	4	4	4	≤0.12	0.5	4	8	8			
MIC_{90}	>4	>4	>4	>4	8	≤0.12	2	4	16	>16			
IPM													
MIC range	8 to 64	4 to 32	8 to 32	16 to 64	16 to 64	4 to 8	≤2						
MIC_{50}	16	8	16	16	32	4	≤2						
MIC_{90}	32	16	32	32	64	8	≤2						
MXF													
MIC range	2 to >8	2 to >8	4 to 8	4 to >8	4 to 8	≤0.25	≤0.25 to 1	≤0.12 to 0.5	0.25 to 4	1 to 8	0.5 to >8	1 to >8	0.5 to >8
MIC_{50}	8	8	4	4	4	≤0.25	≤0.25	≤0.12	1	4	1	4	4
MIC_{90}	>8	>8	>8	8	8	≤0.25	0.5	0.5	4	8	8	8	>8
DOX													
MIC range	>8	2 to >16	>16	0.12 to >16	8 to >16	0.12 to >16	≤0.12 to >16	1 to 16	2 to 16	≥16			
MIC_{50}	>8	>16	>16	>16	>16	0.25	≤0.12	8	2	≥16			
MIC_{90}	>8	>16	>16	>16	>16	>16	>16	16	16	>16			
MIN													
MIC range	>16	4 to >8	>8	≤1 to >8	>8	≤1 to >8	≤1 to >8						
MIC_{50}	>16	8	>8	>8	>8	≤1	≤1						
MIC_{90}	>16	>8	>8	>8	>8	>8	>8						

(Continued on next page)

TABLE 1 (Continued)

Antimicrobial[b]	*M. abscessus* subsp. *abscessus*	*M. abscessus* subsp. *massiliense*	*M. abscessus/massiliense* hybrid	*M. chelonae*	*M. immunogenum*	*M. fortuitum* group	*M. mucogenicum* group	*M. kansasii*	*M. marinum*	*M. simiae*	*M. avium*	*M. intracellulare*	MAC-X
TGC													
MIC range	0.06 to 0.5	0.06 to 0.5	0.06 to 0.25	0.06 to 0.5	0.06 to 0.25	0.03 to 0.12	≤0.25 to 0.25						
MIC_{50}	0.25	0.12	0.25	0.25	0.12	0.06	0.12						
MIC_{90}	0.5	0.5	0.5	0.5	0.25	0.12	0.25						
CLR													
MIC range	≤2 to >16	≤2	0.12 to 0.25	≤2	≤2	≤0.06 to >16	≤2	0.12 to 0.5	0.5 to 2	8 to >64	0.25 to >64	1 to >64	0.5 to 8
MIC_{50}	≥16	≤2	0.06	≤2	≤2	16	≤2	0.25	1	8	2	2	2
MIC_{90}	≥16	≤2	0.5	≤2	≤2	>16	≤2	0.5	2	32	8	>64	4
TOB													
MIC range				≤1 to 4	8 to 16								
MIC_{50}				≤2	8								
MIC_{90}				≤2	16								
RFB													
MIC range								≤0.25	≤0.25	4 to >8			
MIC_{50}								≤0.25	≤0.25	8			
MIC_{90}								≤0.25	≤0.25	>8			
RIF													
MIC range								≤0.12 to 1	0.5 to 1	>8			
MIC_{50}								0.25	0.5	>8			
MIC_{90}								1	1	>8			

[a]All values shown are in μg/ml.

[b]Amk, amikacin; FOX, cefoxitin; SXT, trimethoprim–sulfamethoxazole; LZD, linezolid; CIP, ciprofloxacin; IPM, imipenem; MXF, moxifloxacin; DOX, doxycycline; MIN, minocycline; TGC, tigecycline; CLR, clarithromycin; TOB, tobramycin; RFB, rifabutin, RIF, rifampin.

TABLE 2 MICs and MIC ranges of reference strains tested against SPR719

Reference strain	MIC range (μg/ml)	No. of values at an MIC (μg/ml) of:							
		<0.008	0.015	0.03	0.06	0.12	0.25	0.5	1
Staphylococcus aureus ATCC 29213	<0.008 to 0.12	26	14	1	1	1	0	0	0
Mycobacterium peregrinum ATCC 700686	0.12 to 0.5	0	0	0	0	1	11	5	0
Mycobacterium marinum ATCC 927	0.06 to 0.5	0	0	0	10	7	1	5	0
Escherichia coli ATCC 25922	0.5 to 1	0	0	0	0	0	0	14	2
Enterococcus faecalis ATCC 29212	0.015	0	11	0	0	0	0	0	0

subsp. *abscessus* by sequencing of the *rpoβ* gene and *M. abscessus* subsp. *massiliense* by sequencing of the *erm*(41) gene].

For the MAC isolates in our study ($n = 41$, including 12 *M. avium*, 19 *M. intracellulare*, and 10 MAC-X strains), the SPR719 MIC_{50} range was 0.5 to 1.0 μg/ml, 1 to 2 dilutions higher than the MIC_{50} of 0.23 μg/ml seen in the initial study ($n = 3$, but only *M. avium* was tested). In contrast, the 8 isolates of *M. kansasii* in this study had an SPR719 MIC_{50} value of 0.015 μg/ml compared with the 22 isolates (including two reference strains) in the Locher et al. study that had an MIC_{50} value of 0.06 μg/ml (2).

Studies by Shoen et al. showed the efficacy of the prodrug SPR720 in a chronic murine *M. tuberculosis* infection model with the hypothesis that SPR720 may shorten the duration of treatment for drug-susceptible *M. tuberculosis* and serve as a potent addition to oral regimens to shorten the length of treatment for multidrug-resistant tuberculosis (MDR-TB) (7). In a 2017 report by Rubio et al., SPR720 (prodrug of SPR719) significantly reduced the mycobacterial burden in the lungs, spleen, and liver of severe combined immunodeficiency (SCID) mice acutely infected with *M. abscessus* subsp. *bolletii* (strain 103) after oral dosing of 200, 300, and 400 mg/kg/day after 8 days (8). A subsequent study by Rubio et al., showed the efficacy of SPR720 in a mouse model after oral dosages of 25 to 400 mg/kg/day after 16 days. A daily dosage of 100 mg/kg demonstrated the greatest reduction in bacterial burden of *M. abscessus* in lung, spleen, and liver compared with that in the control group (9). Another 2018 study by Bermudez et al. showed activity of SPR719/720 in strains of "*M. avium* subsp. *hominissuis*" in macrophages, mice, and biofilms (10). The most efficacious regimen with the lowest average organism burden in the lung and the largest number of animals showing clearance of the MAC in the lungs was seen at a daily dosage of 50 mg/kg.

These previous studies along with our study suggest that this compound may be a welcome addition to both the *M. tuberculosis* and NTM treatment arsenal. Further clinical studies to assess the role and efficacy of SPR720 in drug treatment of NTM infections are warranted.

MATERIALS AND METHODS

Isolates. The 161 isolates of NTM submitted to the Mycobacteria/Nocardia Research Laboratory at the University of Texas Health Science Center at Tyler between 2016 and 2018 were tested against SPR719 and other comparative antimicrobials (Table 1). One hundred twenty-three (76%) of the isolates were of respiratory origin, while the remaining 38 isolates (24%) were from skin, soft tissue, blood, and fluids.

The rapidly growing mycobacteria (RGM) belonging to 13 taxa of RGM and slowly growing mycobacteria (SGM) included 33 strains from *M. abscessus* subsp. *abscessus*, 10 from *M. abscessus* subsp. *massiliense*, 10 from *M. abscessus* subsp. *abscessus*/*M. abscessus* subsp. *massiliense* hybrids (identified as *M. abscessus* subsp. *massiliense* by *erm* gene but *M. abscessus* subsp. *abscessus* by *rpoβ* gene), 10 from *M. chelonae*, 10 from *M. immunogenum*, 10 from the *Mycobacterium fortuitum* group, and 10 from the *Mycobacterium mucogenicum* group. The SGM strains included 10 from *Mycobacterium simiae*, 9 from *Mycobacterium marinum*, 8 from *M. kansasii*, 12 from *Mycobacterium avium*, 19 from *Mycobacterium intracellulare*, and 10 from MAC-X species (including *Mycobacterium chimaera*).

Identification. All isolates of NTM were identified by gene sequencing as indicated for each species/group. For the RGM, sequencing of region 5 of the *rpoβ* gene and the *erm*(41) gene (for the *M. abscessus* complex) was performed using previously recommended criteria for identification, including CLSI recommendations (4, 5). The SGM species were identified using partial 16S rRNA gene sequencing along with the CLSI interpretive criteria (4).

Antimicrobial susceptibility testing. Isolates were tested by broth microdilution in cation-adjusted Mueller-Hinton broth using doubling dilutions of antimicrobials (SPR719 concentrations were 0.008 to 16 μg/ml for RGM and SGM in one set of panels and 0.0002 to 64 μg/ml and 0.0002 to 512 μg/ml in another

lot of panels for RGM and SGM, provided by Spero Therapeutics as frozen panels manufactured by Thermo Fisher). MICs for the RGM were read after incubation at 30°C for 3 to 5 days until sufficient growth was evident in the growth control well. The slowly growing NTM were read after incubation at 35°C for 7 to 14 days when sufficient growth was evident in the growth control wells. SPR719 was insoluble at high concentrations (≥32 μg/ml), and, therefore, the MICs of concentrations above this level were not possible. MIC_{50} was defined as the lowest concentration of the antibiotic at which 50% of the isolates were inhibited.

The comparator antimicrobials included amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, minocycline, moxifloxacin, tigecycline, trimethoprim-sulfamethoxazole (TMP-SMX), and tobramycin (for *M. chelonae* and *M. immunogenum*) for the RGM. For the SGM (except the MAC), the antimicrobials included amikacin, ciprofloxacin, clarithromycin, doxycycline, linezolid, moxifloxacin, rifabutin, rifampin, and TMP-SMX. The comparator antimicrobials for MAC included amikacin, clarithromycin, linezolid, and moxifloxacin, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (6). The CLSI-recommended breakpoints are listed in Table 1 (6). All comparator antimicrobial MICs were read on commercial microtiter readers (RAPMYCO and SLOMYCO panels) manufactured by Thermo Fisher.

Quality control. Quality control of susceptibility testing was performed weekly using the CLSI-recommended strains *Mycobacterium peregrinum* ATCC 700686 (RGM) and *M. marinum* ATCC 927 (SGM) for the comparative antimicrobials and *Staphylococcus aureus* ATCC 29213, as recommended by the manufacturer for SPR719. In search for an alternative quality control strain, additional quality control for SPR179 was performed using *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 and the abovementioned mycobacterial reference strains (Table 2).

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REFERENCES

1. Brown-Elliott BA, Nash KA, Wallace RJ, Jr. 2012. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. Clin Microbiol Rev 25:545–582. https://doi.org/10.1128/CMR.05030-11.
2. Locher CP, Jones SM, Hanzelka BL, Perola E, Shoen CM, Cynamon MH, Ngwane AH, Wiid IJ, van Helden PD, Betoudji F, Nuermberger EL, Thomson JA. 2015. A novel inhibitor of gyrase B is a potent drug candidate for treatment of tuberculosis and nontuberculosis mycobacterial infections. Antimicrob Agents Chemother 59:1455–1465. https://doi.org/10.1128/AAC.04347-14.
3. Brown-Elliott BA, Ashcraft M, Bush G, McKinney A, Ritter K, Shipp K, Mooney J, Wallace RJ, Jr, Rubio A. 2018. *In vitro* characterization of a novel gyrase inhibitor (SPR719) against nontuberculous mycobacteria, ASM Microbe, Atlanta, GA.
4. Clinical and Laboratory Standards Institute. 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline. CLSI document MM18-A. Clinical and Laboratory Standards Institute, Wayne, PA.
5. Adékambi T, Colson P, Drancourt M. 2003. *rpo B*-based identification of nonpigmented and late pigmented rapidly growing mycobacteria. J Clin Microbiol 41:5699–5708. https://doi.org/10.1128/JCM.41.12.5699-5708.2003.
6. Clinical and Laboratory Standards Institute. 2011. Susceptibility testing of Mycobacteria, Nocardiae, and other aerobic Actinomycetes: approved Standard, 2nd edition. Document no. M24–A2. Clinical and Laboratory Standards Institute, Wayne, PA.
7. Shoen C, Pucci MJ, DeStefano M, Cynamon M. 2017. Efficacy of SPR 720 and SPR 750 gyrase inhibitors in a mouse *Mycobacterium tuberculosis* infection model, ASM Microbe 2017, New Orleans, LA.
8. Rubio A, Verma D, Ordway D, Pucci MJ, Parr TR, Jr. 2017. Activity of a novel gyrase inhibitor *in vitro* and in an acute SCID mouse model of infection caused by *Mycobacterium abscessus*. ASM Microbe 2017, New Orleans, LA.
9. Rubio A, Stapleton M, Verman D, Ordway D. 2018. Potent activity of a novel gyrase inhibitor (SPR719/720) *in vitro* and in a prolonged acute *Mycobacterium abscessus* mouse model of infection, ASM Microbe 2018, Atlanta, GA.
10. Bermudez LE, Palmer A, Rubio A. 2018. Treatment of *Mycobacterium avium* subspecies *hominissuis* (MAH) infection with a novel gyrase inhibitor (SPR719/SPR720) was associated with a significant decrease in bacterial load as assessed in macrophages, biofilm and in mice, ASM Microbe 2018, Atlanta, GA.