AAC00285 - Pharmacodynamic Evaluation of the Activity of Antibiotics against Hemin and Menadione-Dependent Small-Colony Variants of *Staphylococcus aureus* in Models of Extracellular (Broth) and Intracellular (THP-1 Monocytes) infections.

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Supplemental Material:

Table S1. MICs of antibiotics against bacterial strains and influence of medium supplementation in menadione sodium bisulfite (MSB) or in hemin (24 h)

Antibiotic	MICs (mg/L) at pH 7.4					
	Wild-type	*menD* mutant		*hemB* mutant		*hemB* genetically complemented
		None	+ 2 mg/L menadione	None	+ 2 mg/L hemin	
Oxacillin	> 128	> 128	> 128	> 128	> 128	> 128
Daptomycin	0.5	0.25	0.25	0.5	0.5	1
Vancomycin	1	1	1	1	1	1
Oritavancin	0.25	0.03	0.03	0.125	2	0.25
Ciprofloxacin	0.125	0.25	0.25	0.25	0.25	0.125
Moxifloxacin	0.03	0.125	0.125	0.125	0.06	0.03
Rifampin	0.016	0.016	0.016	0.016	0.016	0.016
Trimethoprim-sulfamethoxazole (80:16 mass ratio)	2	4	8	8	2	2
Gentamicin	0.25	1	1	0.5	0.25	0.25
Quinupristin-dalfopristin (30:70 mass ratio)	0.5	0.125	0.125	0.125	0,5	0.5
Linezolid	2	1	2	2	2	2
Tetracycline	128	64	64	128	128	64
Tigecycline	1	0.5	0.5	0.5	2	2
Azithromycin	1	128	128	128	128	128

Table S2. Pertinent regression parameters[a] (with confidence intervals [CI]) for concentration-effects relationships against extracellular bacteria

antibiotic	Wild-type			*menD* mutant			*menD* supplemented			*hemB* mutant			*hemB* genetically complemented		
	E_{min}[b]	E_{max}[b]	C_s[c]	E_{min}[b]	E_{max}[c]	C_s[d]	E_{min}[b]	E_{max}[c]	C_s[d]	E_{min}[b]	E_{max}[c]	C_s[d]	E_{min}[b]	E_{max}[c]	C_s[d]
Vancomycin	2.90 (1.42 to 4.37)	<-5	0.95	2.66 (0.93 to 4.39)	-2.42 (-4.27 to -0.58)	1.36	3.09 (2.27 to 3.90)	<-5	1.21	2.74 (1.80 to 3.68)	-2.19 (-3.06 to -1.32)	0.91	3.45 (2.30 to 4.61)	<-5	1.26
Daptomycin	3.41 (1.63 to 5.18)	<-5	0.83	2.76 (1.89 to 3.63)	<-5	0.48	2.79 (1.81 to 3.76)	<-5	0.69	3.21 (2.39 to 4.04)	<-5	0.17	3.55 (2.70 to 4.40)	<-5	0.31
Gentamicin	2.58 (1.29 to 3.86)	<-5	1.77	2.69 (1.21 to 4.17)	<-5	0.64	2.56 (1.88 to 3.24)	<-5	1.01	2.86 (1.98 to 3.74)	<-5	0.73	3.33 (1.13 to 5.52)	<-5	1.17
Rifampin	1.88 (0.10 to 3.67)	<-5	3.74	1.99 (0.17 to 3.81)	<-5	2.83	2.04 (1.11 to 2.97)	<-5	4.75	2.35 (1.43 to 3.26)	<-5	3.19	2.60 (1.64 to 3.56)	<-5	4.66
Moxifloxacin	3.81 (1.22 to 6.40)	<-5	1.17	2.80 (2.37 to 3.22)	<-5	0.59	2.73 (1.18 to 4.29)	<-5	0.76	4.22 (1.86 to 6.58)	-4.04 (-5.04 to -3.05)	0.34	3.17 (1.88 to 4.46)	<-5	2.11
Oritavancin	3.23 (1.21 to 5.25)	<-5	2.16	2.70 (1.18 to 4.23)	<-5	4.58	3.97 (2.23 to 3.71)	<-5	4.58	3.25 (2.36 to 4.14)	<-5	0.67	3.58 (2.28 to 4.88)	<-5	1.07

[a] Calculated based on sigmoidal regressions with an Hill coefficient of 1 for extracellular data and for intracellular data with vancomycin, daptomycin, gentamicin, and rifampin, and based on bi-phasic sigmoidal regressions with Hill coefficients of 1 for intracellular data with moxifloxacin and oritavancin;
[b] Increase in CFU (in $\log_{10}$ units) from the corresponding original inoculum as extrapolated for infinitely low concentration of antibiotics (mean with 95% confidence interval). All R^2 for fittings are ≥ 0.79;
[c] Decrease in CFU (in $\log_{10}$ units) from the corresponding original inoculum as extrapolated for infinitely large concentration of antibiotics (mean with 95% confidence interval; values lower than -5 are not shown as this is our lowest limit of detection);
[d] Concentration (x MIC) resulting in no apparent bacterial growth as determined by graphical interpolation. MIC values used: values at pH 7.4 for extracellular activity; values at pH 5.5 for intracellular activity for all strains by *hemB* mutant, value at pH 5.5 in the presence of hemin for *hemB* mutant based on data of Figure 2 suggesting availability of hemin-like compounds in the cellular medium.

Table S3. Pertinent regression parameters[a] (with confidence intervals [CI]) for concentration-effects relationships against intracellular bacteria

antibiotic	Wild-type				*menD* mutant				*menD* complemented				*hemB* mutant				*hemB* genetically complemented			
	E_{min}[b]	E_{max}[c]	C_s[d]	Frac[e]	E_{min}[b]	E_{max}[c]	C_s[d]	Frac[e]	E_{min}[b]	E_{max}[c]	C_s[d]	Frac[e]	E_{min}[b]	E_{max}[c]	C_s[d]	Frac[e]	E_{min}[b]	E_{max}[c]	C_s[d]	Frac[e]
Vancomycin	3.45 (2.80 to 4.09)	-0.31 (-0.85 to 0.22)	5.27	na	1.36 (1.14 to 1.58)	-0.74 (-0.88 to -0.60)	0.34	na	3.32 (2.94 to 3.71)	-0.35 (-0.69 to -0.01)	5.94	na	3.34 (3.11 to 3.57)	-0.69 (-0.84 to -0.53)	1.11	na	3.06 (2.16 to 3.96)	-1.19 (-2.04 to -0.34)	2.03	na
Daptomycin	3.14 (2.88 to 3.41)	-0.85 (-1.01 to -0.69)	1.09	na	1.07 (0.27 to 1.86)	-1.59 (-2.10 to -1.09)	0.23	na	3.32 (2.79 to 3.84)	-0.25 (-0.46 to -0.05)	0.87	na	4.08 (3.37 to 4.78)	-0.27 (-0.56 to 0.03)	1.21	na	3.23 (2.94 to 3.51)	-0.86 (-1.04 to -0.68)	0.31	na
Gentamicin	3.57 (2.98 to 4.16)	-0.99 (-1.24 to -0.74)	0.15	na	0.88 (0.50 to 1.26)	-1.86 (-2.38 to -1.33)	0.03	na	3.13 (2.89 to 3.37)	-0.82 (-1.02 to -0.62)	0.05	na	3.52 (3.01 to 3.94)	-0.49 (-0.67 to -0.30)	0.35	na	3.29 (2.85 to 3.74)	-0.73 (-0.93 to -0.53)	0.23	na
Rifampin	2.50 (2.18 to 2.81)	-1.75 (-1.93 to -1.58)	66.76	na	0.51 (0.07 to 1.10)	-1.43 (-1.78 to -1.07)	25.60	na	2.82 (2.36 to 3.28)	-0.53 (-0.66 to -0.39)	37.41	na	3.02 (2.24 to 3.80)	-0.91 (-1.15 to -0.67)	27.18	na	2.28 (1.26 to 3.29)	-1.61 (-2.18 to -1.04)	76.78	na
Moxifloxacin	3.34 (2.38 to 4.30)	<-2	0.91	0.75	0.85 (0.52 to 1.18)	<-2	0.07	0.72	4.55 (13.8 to 20.1)	<-2	0.18	0.35	3.54 (-198 to 204)	-0.99 (-1.24 to -0.67)	0.26	0.96	3.15 (13.8 to 20.1)	<-2	0.16	0.36
Oritavancin	2.98 (2.77 to 3.20)	<-2	2.51	0.63	0.77 (0.41 to 1.06)	<-2	0.63	0.36	3.99 (24.3 to 32.2)	<-2	16.6	0.27	3.51 (3.24 to 3.78)	<-2	0.31	0.37	2.54 (2.13 to 2.94)	<-2	2.14	0.64

[a] Calculated based on sigmoidal regressions with an Hill coefficient of 1 for extracellular data and for intracellular data with vancomycin, daptomycin, gentamicin, and rifampin, and based on bi-phasic sigmoidal regressions with Hill coefficients of 1 for intracellular data with moxifloxacin and oritavancin;

[b] Increase in CFU (in $\log_{10}$ units) from the corresponding original inoculum as extrapolated for infinitely low concentration of antibiotics (mean with 95% confidence interval). All R^2 for fittings are ≥ 0.88;

[c] Decrease in CFU (in $\log_{10}$ units) from the corresponding original inoculum as extrapolated for infinitely large concentration of antibiotics (mean with 95% confidence interval). For moxifloxacin and oritavancin, the values are not shown as we did not have enough data points close to a potential second plateau, making therefore the calculation of E_{max} values somewhat unreliable;

[d] Concentration (x MIC) resulting in no apparent bacterial growth as determined by graphical interpolation. MIC values used: values at pH 7.4 for extracellular activity; values at pH 5.5 for intracellular activity for all strains by *hemB* mutant, value at pH 5.5 in the presence of hemin for *hemB* mutant based on data of Figure 2 suggesting availability of hemin-like compounds in the cellular medium;

[e] Proportion of the total response that could be ascribed to the first wave of CFU decrease in the biphasic curve.