

**AAC1858-13**

**Antibiotic activity against naive and induced *Streptococcus pneumoniae*  
biofilms in an *in vitro* pharmacodynamic model**

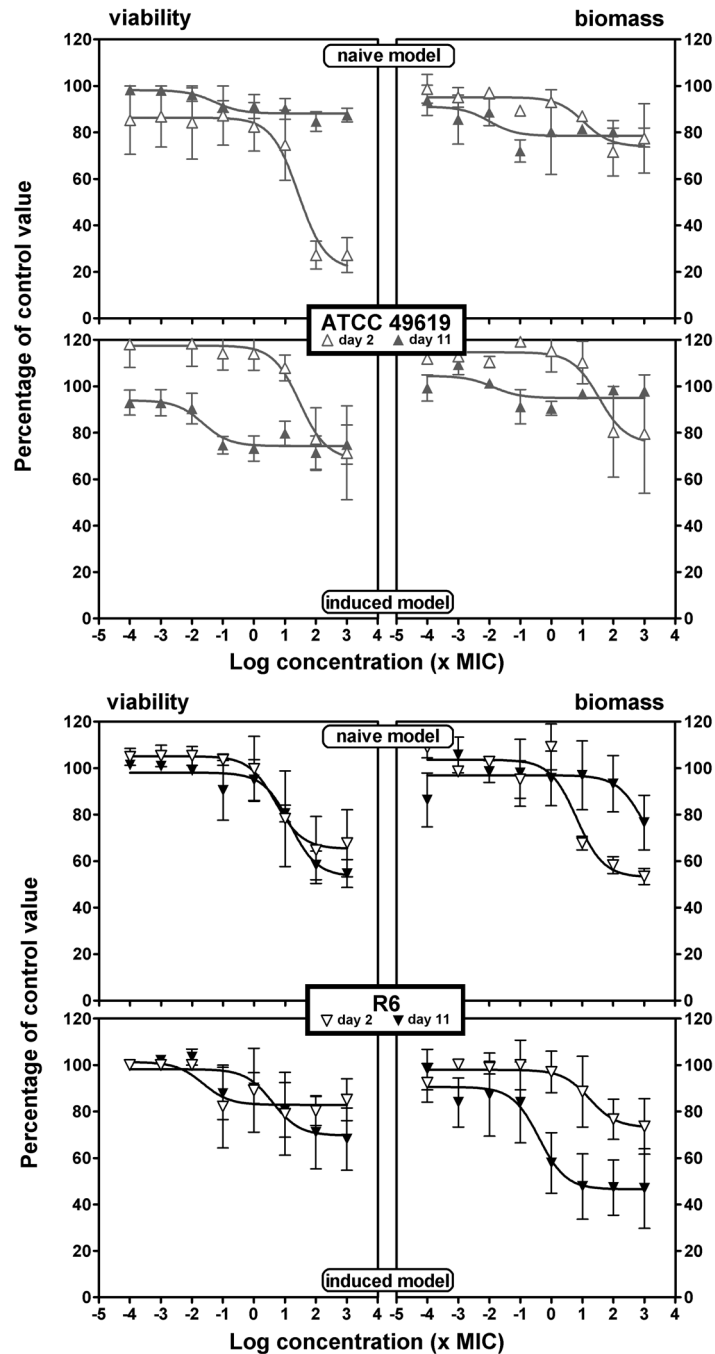
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**SUPPLEMENTARY MATERIAL**

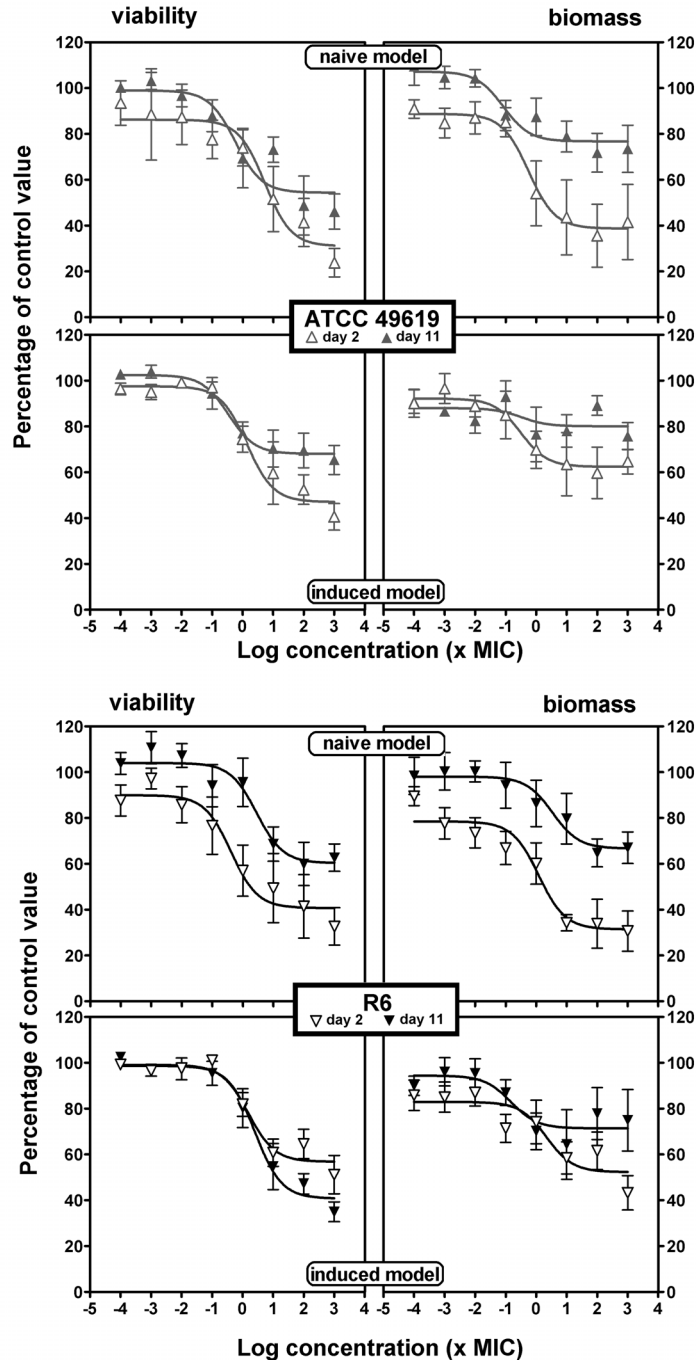
Figure S1

Concentration-response activity of clarithromycin against biofilms of ATCC 49619 (top) or R6 (bottom). Two-day- (open symbols) or 11-day- (closed symbols) old biofilms from the naive model (upper panels for each strain) or the induced model (lower panels for each strain) were incubated with increasing concentrations of clarithromycin for 24h. The ordinate shows the change in viability (measured by the decrease in resorufin fluorescence; left panels) or in biofilm mass (measured by the decrease in crystal violet absorbance; right panels) in percentage of the control value (no antibiotic present). All values are means  $\pm$  SEM of 4-10 independent experiments performed in quadruplicates (when not visible, the bars are smaller than the size of the symbols). The pertinent pharmacological descriptors of the curves are presented in Tables S1-S2.



**Figure S2**

Concentration-response activity of levofloxacin against biofilms of ATCC 49619 (top) or R6 (bottom). Two-day- (open symbols) or 11-day- (closed symbols) old biofilms from the naive model (upper panels for each strain) or the induced model (lower panels for each strain) were incubated with increasing concentrations of levofloxacin for 24h. The ordinate shows the change in viability (measured by the decrease in resorufin fluorescence; left panels) or in biofilm mass (measured by the decrease in crystal violet absorbance; right panels) in percentage of the control value (no antibiotic present). All values are means  $\pm$  SEM of 4-10 independent experiments performed in quadruplicates (when not visible, the bars are smaller than the size of the symbols). The pertinent pharmacological descriptors of the curves are presented in Tables S1-S2.



**Table S1. Pertinent regression parameters<sup>a</sup> (with 95% confidence intervals) and statistical analysis<sup>c</sup> for strain ATCC 49619**

AB	Biofilm model	Effect on viability within the matrix					Effect on biofilm thickness				
		E <sub>max</sub> <sup>b</sup> % loss of viability (CI at 95%)	Concentration yielding specified effect		R <sup>2</sup>	E <sub>max</sub> <sup>b</sup> % loss of matrix (CI at 95%)	Concentration yielding specified effect		R <sup>2</sup>		
			20% reduction (in X MIC)	50% reduction (in mg/L)			20% reduction (in X MIC)	50% reduction (in mg/L)			
AMX	2 days; naive	68.89 (51.80 to 85.98) / A <sup>c</sup>	16.5 (9.7 to 23.5) / A <sup>c</sup>	1.1	111.3	0.648	50.13 (40.71 to 59.55) / A	0.1 (-11.8 to 12) / A	< 0.1	101.3	0.430
	2 days; induced	40.21 (26.96 to 53.46) / B	7.6 (-3.4 to 22.5) / A	0.5	>10 <sup>4</sup>	0.589	45.83 (29.74 to 61.92) / A	0.2 (-27.8 to 29) / A	< 0.1	>10 <sup>4</sup>	0.413
	11 days; naive	35.01 (25.31 to 44.71) / B	0.9 (12.23 to 14.43) / B	0.1	>10 <sup>4</sup>	0.500	25.56 (11.78 to 39.34) / B	35.8 (-2.8 to 74.4) / B	2.3	>10 <sup>4</sup>	0.206
	11 days; induced	37.17 (29.57 to 44.77) / B	0.5 (-12.23 to 13.23) / B	0.1	>10 <sup>4</sup>	0.626	6.72 (-0.78 to 14.22) / C	> 10 <sup>4</sup> / C	> 640	>10 <sup>4</sup>	0.147
CLR	2 days; naive	79.01 (67.91 to 90.11) / A	2.9 (-20.1 to 26.13) / A	< 0.1	33.5	0.648	26.08 (14.72 to 37.44) / A	28.2 (-14.4 to 70.8) / A	60.9	>10 <sup>4</sup>	0.396
	2 days; induced	31.46 (21.04 to 41.88) / B	98.6 (70.6 to 124.9) / B	3.2	>10 <sup>4</sup>	0.552	24.59 (1.54 to 47.64) / A	260 (88.2 to 431.2) / B	8.3	>10 <sup>4</sup>	0.553
	11 days; naive	11.87 (10.27 to 13.47) / C	> 10 <sup>4</sup> / C	> 320	>10 <sup>4</sup>	0.427	21.50 (13.76 to 29.24) / A	0.1 / C	< 0.1	>10 <sup>4</sup>	0.137
	11 days; induced	25.74 (22.9 to 28.58) / B	0.06 (-33.5 to 33.5) / A	< 0.1	>10 <sup>4</sup>	0.367	5.00 (0.47 to 9.53) / B	> 10 <sup>4</sup> / C	> 320	>10 <sup>4</sup>	0.325
SOL	2 days; naive	63.03 (50.63 to 75.43) / A	3.5 (-15.25 to 21.4) / A	< 0.1	9.2	0.746	63.88 (56.51 to 71.25) / A	0.1 (-4 to 4.3) / A	< 0.1	0.4	0.718
	2 days; induced	21.86 (12.71 to 31.01) / B	192.5 (170.3 to 220) / B	- 1.5	>10 <sup>4</sup>	0.499	59.48 (50.78 to 68.18) / A	0.1 (-15.7 to 15.7) / A	< 0.1	1.4	0.634
	11 days; naive	36.4 (25.56 to 47.24) / C	17.7 (9.3 to 34.9) / C	- 0.1	>10 <sup>4</sup>	0.491	34.26 (15.41 to 53.11) / B	58.6 (33.9 to 83.3) / B	0.5	>10 <sup>4</sup>	0.277
	11 days; induced	35.74 (31.20 to 40.28) / C	0.6 (-5 to 6.3) / A	< 0.1	>10 <sup>4</sup>	0.733	11.64 (0.36 to 22.52) / C	> 10 <sup>4</sup> / C	> 80	>10 <sup>4</sup>	0.050
LVX	2 days; naive	69.06 (51.60 to 86.52) / A	0.7 (-14.8 to 16.1) / A	0.7	10.0	0.434	61.22 (47.73 to 74.71) / A	0.1 (-11.5 to 11.7) / A	0.1	2.0	0.408
	2 days; induced	52.90 (46.22 to 59.58) / B	0.7 (-4 to 5.4) / A	0.7	21.6	0.829	37.56 (27.42 to 47.70) / B	0.2 (21.4 to 21.7) / A	0.2	>10 <sup>4</sup>	0.334
	11 days; naive	45.63 (35.23 to 56.03) / B	0.4 (-10.4 to 11.2) / A	0.4	>10 <sup>4</sup>	0.495	23.23 (15.46 to 31.00) / C	0.7 (-16.5 to 18) / A	0.7	>10 <sup>4</sup>	0.378
	11 days; induced	31.92 (25.59 to 38.25) / C	0.7 (-7.8 to 9.2) / A	0.7	>10 <sup>4</sup>	0.598	19.93 (11.86 to 28.00) / C	> 10 <sup>4</sup> / B	> 10 <sup>4</sup>	>10 <sup>4</sup>	0.073
MXF	2 days; naive	74.07 (69.76 to 78.38) / A	0.1 (-6 to 6.3) / A	< 0.1	0.1	0.569	81.25 (70.63 to 91.87) / A	NA <sup>d</sup>	NA <sup>d</sup>	0.1	0.620
	2 days; induced	64.62 (58.22 to 71) / A	1.0 (-5 to 7.2) / A	0.1	5.9	0.765	73.19 (63.55 to 82.83) / A	NA <sup>d</sup>	NA <sup>d</sup>	0.9	0.655
	11 days; naive	42.18 (39.19 to 45.17) / B	1.3 (-4.2 to 7) / A	0.2	>10 <sup>4</sup>	0.801	20.87 (13.59 to 28.15) / B	3.9 (-17.2 to 25) / A	0.5	>10 <sup>4</sup>	0.454
	11 days; induced	45.14 (41.85 to 48.42) / B	4.7 (-0.47 to 10) / A	0.6	>10 <sup>4</sup>	0.847	17.21 (10.37 to 24.05) / B	> 10 <sup>4</sup> / B	> 1250	>10 <sup>4</sup>	0.193

<sup>a</sup> Calculated based on sigmoidal regressions with a Hill coefficient of 1

<sup>b</sup> Decrease in viability and matrix thickness from the original values obtained under control conditions (growth without antibiotic) as extrapolated for an infinitely large concentration of antibiotic (means with 95% confidence intervals).

<sup>c</sup> Statistical analysis: One-way ANOVA with Tukey post test for multiple comparisons between different types of biofilms for each drug, values with different letters are significantly different from each other (P<0.05); see figures 6 and 7 for comparisons between antibiotics for each type of biofilm.

<sup>d</sup> not applicable (TOP of the Hill equation close to 80%)

**Table S2. Pertinent regression parameters<sup>a</sup> (with 95% confidence intervals) and statistical analysis<sup>c</sup> for strain R6**

AB	Biofilm model	Effect on viability within the matrix					Effect on biofilm thickness				
		E <sub>max</sub> <sup>b</sup> % loss of viability (I at 95%)	Concentration yielding specified effect		R <sup>2</sup>	E <sub>max</sub> <sup>b</sup> % loss of matrix (CI at 95%)	Concentration yielding specified effect		R <sup>2</sup>		
			20% reduction (in X MIC)	(in mg/L)			50% reduction (in X MIC)	20% reduction (in X MIC)		(in mg/L)	50% reduction (in X MIC)
AMX	2 days; naive	71.60 (57.16 to 86.04) / A <sup>c</sup>	11.1 (3.6 to 17.5) / A <sup>c</sup>	0.4	79.08	0.748	41.45 (29.26 to 53.64) / A	1.6 (-27 to 30.2) / A	0.1	>10 <sup>4</sup>	0.294
	2 days; induced	41.11 (21.52 to 60.70) / B	1.1 (70.1 to 72.3) / A	0.1	>10 <sup>4</sup>	0.258	35.41 (22.70 to 48.12) / A	0.6 (39.4 to 31.1) / A	< 0.1	>10 <sup>4</sup>	0.417
	11 days; naive	21.3 (12.38 to 30.22) / B	48.3 (4.1 to 92.4) / B	1.5	>10 <sup>4</sup>	0.298	25.36 (18.23 to 32.49) / B	0.4 (-88.2 to 89) / A	< 0.1	>10 <sup>4</sup>	0.189
	11 days; induced	31.04 (21.62 to 40.46) / B	3.5 (-23.9 to 36.3) / A	0.1	>10 <sup>4</sup>	0.589	24.24 (18.81 to 29.67) / B	> 10 <sup>4</sup> / B	> 320	>10 <sup>4</sup>	0.826
CLR	2 days; naive	34.75 (26.87 to 42.63) / A	8.5 (-21.9 to 39) / A	0.1	>10 <sup>4</sup>	0.503	46.03 (38.48 to 53.58) / A	5.9 (-4.7 to 16.9) / A	0.4	>10 <sup>4</sup>	0.881
	2 days; induced	17.82 (11.26 to 24.38) / B	> 10 <sup>4</sup> / B	> 640	>10 <sup>4</sup>	0.472	26.84 (11.01 to 42.67) / B	44.0 (-16.8 to 104.6) / B	2.8	>10 <sup>4</sup>	0.332
	11 days; naive	46.59 (41.35 to 51.83) / A	9.9 (-2.2 to 22.8) / A	0.6	>10 <sup>4</sup>	0.767	44.59 (-304.62 to 393.80) / A	715.1 / C	45.6	>10 <sup>4</sup>	0.132
	11 days; induced	30.34 (23.63 to 37) / A,B	7.1 (-39.3 to 53.6) / A	0.5	>10 <sup>4</sup>	0.411	53.50 (37.71 to 62.29) / A	0.1 (-43.4 to 43.6) / A	< 0.1	1.17	0.478
SOL	2 days; naive	47.29 (33.04 to 61.54) / A	33.8 (27.5 to 46.8) / A	0.1	>10 <sup>4</sup>	0.572	55.40 (-127.4 to 171.8) / A,B	NA <sup>d</sup>	NA <sup>d</sup>	5307.13	0.043
	2 days; induced	24.78 (41.50 to 8.06) / B	29.8 (-119.6 to 178.6) / A	0.1	>10 <sup>4</sup>	0.105	28.38 (21.27 to 35.5) / A	0.9 (0.5 to 2.3) / A	< 0.1	>10 <sup>4</sup>	0.099
	11 days; naive	34.26 (15.41 to 53.11) / A	59.8 (33 to 84.4) / A	0.2	>10 <sup>4</sup>	0.277	12.93 (7.92 to 17.94) / B	> 10 <sup>4</sup> / B	> 40	>10 <sup>4</sup>	0.042
	11 days; induced	45.18 (34.74 to 55.62) / A	24.6 (18.6 to 33.6) / A	0.1	>10 <sup>4</sup>	0.688	14.84 (10.77 to 18.91) / B	> 10 <sup>4</sup> / B	> 40	>10 <sup>4</sup>	0.052
LVX	2 days; naive	59.24 (45.59 to 72.89) / A	0.1 (-9.8 to 10) / A	0.1	1.77	0.371	68.52 (62.93 to 74.1) / A	NA <sup>d</sup>	NA <sup>d</sup>	1.86	0.511
	2 days; induced	43.21 (36.62 to 49.80) / B	1.3 (-6 to 8.6) / A	0.7	>10 <sup>4</sup>	0.800	47.72 (42.2 to 53.24) / B	0.2 (-17.1 to 17.5) / A	0.1	>10 <sup>4</sup>	0.306
	11 days; naive	39.64 (28.75 to 50.53) / B	3.5 (-6.1 to 13.9) / A	1.8	>10 <sup>4</sup>	0.530	33.28 (27.37 to 39.2) / C	4.6 (-6.1 to 13.9) / A	2.3	>10 <sup>4</sup>	0.328
	11 days; induced	59.21 (51.05 to 67.37) / A	1.2 (-4.9 to 7.3) / A	0.6	13.49	0.808	28.63 (23.58 to 33.68) / C	0.3 (-63.3 to 63.9) / A	0.2	>10 <sup>4</sup>	0.212
MXF	2 days; naive	81.18 (68.82 to 95.54) / A	0.3 (-7.5 to 7.9) / A	< 0.1	2.56	0.694	59.06 (48.27 to 69.85) / A	NA <sup>d</sup>	NA <sup>d</sup>	1.78	0.513
	2 days; induced	47.73 (32.28 to 63.18) / B	7.9 (-1.9 to 20.1) / A	0.5	>10 <sup>4</sup>	0.638	47.88 (32.02 to 63.74) / A	14.5 (2.9 to 29.9) / A	0.5	>10 <sup>4</sup>	0.609
	11 days; naive	30.82 (18.16 to 43.48) / C	14.9 (0.7 to 29) / A	1.0	>10 <sup>4</sup>	0.435	23.67 (17.95 to 29.39) / B	5.2 (-12.5 to 22.9) / A	0.3	>10 <sup>4</sup>	0.365
	11 days; induced	48.73 (37.21 to 60.25) / B	4.8 (-3.3 to 13.3) / A	0.3	>10 <sup>4</sup>	0.636	9.66 (5.64 to 13.68) / C	> 10 <sup>4</sup> / B	> 640	>10 <sup>4</sup>	0.282

<sup>a</sup> Calculated based on sigmoidal regressions with a Hill coefficient of 1

<sup>b</sup> Decrease in viability and matrix thickness from the original values obtained under control conditions (growth without antibiotic) as extrapolated for an infinitely large concentration of antibiotic (means with 95% confidence intervals).

<sup>c</sup> Statistical analysis: One-way ANOVA with Tukey post test for multiple comparisons between different types of biofilms for each drug, values with different letters are significantly different from each other (P<0.05); see figures 6 and 7 for comparisons between antibiotics for each type of biofilm.

<sup>d</sup> not applicable (TOP of the hill equation close to 80 %)