Activity of macrolides, ketolides, and fluoroquinolones against *S. pneumonaie* in an in vitro pharmacodynamic model of biofilm

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Objectives: Persistent infections by *S. pneumoniae* like chronic sinusitis or otitis are associated with in situ formation of biofilms. This makes eradication difficult due to the protective role of the matrix in which bacteria are embedded. Our objective was to develop an in vitro model of young and aged biofilms of *S. pneumoniae* to study the effect of antibiotics on biofilm mass and intra-matrix bacterial survival.

Methods: Biofilms were obtained by culture of *S. pneumoniae* (capsulated [ATCC49619] and non capsulated [R6] strains) in 96-well plates for 2, 4, 7 and 11 days. Antibiotic activity was evaluated after 24 h of incubation at concentrations ranging from 0.0001 to 1000-fold the MIC in broth. Total biofilm mass (matrix + bacteria) was quantified by staining with crystal violet (CV) followed by OD measurement at 620 nm, and bacterial viability using the redox indicator resazurin (reduced in situ to fluorescent resorufin [RF] by living cells; [Lett. Appl. Microbiol. 2008, 49:249-54]). A Hill equation was fitted to the data to calculate maximal relative activity (Emax [infinitely large antibiotic concentration]).

Results: In the absence of antibiotic, CV OD increased from 0.6-0.9 to 32-33 with both strains between day 2 and day 11. The Table shows that the activity of most antibiotics against bacteria (survival) was lower with R6 than with ATCC49619 in young biofilms, and markedly decreased for ATCC49619 in mature biofilms with both strains reaching globally similar low values. For biofilm mass, activity was globally similar for macrolides/ketolides in young and aged biofilms for ATCC49619 but reduced in old biofilms for fluroquinolones, and globally lower for all antibiotics in aged biofilms for R6. The most constant decreases amongst all antibiotics were observed with moxifloxacin and amongst macrolides/ketolides with solithromycin.

Conclusion: Biofilm production is independent of the non-capsulated or capsulated phenotype, and is accompanied over aging by a global decrease of antibiotic activity that, in proportion, affects more the strain (capsulated) for which a better activity was observed in young biofilms (perhaps related to differences in biofilm composition). The constantly higher activity of moxifloxacin and, to a lesser extent, solithromycin, (perhaps due to combination of a low MIC and a bactericidal activity) may be worth further exploration.

			Viability (% decrease from control value)				Biofilm mass (% decrease from control value)			
strain	**Antibiotic [a]**	**MIC (µg/ml)**	**Day 2**		**Day 11**		**Day 2**		**Day 11**	
			E_{10xMIC} [b]	**E_{max} [c]**	**E_{10xMIC} [b]**	**E_{max} [c]**	**E_{10xMIC} [b]**	**E_{max} [c]**	**E_{10xMIC} [b]**	**E_{max} [c]**
ATCC 49619	CLR	0.032	-25	-73	-10	-13	-13	-7	-19	-20
	AZM	0.064	-34	-80	-5	-20	+2	-15	-7	-15
	TEL	0.016	-0	-80	-11	-40	-10	-30	-26	-62
	SOL	0.032	-67	-70	-25	-38	-47	-55	-39	-55
	MXF	0.125	-90	-90	-51	-45	-76	-75	-25	-30
	LVX	1	-45	-75	-25	-35	-27	-65	-9	-10
R6	CLR	0.063	-22	-35	-5	-50	-26	-35	-3	-10
	AZM	0.5	+4.00	-35	+4	-45	-14	-20	-9	-10
	TEL	0.008	-15	-45	-5	-40	-23	-40	-9	-12
	SOL	0.004	-31	-45	-15	-35	-35	-50	-7	-0
	MXF	0.063	-91	-90	-17	-25	-75	-70	-40	-40
	LVX	0.5	-20	-65	-31	-5	-65	-60	-10	-35

[a] CLR: clarithromycin, AZM: azithromycin; TEL: telithromycin; SOL: solithromycin (CEM-101); LVX: levofloxacin: MXF: moxifloxacin

[b] values interpolated from the Hill equation of the sigmoidal dose-response for experiments following crystal violet absorbance or resazurin fluorescence as a function of the drug concentration, with all data expressed as percentage of control values

[c] values extrapolated from the Hill equation of the same sigmoidal dose-response, for an infinitely large antibiotic concentration