

Activity of 9 Antibiotics Against Intracellular Forms of *S. pneumoniae*

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Objectives: Although often considered as an extracellular organism only, *S. pneumoniae* has been shown to invade and survive within eukaryotic cells in vitro (Jonsson et al., JID 1985,152:4-13; Peppoloni et al., Microbes infect 2010,12:990-1001). Intracellular foci of *S. pneumoniae* have also been observed in patients with persistent streptococcal infections (Coates et al. Otolaryngol Head Neck Surg 2008,138:778-81). We have set up an in vitro model of macrophages infected by *S. pneumoniae* in order to quantitatively assess and compare the activity of antibiotics against these intracellular forms.

Methods: *S. pneumoniae* ATCC 49619 and THP-1 myelomonocytic cells were used throughout. MICs were determined in cation-adjusted Mueller Hinton broth supplemented with 2.5% lysed horse blood. Infection of THP-1 cells was performed with opsonized *S. pneumoniae* incubated for 2 h at 37°C at a 10:1 bacteria:cell ratio. Non-phagocytized bacteria were eliminated by incubation with gentamicin (50 mg/L; 1 h) and 4 successive washings with PBS. Infected cells were then transferred for 24 h in fresh culture medium containing antibiotic concentrations ranging from about 1/100 to 100 x the MIC (full concentration-dependent effects), collected, lysed and used for cfu counting. The change in cfu was plotted against antibiotic concentration and used for fitting a Hill equation to determine the static concentration (C_s) and the maximal relative efficacy (E_{max}) of each antibiotic (Barcia-Macay et al. AAC 2006, 50:841-51).

Results: The table shows the MICs and the intracellular C_s and E_{max} of the antibiotics ordered by increasing maximal relative efficacy (increasingly negative E_{max}). C_s varied from values close to the MIC (LZD, MXF, DAP, Q-D) to large multiples of MIC (AZM, CEM-101, RIF), and E_{max} from -1 \log_{10} (AMX) to -3 \log_{10} (Q-D).

Conclusions: This model shows that the intracellular activity of antibiotics can only partially be predicted from the determination of their MIC in broth, and that only one antibiotic (Q-D) yields a truly intracellular bactericidal effect (defined as 3 \log_{10} cfu decrease). Further studies examining the subcellular localization of the phagocytized bacteria and of the antibiotics may help in rationalizing these observations.

Antibiotic	MICs (mg/L)	Intracellular Activity (24 h)		
		C_s^a		E_{max}^b (CI)
		mg/L	x MIC	
Amoxicillin (AMX)	0.03	~ 0.32	~ 11	-1.03 (-1.50 to -0.56)
Linezolid (LZD)	1	~ 2.09	~ 2	-1.41 (-2.19 to -0.62)
Azithromycin (AZM)	0.004	~ 0.23	~ 58	-1.85(-2.24 to -1.47)
Rifampicin (RIF)	0.002	~ 0.38	~ 190	-1.96 (-2.04 to -1.88)
Moxifloxacin (MXF)	0.125	~ 0.46	~ 3	-2.1 (-2.56 to -1.64)
Fluoroquinolone (FNX)	1	~ 1.13	~ 1	-2.21 (-2.82 to -1.59)
Solithromycin (CEM-101)	< 0.0001	~ 0.03	> 300	-2.25 (-2.71 to -1.79)
Daptomycin (DAP)	0.5	~ 1.86	~ 4	-2.4 (-3.42 to -1.39)
Quinupristin-dalfopristin (Q-D)	0.5	~ 0.52	~ 1	-3.11 (-3.58 to -2.64)

^a concentration (in mg/L or in X MIC) resulting in no apparent bacterial growth

^b relative maximal efficacy (decrease [in \log_{10} scale] in the number of cfu from the post-phagocytosis inoculum, as extrapolated for infinitely large concentration of antibiotics; Hill equation, slope factor of 1) with 95 % confidence interval