Activities of amoxicillin (AMX), clarithromycin (CLR) and moxifloxacin (MXF) towards intracellular forms of Streptococcus pneumoniae with increasing mutant inhibitory concentrations (MICs): attempt at defining an intracellular susceptibility breakpoint

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Background: S. pneumoniae has developed strategies for invasion and survival within eukaryotic cells, favoring its dissemination and the establishment of persistent foci. Previously, we showed that these forms are less susceptible to antibiotics than their extracellular counterparts (Lemaire et al, poster P781, 21th ECCMID). In this context, we evaluated the intraphagocytic activities of AMX, CLR and MXF using strains with increased MICs to these antibiotics in an attempt to define an intracellular breakpoint.

Methods: Isolates with MICs (microdilution; cation-adjusted broth; CLSI recommendations) ranging from 0.008 to 16 mg/L (AMX [n=9]; CLR [n=8]) and 0.06 to 2 mg/L (MXF [n=10]) were selected. Phagocytosis by THP-1 macrophages was performed at a bacteria-to-cell ratio of 20 for 2 h. Cells were incubated with antibiotics for 24 h at increasing antibiotic concentrations (0.001 to 100 mg/L) and intracellular activities were measured by recording the change in CFUs (in log10 units) compared to post-phagocytosis values. The change observed for an extracellular concentration corresponding to the human Cmax (ECmax; AMX: 10; CLR: 1; MXF: 4 mg/L) was determined by direct reading for cells incubated at those concentrations. Static concentration (Cs; in mg/L) was determined by graphical intrapolation using Hill functions (slope factor = 1) fitted to the data (change in CFU vs. log10 of the drug concentrations). Recursive partitioning was used to classify responses based on dichotomous split of MICs (separating low and high responses) to determine a breakpoint. Results: There was a gradual shift of the concentration-dependent responses of phagocytozed isolates towards decreased efficacy as a function their MICs, with ECmax moving from negative to positive values and increased Cs (see Figure). For both parameters, recursive partitioning indicated a dichotomous split at an MIC of 0.5 mg/L (vertical dotted line; LogWorth values: 5.77 and 16.28 [values > 2 indicate that the variable used in the branch is significant and should be included in the decision tree]). Conclusions: Antibiotics control the intracellular growth of S. pneumoniae for strains for which their MICs are up to 0.5 mg/L, irrespective of their pharmacological class. This intracellular breakpoint is similar or close to the clinical susceptibility breakpoints set by EUCAST for human infections caused by S. pneumoniae (AMX: 0.5; CLR: 0.25; MXF: 0.5 mg/L)
Analysis of the concentration-dependent activities of antibiotics against intraphagocytic forms of *S. pneumoniae*. The graphs show the changes in $E_{\text{cmax}}$ and $C_s$ as a function of the MICs of the corresponding isolates. The horizontal dotted lines refer to the value of the post-phagocytosis CFUs (left diagram) and to the MIC (right diagram). The vertical dotted line shows the value of the dichotomous split as determined by recursive partitioning (JMP®, Version 9.03. SAS Institute Inc., Cary, NC 1989-2007).