### Method

1. **Culture**
   - 2, 4, 7 and 11 days
   - S. pneumoniae non capsulated strain R6
capsulated strain ATCC 49619

2. **Exposure to antibiotics**
   - 24h incubation
   - Macrolides/Ketolides/Quinolones
   - Concentrations: 0.0001 to 1000-fold the MIC in broth

3. **Quantification of antibiotic activity (24h)**
   - Fluorimetric quantification
   - Spectrophotometric quantification
   - Experiments were performed 3 to 6 times independently

### Results

- In the absence of antibiotic, the resorufin fluorescence signal increased from 3000 to 5000 (intensity values) to 5000-9000 between day 2 and 11, and the optical density of CV increased from 0.5 to 32-33 between day 2 and day 11 for both strains.

- Concentration-effect curves (for selected antibiotics; upper graphs) and maximal effects (for all antibiotics; lower graphs) show that:
  - activity is markedly lower against old than young biofilms with respect to both viability and matrix;
  - for young biofilms, MXF is the most potent antibiotic (lowest EC<sub>50</sub>) among all antibiotics tested, whereas ketolides and fluoroquinolones (only for R6) show higher efficacy on matrix.

- For old biofilms, all antibiotics show similar activity when expressed in multiples of their MIC (note that the MIC of solithromycin is lower than that of all other antibiotics including telithromycin).

### Conclusion

- The amount of biofilm produced over time is independent of the non capsulated or capsulated phenotype and is accompanied over aging by a global decrease of antibiotic activity.

- Taking also into account MIC values in broth, the most efficient antibiotics (on a weight basis) are solithromycin amongst macrolides/ketolides, and moxifloxacin amongst fluoroquinolones.

- The combined high potency and efficacy of moxifloxacin may result from a combination of its bactericidal character (including cell lysis) and its high intrinsic activity.

### References