Aspects on antimicrobial resistance in the respiratory tract pathogen *Haemophilus influenzae*

**Background:** The number of isolates of *Haemophilus influenzae* with antimicrobial resistance towards beta-lactam antibiotics, mainly aminopenicillins, has increased over the last years. The principal reason behind this increase is the spread of isolates with mutations in penicillin binding protein 3 (PBP3). There is also a stable fraction of isolates expressing beta-lactamase. In this thesis, we want to investigate several different aspects of antimicrobial resistance in *H. influenzae*, including antibiotic tolerance. Methodological and clinical consequences of reduced aminopenicillin susceptibility in this respiratory tract pathogen will be evaluated.

**Methods:** In paper I, we investigated a clinical isolate that was initially categorized as resistant to aminopenicillins but lacked previously described amino acid substitutions in PBP3 that contribute to resistance. Instead, the significance of an alternative amino acid substitution, Y528H, was studied by introducing it into a susceptible isolate through site-directed mutagenesis. In paper II, a cohort of adult patients receiving benzylpenicillin as empirical treatment for severe respiratory tract infections with *H. influenzae* was retrospectively compared to patients receiving wide-spectrum beta-lactams, with 30-day mortality, 30-day readmission rate and early clinical response as the main outcomes.

**Results:** In paper I, introduction of the Y528H substitution rendered a susceptible strain to be classified as resistant in the screening algorithm for beta-lactam resistance. MICs to aminopenicillins were increased in the mutant strain. However, the mutant remained susceptible to aminopenicillins according to clinical breakpoints and MICs did not increase to the same levels as seen in the original clinical strain, suggesting alternative contributing mechanisms. In paper II, empirical treatment with benzylpenicillin was not associated with a significantly worse clinical outcome compared to treatment with wide spectrum antibiotics, even after adjustment for confounders in a logistic regression model and a propensity score matching.

**Significance:** We have identified a new mutation (Y528H) that affects aminopenicillin susceptibility in *Haemophilus influenzae* (Paper I). Our work also supports the current recommendations of using benzylpenicillin as empirical treatment for community acquired
pneumonia (CRB-65 score ≤ 2), even in the light of a potential increase in the proportion of cases caused by *H. influenzae* (Paper II).

**List of papers:**


II. Thegerström J, Månsson V, Riesbeck K, Resman F. Benzylpenicillin versus wide-spectrum beta-lactam antibiotics as empirical treatment of *Haemophilus influenzae*-associated severe lower respiratory tract infections in adults; a retrospective propensity score matched study. Under review in EJCMID.