

# **Ampicillin-resistant $\beta$ -lactamase-negative *Haemophilus influenzae* strains isolated in the Czech Republic in 2010-2018**

According to the results of regular surveillance of resistance of bacterial pathogens of respiratory tract infections, the number of *Haemophilus influenzae* strains with non-enzymatic resistance to  $\beta$ -lactam antibiotics is increasing in the Czech Republic. Non-enzymatic resistance is caused by mutations in the *ftsI* gene encoding penicillin-binding protein (PBP3), which result in a reduced ability to bind  $\beta$ -lactam antibiotics. Demonstration of this type of resistance is more difficult than detection of  $\beta$ -lactamase, which is still the most common cause of aminopenicillin resistance in *H. influenzae*.

Analysis of a set of 228 *H. influenzae* strains revealed that the highest possible capture (99.5%) of isolates with non-enzymatic resistance due to mutations in the *ftsI* gene can be achieved in routine practice by simultaneous examination of penicillin, ampicillin, amoxicillin and cefuroxime disc diffusion method according to the EUCAST methodology. The currently recommended EUCAST method using only penicillin had a lower mutation detection rate of 95.7%.

Sequence analysis showed considerable variability in *ftsI* gene mutations. In the set of 228 strains, a total of 37 different combinations of amino acid substitutions were found to occur at 23 positions in the PBP3 protein (V329I, D350N, S357N, A368T, M377I, S385T, A388V, L389F, P393L, A437S, I449V, G490E, I491V, R501, A502V, V511A, R517H, I519L, N526K, A530S and T532S). The most common combination (35%) of amino acid substitutions was the combination D350N, M377I, A502V, N526K. Epidemiological typing does not indicate a spread of particular MLST type/clone. A total of 74 STs were found, forming six clonal complexes (CCs) with a founder (ancestor) found, eight CCs without a founder found, and 33 singletons. The most widespread ST 1034 was associated mainly with a combination of mutations D350N, M377I, A502V, N526K.