Rifampicin resistant strains in [i]Neisseria meningitidis[i] – Mechanism of acquisition, reversion [i]in vitro[i] and impact on fitness.

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The molecular mechanism leading to Rifampicin resistance in meningococci has been characterised previously. Responsible for acquisition of resistance is a single point mutation within a defined subgenic region of [i]rpoB[i], the gene locus coding for the prokaryotic RNA polymerase. Using increasing selection pressure we have selected a collection of strains which displayed Rifampicin resistance following a couple of steps of slightly increasing minimal inhibitory concentrations (MIC). These strains were found to harbour –however- only a single point mutation, too, mainly the most common amino acid substitution described so far. PCR of the [i]mtrR[i] promoter region (carrying an insertion sequence in meningococci) did not reveal any change in length of the amplicon.
Four strains (one sensitive control strain, two of the strains selected for resistance and one strain which was isolated already resistant from a patient) were chosen for a long term experiment to find out more about the stability of Rifampicin resistance [i]in vitro[i]. The strains were propagated in cell culture medium for up to 420 generations (previous experiments have shown a generation time of ap. 2.3 hrs in the medium chosen). One of the strains reverted to a sensitive phenotype during the experiment. Sequence analysis of the subgenic [i]rpoB[i] fragment revealed that the known amino acid substitution was simply reverted. E-test which were performed on every fifth day of the long term experiment indicated a shorter [i]in vitro[i] generation time for the Rifampicin sensitive strain.
These data indicate that Rifampicin resistance is spontaneously revertible and gives further proof that only a single point mutation causes resistance.