Rifampicin resistant strains in *Neisseria meningitidis* - Mechanism of acquisition, reversion *in vitro* and impact on fitness.

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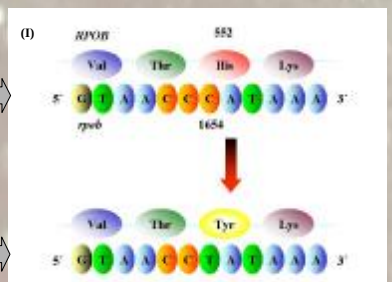
Introduction to the molecular basis of rifampicin resistance in meningococci:

Meningococci (*Neisseria meningitidis*) can acquire rifampicin resistance spontaneously under treatment. The molecular mechanism has been described as a single point mutation in the *rpoB* gene (I) coding for the ß-subunit of DNA-directed RNA-Polymerase (CARTER et al 1994, NOLTE 1997).

Rifampicin susceptible strain

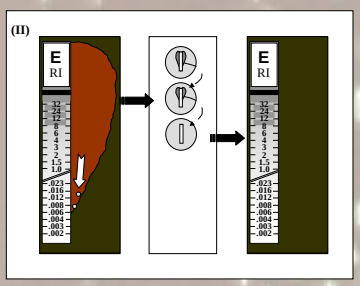
Rifampicin resistant strain



(I): The most common point mutation (His^{552} >> Tyr^{552}) in *RPOB* leading to rifampicin resistance in meningococci.

Question 1: What`s about stepwise acquisition of rifampicin resistance?

Experimental outline (II) to select strains by applying selection pressure:



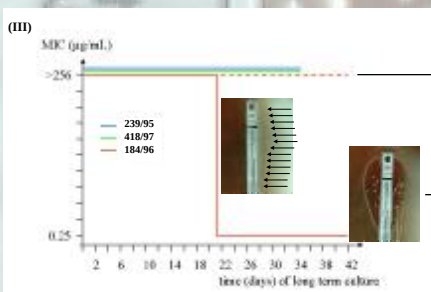
14 strains selected in vitro towards rifampicin resistance

(data from NOLTE et al (submitted (b))

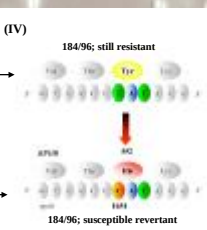
Following a mean of 5.43 +/- 0.51 passages over E-tests 13 of the 14 strains displayed only one amino acid substitution in the subgenic *rpoB* fragment when compared to their susceptible parental strains The remaining strain displayed two amino acid substitutions.

Question 2: What`s about stability of rifampicin resistance in vitro?

Experimental outline to study stability:
Three resistant strains were kept in long term culture over a minimum of 36 days with an average of about 10 generations per day (III).
The meningococci were propagated in liquid cell culture medium (RPMI1603) with passages every 24 hrs.
On every fifth day susceptibility of the strains was assayed by E-test. *rpoB* amplicons of each strain were sequenced (IV).



(III): Result of long term culture: One of three strains tested reverted to the susceptible phenotype.

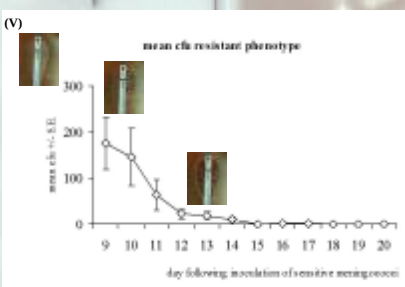


(IV): The strain which lost it's rifampicin resistance displayed the wildtype genotype in *rpoB*. The resistance determining mutation was reverted.

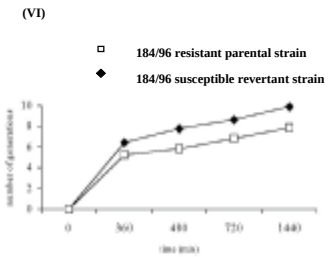
(data from NOLTE et al (submitted)

Question 3: What`s about differences in the fitness of rifampicin resistant and susceptible meningococci?

Experimental outline to study fitness:
The resistant strain 184/96 was grown for two days (passage after 24 hrs) before one colony of it's susceptible revertant was added to the culture.
E-test`s were performed directly from the culture daily.
As soon as the number of colonies within the inhibition ellipse was countable this number was recorded (V).
Both strains were cultured in separate containers to determine the generation time and the number of generations per 24 hrs (VI).



(V): Demonstration of higher fitness: The susceptible revertant overgrew it's resistant parental strain.



(VI): The resistant strain displayed lower number of generation within 24 hrs when growing in liquid cell culture medium. The growth rate was normalised to the initial inoculum.

(data from NOLTE et al (submitted)

Conclusions:

The main mechanism of acquiring rifampicin resistance is a one step mutation in *rpoB* leading to an amino acid substitution. Even strains which were selected by a number of passages (displaying increasing MIC's during this procedure) were found to harbour only one point mutation. However, these strains approached to a MIC of 4 µg/mL over various numbers of passages, becoming fully resistant with the next passage.
High range rifampicin resistance seems to be not necessarily associated with membrane properties as a high range resistant strain spontaneously reverted to the susceptible phenotype by a simple "back-mutation" in the *rpoB* gene.
The fitness of the rifampicin resistant phenotype is lower (in terms of growth rate) compared to the susceptible parental one. This finding may explain the low frequency with which rifampicin resistant strains are isolated from the population.

Cited literature:
Carter et al (1994): Molecular characterisation of Rifampin-resistant Neisseria meningitidis. *Antimicrobial Agents & Chemotherapy* 38,1256-1261;
Nolte, O. (1997): Rifampicin resistance in *Neisseria meningitidis*: evidence from a study of sibling strains, description of new mutations and notes on population genetics. *Journal of Antimicrobial Chemotherapy* 39,747-755;
Nolte et al (submitted): High level Rifampicin resistance in *Neisseria meningitidis* can revert spontaneously in vitro and is associated with reduced growth rate. *Journal of Antimicrobial Chemotherapy*;
Nolte et al submitted (b)): Description of new mutations in the *rpoB* gene in Rifampicin resistant *Neisseria meningitidis* selected *in vitro* in a step wise manner. *Journal of Antimicrobial Chemotherapy*