Summary

The development of fluoroquinolone resistance in *E. coli* is associated with the overexpression of AcrAB/TolC efflux pump. The functionality of this pump seems to be required for the emergence of mutants with *target* mutations in genes coding for gyrase (e.g. *gyrA*). While single point mutations in *gyrA* do not lead to fluoroquinolone resistance of clinical relevance (i.e. MIC ≥ 4 µg/mL) a combination of at least three mutations in different genes does. During the selection, acquisition of *non target* mutations affecting the upregulation of the AcrAB/TolC efflux pump, e.g. *acrR*, *marO* and *marR*, are involved.

In this work reporter gene systems were developed to enable quick, easy and sensitive determination of alterations in the expression of *acrAB* and *acrEF*. This allowed to determine the impact of several factors (permanent, like mutations or transient, like induction phenomena) on the expression of *acrAB* in the development of fluoroquinolone resistance in *E. coli*. Furthermore the role of homologous efflux pump AcrEF/TolC was to be investigated.

These reporter gene plasmids were constructed by fusing the respective promotors *pacrAB* and *pacrEF* to the reporter gene *luc* encoding firefly luciferase. After transformation of different *E. coli* strains as well as *Salmonella* wildtype and mutant cells with the resulting recombinant plasmids the activity of promotors were quantitated by determining the intensity of luciferase induced light emission.

Subinhibitory concentrations of ciprofloxacin and ofloxacin showed a slight reduction of the activity of *pacrAB* (15-20 %). This is contrary to the assumption that the funcionality of AcrAB in the emergence of *target* mutations is due to the induction by fluoroquinolones. However, for *gyrA* single mutants an increasing acitivity of *pacrAB* in correlation with known results (reduced ciprofloxacin accumulation) was shown. This supports the idea that overexpression of *acrAB* has an impact on the emergence of subsequent *target* mutations in genes coding for gyrase and topoisomerase IV. A reduced expression of *acrAB* of about 40-50 % was shown for the *gyrB*-mutant KD112 and in the presence of subinhibitory concentrations of novobiocin. These results point to a role of variations in local supercoiling for the expression of *pacrAB*.

The reporter gene systems were used for identification and characterization of new mutations in the *mar*-operon (*marO*, *marR*). Variations in the expression of *acrAB* genes determined in the different mutants were not associated with differences in MICs (phenotypical). Adding salicylate as an inductor of the *mar*-operon to *E. coli* wildtype WT and *marR*-mutant MII resulted in an increased activity of *pacrAB* in both strains. Due to the finding that salicylate has an effect on the expression of *acrAB* in a strain with a *marR*-deletion, too, these findings point to the existence of *mar*-dependent as well as a *mar*-independent factors for the induction of the *acrAB* expression.

Using structural data retrieved from the Brookhaven PDB of MarR-dimer as a template, several structural 3D-models of *marR*-point mutants were calculated, revealing additional information to explain the molecular basis for the resistance phenotype.

Furthermore, the activity of *pacrAB* was determined for two fluoroquinolone resistant *marR*-deletion mutants showing a reduced fitness as well as for derivatives obtained from these mutants after incubation in the absence of selective pressure by fluoroquinolones. These latter strains show a compensation of the reduced fitness and enhanced susceptibility. Both have been shown to carry a single *marA*-mutation resulting in an amino acid exchange (I58N or N21Y). To investigate a possible role of

mar-regulated functions in the fitness of antibiotic resistant cells, *marR* and *marA* genes were provided in trans for complementation assays.
The results of the susceptibility testing confirmed that the *marR*-deletion in the parental strains contributes to the multiple antibiotic resistance (*mar*) phenotype. This effect of the *marR* deletion is lost in the *marR*-*marA*-double mutants. Using the reporter gene systems it was demonstrated that the *marA*-mutation was associated with a reduced activity of *pacrAB* down to the level of the wildtype. Providing a *marA*-overexpression vector in trans this effect as well as the reduced susceptibility to different antibiotics was partially reversed compared to the parental strains (Δ*marR*-mutants). A possible reason for the incomplete complementation could be that a mutated and an intact MarA monomere compete for the formation of binary complex with RNA-polymerase. Another although rather hypothetical explanation might be the presence of an additional unknown mutation. 3D-models of MarA bound to the marbox for the two *marA*-point mutants were calculated based on strucutral data from the PDB of the MarA-monomer.
For investigation of regulatory interaction between AcrAB/TolC and AcrEF/TolC deletion mutants of *E. coli* were used lacking either *acrAB* or *acrEF*. These mutants were transformed with either reporter gene plasmids. Only for the *acrAB*-deletion mutant a slight increase in *pacrAB*-activity was detectable (150 %). The expression of *acrEF* is equivalent to the background activity of the reporter gene systems in both strains and, hence, seems to play a minor role for the mdr efflux of the cells. It seems that in response to the *in vitro*-generated *acrAB*-deletion an increase of *acrAB* expression is a compensatory reaction, implying that the cells are able to sense the content of functional AcrAB. Maybe in a subsequent selection step the mutant acquires compensatory mutations.
An increase in the activity of *pacrEF* was shown in two *marR*-deletion mutants (150 % and 290 %, respectively) as well as in the presence of salicylate (180 %). But these results are of unknown relevance, because of very low absolute values for *pacrEF* activity. Due to this very low level of the expression of *acrEF* it seems that mdr efflux due to this pump plays a minor role in these *E. coli*-strains.
The newly developed reporter gene systems provide a powerful tool for the determination of the *pacrAB*- and *pacrEF*-activity in various mutants, e.g. to monitor *in vivo* the expression in infection models. In addition, they offer the possibility to screen for inhibitors of the expression of *acrAB* resulting at least in the - partial - reversion of antibiotic resistance.