P0204
Poster Session I
Basic science: pathogenesis of staphylococci

MUTATIONS IN THE FABI PROMOTER OFTEN ASSOCIATE WITH ALTERNATIVE MECHANISMS OF TRICLOSAN RESISTANCE IN STAPHYLOCOCCUS AUREUS

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Objective. The enoyl-acyl carrier protein (ACP) reductase enzyme (FabI) is the target for a series of antimicrobial agents including novel compounds in clinical trial and the biocide triclosan. Mutations in *fabI* and heterodiploidy for *fabI* have been shown to confer resistance in *S.aureus.* We investigated the molecular nature of *fabI* overexpression in *S. aureus* isolates and in *in vitro* selected mutants with reduced susceptibility to triclosan.

Methods. Promoter analysis was performed on 38 triclosan resistant and 19 susceptible clinical strains, as well as 23 laboratory mutants selected after exposure to triclosan. The promoter region was amplified by PCR and sequenced. Furthermore, 7 laboratory mutants and their 3 corresponding parental strains were subjected to all genome sequencing. The gene expression profile of 4 clinical strains and 4 laboratory mutants with mutations in the promoter regions was compared to their wild type, triclosan susceptible isogenic or prototypical strains, using a self-designed custom array containing probes targeting genes from 2 *S.aureus* strains and a variety of genes of plasmid origin.

Results. Mutations in the *fabI* promoter were found in 18.4 % of triclosan resistant clinical isolates, regardless the molecular mechanism conferring resistance. This suggests that mutations in the *fabI* promoter do not confer resistance by themselves, but are associated with other mechanisms of resistance. Some of the mutations identified in the clinical isolates were also detected in a series of laboratory mutants, indicating that the *in vitro* model used in this study mimics selective pressure in the field. The number of mutations detected by whole genome sequencing in laboratory mutants selected by multiple passages in triclosan varied from one to nine mutations per genome. Only mutations in the *fabI* locus were shared by all strains and none of the other mutations were common to more than one strain. To investigate the effect of *fabI* promoter mutations on gene expression, microarray analysis of laboratory mutants and clinical isolates were performed in the absence of triclosan. The comparisons between *in vitro* selected mutants and their isogenic wild type strains identified *fabI* as one of the few significantly up-regulated genes. A similar comparison between triclosan resistant clinical isolates containing mutations in the *fabI* promoter and sensitive prototypical strains also demonstrated a significant up-regulation of *fabI* gene. 37 Genes were found to be up-regulated and 6 genes down-regulated in all 4 clinical strains. Importantly, none of these genes were involved in efflux mechanisms, a known determinant of triclosan resistance in gram negative organisms.

Conclusion. In conclusion, we have shown that mutations in the *fabI* promoter region confer *fabI* up-regulation in both clinical isolates and/or laboratory mutants. These data provide the first genetic evidence linking promoter mutations with up-regulated expression of the *fabI* gene.