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Poster Session VI
New quinolones, oxazolidones, and a chimera of both

MODE OF ACTION OF MCB3681 - ANALYSIS OF MCB3681 PROTEOME SIGNATURE

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Objectives: The investigational agent MCB3681 is a novel quinolone-oxazolidinone antibacterial interacting with bacterial type II topoisomerases and inhibiting protein synthesis. MCB3681 is antibacterially active against Gram-positive pathogens irrespective of whether the isolates are methicillin-, and/or vancomycin-, quinolone-, and/or linezolid-resistant. As MCB3681 is active against ciprofloxacin and linezolid double-resistant mutants whereas a fixed 1:1 combination of ciprofloxacin and linezolid is not active, it was hypothesized that MCB3681 interacts with an additional target other than the typical quinolone and oxazolidinone targets.

Methods: The effect of MCB3681 on growing *S. aureus* RN1HG001 cells has been analyzed; 2D gel electrophoresis combined with MALDI-TOF-MS/MS for protein identification was used to analyze the adjustment of the soluble proteome of *S. aureus* RN1HG001 to short-term exposure (10 min) to MCB3681. Proteins were pulse labeled (5 min) with L-[^{35}S] methionine and the cytosolic proteins were analyzed. Proteins for preparative 2D gels were obtained from cells exposed for 30 min to 4 mg/L MCB3681. For protein identification, spots were cut from preparative gels. Spots were identified by mass spectrometry and quantified using the Delta 2D software. The labeling experiments were repeated three times.

Results: Synthesis of 29 proteins was reprogrammed by short-term exposure of *S. aureus* to supra-inhibitory concentrations of MCB3681: synthesis of 13 protein spots was increased, and synthesis of 16 spots was decreased; 27 out of the 29 protein spots with altered synthesis could be identified. All identified spots were cytosolic proteins and only two had no function assigned to them. Among the proteins upregulated in MCB3681 treated cells were three ribosomal proteins and PyrR, a bifunctional protein which functions as uracil phosphoribosyltransferase and as regulatory protein of pyrimidine synthesis. Downregulated in MCB3681 treated cells were proteins from different amino acid synthesis pathways (GlyA, MetE, GlnA) and two aminoacyl-tRNA synthetases (IleS, AspS). Furthermore, synthesis of methicillin-resistance factor protein FemB was significantly lower in *S. aureus* cells treated with MCB3681.

Conclusions: MCB3681 interferes with the established quinolone- and oxazolidinone-typical targets, i.e. DNA-gyrase and topoisomerase IV as well as protein synthesis. Transcriptional response of *S. aureus* RN1HG001 to MCB3681 revealed that: 1) MCB3681 was interacting with a fourth target, i.e. two aminoacyl-t-RNA synthetases. 2) In addition, the synthesis of the gene product mediating methicillin resistance (FemB) was inhibited by MCB3681. The interaction with four targets may explain the low propensity for resistance development, the maintained activity against quinolone-/oxazolidinone-double-resistant strains and marked activity against multidrug-resistant isolates; reduced FemB-synthesis may contribute to the pronounced activity against methicillin-resistant staphylococci.