ANTIBIOTICS DRIVEN DYSBIOSIS MEDIATE INTRALUMINAL AGGLUTINATION AND ALTERNATIVE SEGREGATION OF *ENTEROCOCCUS FAECIUM* FROM THE INTESTINAL EPITHELIUM

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Background and objective. The microbiota is a complex bacterial ecosystem within the mammalian gastrointestinal tract and is important for colonization resistance towards pathogens. Patients are often administered antibiotics that perturb the microbiota, leading to a decrease in colonization resistance and intestinal proliferation of drug-resistant pathogens like *Enterococcus faecium* (*Efm*). This process is not fully understood yet. Therefore, the objective was to unravel the host events that occur after antibiotics induced dysbiosis and subsequent outgrowth of *Efm* in mice.

Methods. Group A + B mice were treated 2 days with ceftriaxone, inoculated with *Efm* E980 or E1162, and left on cefoxitin for 10 days. Group C + D were mock treated and inoculated with E980 or E1162. Control mice (E + F) were treated with antibiotics only or left untreated. Microbial composition and *Efm* was monitored by 16S rRNA gene sequencing and by enumeration of CFU's from faeces. Colon/cecum tissue was analyzed by light (LM), scanning electron microscopy (SEM) and immune fluorescence (IF).

Results and Discussion. 16S rRNA gene sequencing and plating CFU's from faeces demonstrated perturbation of the microbiota of Group A + B mice and outgrowth of E980 and E1162 in the cecum/colon, while C + D mice were low-level colonized with *Efm* and E + F not. SEM + LM on cecum/colon tissue showed agglutinated *Efm* in an extracellular matrix apically of intestinal epithelial cells (IECs; A + B), while C + D + F mice revealed diverse microbiota. LM on H&E-, Gram- or PAS-stained cecum/colon sections and IF revealed a reduced colon wall and mucus-associated microbiota layer, a diminished Muc2 mucus layer and Muc2 protein in group A + B + E mice compared to untreated C + D + F mice. IF on cecum/colon sections revealed deformed junctions and a spatial segregation of *Efm* from IECs in a matrix consisting of secretory IgA (sIgA), polymeric immunoglobulin receptor (pIgR) and E-cadherin (Ecad) *in vivo*, while the mucus layer was diminished. The host proteins were also detected in the *Efm* agglutinations in the cecum/colon while recombinant pIgR, Ecad and IgA agglutinated *Efm in vitro*. Thus, cephalosporin-driven microbiota perturbation diminished the mucus layer and allowed *Efm* to proliferate in the gut. While the mucus layer is reduced, the host devised an alternate mechanism to separate *Efm* from host IECs by agglutinating *Efm* in a matrix of sIgA, pIgR and Ecad.