

Impact of *per os* antibiotic treatments on the gastrointestinal colonization with a multiresistant *Klebsiella pneumoniae* strain

Colonization resistance refers to the ability of microbiota to prevent expansion and persistence of exogenously acquired bacterial species. It is a pivotal defense mechanism that can be impaired by antibiotic treatment. Previous research shows that changes in microbiota composition, mainly the elimination of specific groups of anaerobic bacteria lead to multi-drug resistant bacteria domination of the gastrointestinal tract in antibiotic-treated mice.

In this study our goal was to determine the effect of different *per os* antibiotic treatments on colonization rate and resistance gene copy numbers in intestinally carried *K. pneumoniae* (KP5825) in mice. This strain expressed a PMQR gene (*aac(6')-Ib-cr*) an extended-spectrum β -lactamase enzyme (CTX-M-15) as well as a carbapenemase enzyme.

All experiments were carried out using 6–8 weeks-old C57BL/6 male mice. For colonization, 5×10^6 CFU of KP5825 was administered orally on the 14th and 15th days of ampicillin pretreatment. After the colonization mice were divided into 6 groups. These groups received the following antibiotic treatments for 15 days: 0.5g/L ampicillin (AMP), 0.1g/L ceftazidime (CAZ), 0.5g/L ceftazidime, 0.1g/L ciprofloxacin (CIP) and 0.5g/L ciprofloxacin was administered in the drinking water (6th group: control). Fresh fecal pellets were collected from the animals every 3 days throughout the 15 days period. From the fecal samples microbial DNA was extracted.

In the second phase of our experiments the V3-V4 region of the 16S rRNA gene was amplified and sequenced with the Illumina MiSeq platform to determine the microbiota composition in the intestine of colonized mice. We used qPCR technique to determine the KP5825 DNA quantities and assess the resistance gene copy numbers.

Our results show a significant perturbation in the intestinal microbiota. We could also observe that after 15 days KP5825 decreased significantly ($p < 0,004$) in the control group and in the CIP treatment groups ($p < 0,0001$, $p < 0,002$). In the AMP and CAZ treated groups the amount of KP5825 DNA did not change.

Furthermore, our qPCR results show that higher dose CAZ treatment lead to 4-fold increase in quantities of plasmids with CTX-M. Thus, the CAZ had higher power on the dissemination of resistance genes than CIP. This raises the possibility of in vivo plasmid transfer, which we intend to investigate in our future experiments.