

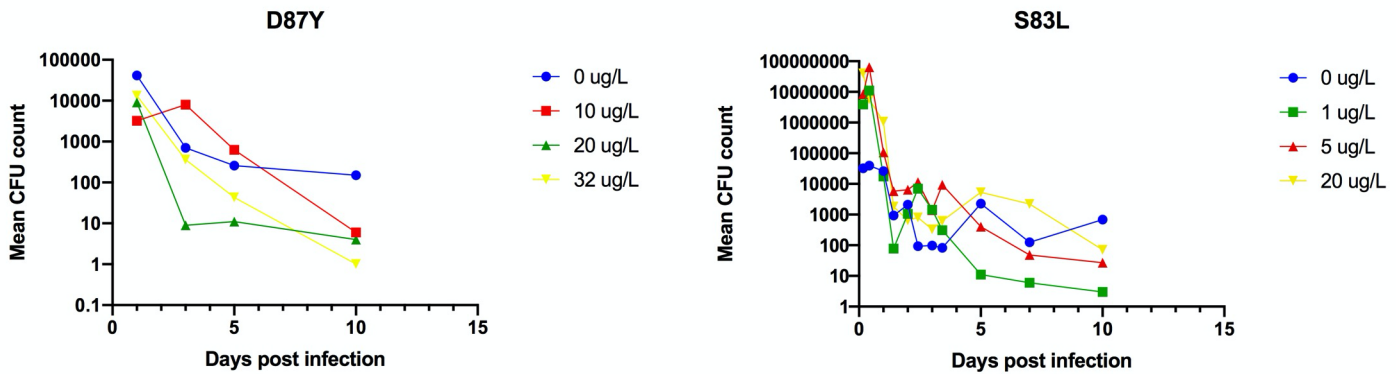
Supplementary Information

Water levels of ciprofloxacin

The limit of quantification (LOQ) of the ciprofloxacin analysis was 10 ng/L, the linearity (R²) of the calibration curve ranged between 0.9322 and 0.9998, and the relative standard deviation between triplicates for both 0h and 24h samples (triplicate pre-treatment and analysis of one water samples collected) ranged from 1 to 30% in total for all six experiments. The average daily exposure level of ciprofloxacin (calculated using the results of the 24h samples including pseudo triplicate samples) of each experiment was 10 (n=19), 20 (n=14), 35 (n=14), 196 (n=14), 1 990 (n=18) and 23 814 (n=14) ng/mL with a relative standard deviation of 17, 22, 10, 5, 7 and 7%, respectively. The difference between the average daily water concentrations measured in 0h samples and 24h samples, for the six experiments, varied from almost none ($\pm 0.7\%$) to a 18% decrease and a 13% increase, respectively. Thus, the concentration differences between freshly prepared water and after 24h were minor with no clear trend and could have been the results of differences in the preparation of the experimental water, the water sampling, adsorption to surfaces/particles and water evaporation during the experiments as well as differences in pre-treatment and analysis of the samples.

Mallard competition experiments, CFU counts

Fig S1.



In the *in vivo* experiments, colony forming units (cfu) of the combined amount of Cip^R and Cip^S mutants on 500 ug/ml Streptomycin EMBL plates were counted. From $gyrA^{wt}/gyrA^{D87Y}$ (A) and $gyrA^{wt}/gyrA^{S83L}$ (B), the mean value of each group and day are plotted with inserted connecting lines.

Table S1A/B. The patching results from *in vivo* $gyrA^{wt}/gyrA^{D87Y}$ and $gyrA^{wt}/gyrA^{S83L}$ competitions. Both mutants were selected on EMBL plates supplemented with 500 μ g/ml streptomycin. 50 colonies from each streptomycin plate were patched to a 0.04 μ g/ml ciprofloxacin EMBL plate. Cip^R = ciprofloxacin resistant mutant, Cip^S=ciprofloxacin susceptible mutant. If there was less than 50 cfu on streptomycin plates, all available colonies were patched. X = no growth on streptomycin EMBL plates. To enable ratio calculations, 0 in the nominator or denominator was changed to 1 in relevant cases (*). Index=index bird, T=transmission bird.