

Antibiotics and pathogens, when the natural bacterial community resists!

The spread and persistence of antibiotic resistances (AR) in waters is a major threat for the environmental management, with direct risks for human health. Although antibiotic resistant bacteria (ARB) are found everywhere, their ecological success is related to anthropic impact, and to disturbed natural communities. In the same waters where AR can be a problem, other disturbances promoted by human activities can interfere with natural communities, e.g. the input of allochthonous (and potentially pathogenic) bacteria. The concomitant impact of those disturbances can have dramatic effects on the persistence and resistance of natural communities. With an experimental system on continuous cultures, we tested the impact on the microbial community from anthropized Lake Maggiore of enhanced tetracycline concentrations and of a subsequent invasion by a mixed population of *E. coli*. We measured the response in terms of bacterial and eukaryotic abundances, phenotypical distribution, bacterial community composition, spread and persistence of tetracycline resistance genes, and the fate of *E. coli* strains, by applying a number of techniques including flow-cytometry, microscopy, qPCR, and Illumina sequencing. We observed the preservation of AR genes, even when antibiotics were not in the system, suggesting a relatively small cost of maintenance of these genes, or their involvement in other genetic pathways. However, we did not see a general increase of e.g. *tetA* resistance genes, following the addition of antibiotics. In fact, it rather seemed that resistance to tetracycline was rather acquired by morphological changes and species interactions; we observed many more aggregated cells when the antibiotic was added. On the other hand, the invasion with *E. coli* seemed to generally promote the overall productivity of the systems without and with little antibiotic. Our results demonstrate that despite the heavy disturbance the natural microbial communities can develop a number of mechanisms of resistance in order to reduce the impact (e.g. aggregation), complicating the system modelling, thus reducing our forecasting possibilities.

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