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ID412 | Bacterial population variability in wastewater treatment and thpotential persistence of specific lineages

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Wastewater treatment plants (WWTPs) are essential barriers to attenuate the emission of contaminants, such as antibiotic-resistant bacteria (ARB) and resistance genes (ARGs), which monitoring is becoming a requirement (Directive (EU) 2024/3019). Despite the urgent need to improve wastewater treatment to remove ARB&ARGs, the complexity of microbial community dynamics during treatment remains poorly understood. This work discusses the bacterial community variations in full-scale real-world systems: i) between inlet and outlet of a WWTP with three full-scale activated sludge treatment lines, ii) due to ozonation, and iii) impact on the receiving river. Analyses included total coliforms and *E. coli* counts, quantitative PCR of selected genes (e.g. 16S rRNA, IS26, blaOXA-58, blaVEB), 16S rRNA gene metabarcoding, isolates identification by Maldi-TOF, and genome-based inference of ARB dissemination.

Activated sludge treatment led to important variations on bacterial community composition, being genera affiliated to the phylum Candidatus Saccharibacteria, followed by those affiliated to the class Betaproteobacteria those that most frequently increased after treatment. Members of the genera *Acinetobacter*, *Aeromonas* and *Aliarcobacter* were among the most abundant in the inlet and persisted among the most abundant in the outlet. Other genera such as *Bacteroides*, *Enterococcus*, *Parabacteroides*, *Streptococcus* or *Trichococcus* decreased with the treatment. Ozonation was associated with the increase of members of the genera *Acidovorax*, *Flavobacterium* and *Romboutsia* and post-storage reactivation with the increase of *Cloacibacterium* and *Flavobacterium* and decrease of *Romboutsia*. Considering a group of 12 ARGs and MGEs, abundance decrease was observed after the activated sludge treatment and no impact was observed in the receptor river. The abundance (copies/mL) of the genes *sul1* and *blaOXA-58* increased after storage of the ozonated effluent up to 0.9 log-units.

Presumptive coliforms isolated (~400) on CCA (Chromogenic Coliform Agar) or on CCA supplemented with cefotaxime (4 mg/L) were identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter* spp., and other and 16.4% (72/439) were multidrug resistant. The whole genome sequence of *Klebsiella pneumoniae* and *Citrobacter freundii* recovered from the inlet, outlet, after ozonation and river suggest clonal dissemination, for example a *K. pneumoniae* ST29 with plasmids ColRNAI and incFIB(K) from the WWTP to the river.