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Proteobacteria become predominant during regrowth after water disinfection☆

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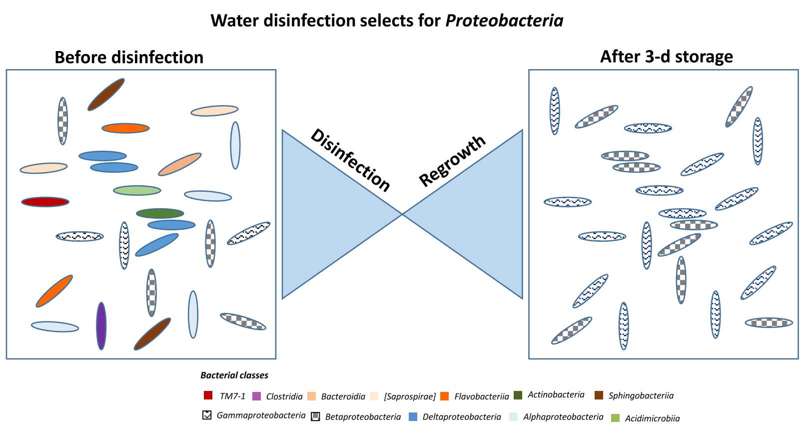
HIGHLIGHTS

- Surface water and secondarily treated urban wastewater were disinfected.
- UV, ozonation and photocatalytic ozonation processes were used.
- Bacterial regrowth occurred after water disinfection.
- Stored disinfected water had higher proportion of *Gamma-* and *Betaproteobacteria*.
- *Pseudomonas*, *Acinetobacter* or *Rheinheimera* were among the selected groups.

GRAPHICAL ABSTRACT



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ABSTRACT

Disinfection processes aim at reducing the number of viable cells through the generation of damages in different cellular structures and molecules. Since disinfection involves unspecific mechanisms, some microbial populations may be selected due to resilience to treatment and/or to high post-treatment fitness. In this study, the bacterial community composition of secondarily treated urban wastewater and of surface water collected in the intake area of a drinking water treatment plant was compared before and 3-days after disinfection with ultraviolet radiation, ozonation or photocatalytic ozonation. The aim was to assess the dynamics of the bacterial communities during regrowth after disinfection.
In all the freshly collected samples, *Proteobacteria* and *Bacteroidetes* were the predominant phyla (40–50% and 20–30% of the reads, respectively). Surface water differed from wastewater mainly in the relative abundance of *Actinobacteria* (17% and <5% of the reads, respectively). After 3-days storage at light and room temperature, disinfected samples presented a shift of *Gammaproteobacteria* (from 8 to 10% to 33–65% of the reads) and *Betaproteobacteria* (from 14 to 20% to 31–37% of the reads), irrespective of the type of water and disinfection

☆ "We will always remember Cristina not only as an excellent professional, but above all as an extraordinary and lovely person. With esteem and deep affection from all Cristina's colleagues from Porto."

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Surface water
Selective advantage

process used. Genera such as *Pseudomonas, Acinetobacter* or *Rheinheimera* presented a selective advantage after water disinfection. These variations were not observed in the non-disinfected controls. Given the ubiquity and genome plasticity of these bacteria, the results obtained suggest that disinfection processes may have implications on the microbiological quality of the disinfected water.

1. Introduction

Water quality management plays a central role on the Millennium Development Goals, since it contributes, directly or indirectly, to the access to safe drinking water and basic sanitation, the sustainability of the environmental resources and the wellbeing of populations (GEMS-Water, 2014; UNICEF/WHO, 2015). Proper wastewater treatment is then a crucial step to prevent the environmental contamination of the receptor water bodies, either surface or groundwater, particularly in geographic regions with intense demographic growth and scarcity of freshwater, where the same water body (e.g. river) receives wastewater discharges and serves as source for drinking water production.

Sewage contains a multitude of chemical and biological pollutants, including pathogens, which removal by the so-called conventional wastewater treatment processes cannot be assured, raising the risks of contamination of the drinking water resources (Fatta-Kassinos et al., 2011a; UNICEF/WHO, 2015). Conventional processes for water treatment were designed about one century ago aiming at reducing the organic load of the wastewater, as well as the pathogens, which by competition with the microorganisms responsible for the secondary treatment are expected to be eliminated upon treatment (Tchobanoglous et al., 2003). However, the profound change in the society, particularly the dramatic demographic increase in some regions and the intense development of the pharmaceutical industry, has made the conventional treatment ineffective (Melvin and Leusch, 2016). For instance, harmful loads of both anthropogenic chemical organic compounds and microorganisms, such as pathogenic bacteria, viruses, fungi, protozoa or helminth parasites, and antibiotic resistant bacteria, are known to persist in secondarily treated wastewater (Fatta-Kassinos et al., 2011a; Fatta-Kassinos et al., 2011b, Manaia et al., 2016; Melvin and Leusch, 2016, Varela and Manaia, 2013). Indeed, a broad range of full or opportunistic pathogenic and commensal bacteria harbouring acquired genetic determinants of resistance or virulence are commonly found in treated wastewater (Cai and Zhang, 2013; Stevik et al., 2004; Varela and Manaia, 2013).

Advanced treatment technologies emerged as a way to tackle these risks, aiming to reduce the discharge of chemical emerging contaminants into the receiving water bodies, as well as to reduce the microbial load in the final effluent to levels compatible with local regulations and directives. For example, environmental quality standards, such as priority substances concerning aquatic ecosystems, were recently updated at the European Union level in Directive, 2013/39/EU (Directive, 2013; Ribeiro et al., 2015a), and a list of substances for Union-wide monitoring in the field of water policy was defined in the Watch List of Decision 2015/495/EU (Decision, 2015; Barbosa et al., 2016). Guidelines the quality of wastewater for reuse have been also established in different regions of the world and include recommended levels of different physicochemical parameters and indicator bacteria (Becerra-Castro et al., 2015). Ultraviolet radiation (UV) and ozonation (O_3) are examples of technologies commonly used for water or wastewater disinfection (EPA, 1999a; EPA, 1999b; Victoria, 2002), but they are still considered less conventional than other processes such as chlorination in the case of drinking water disinfection (Victoria, 2002). While UV inactivates microbial cells through mutagenic activity, O_3 (a chemical oxidation technology) promotes oxidation of both organic molecules and cellular structures. Improved technologies conceptually based on the generation of highly reactive species, such as hydroxyl radicals (Comninellis et al., 2008), are designated generically as advanced oxidation processes (AOPs). The Fenton process and titanium dioxide (TiO_2) photocatalysis (under solar or artificial radiation) are some well-known AOPs, and the coupling or integration of processes is also possible, such as photo-Fenton or photocatalytic ozonation (Beltrán et al., 2012; Moreira et al., 2015; Moreira et al., 2016; Spasiano et al., 2015). However, some of these processes, such as photocatalytic ozonation, was not applied yet at full-scale. Nevertheless, efforts are needed to develop more advanced technologies to face actual water pollution concerns, such as organic micropollutants and microorganisms. For instance, the Swiss parliament approved in 2011 a strategy to reduce the micropollutants by 80% at WWTP effluents, and large-scale pilot advanced treatments have been tested in this direction (Bui et al., 2016; Margot et al., 2013).

In any case, although known to be effective on the inactivation of microorganisms (e.g., EPA, 1999a; EPA, 1999b; Victoria, 2002; Garvey and Rowan, 2015, Norton-Brandao et al., 2013; Rizzo et al., 2013; Rizzo et al., 2014; Zapata et al., 2010), less attention has been given to the effect of advanced treatments on the dynamics of the bacterial communities, i.e. the variations on the whole bacterial microbiome composition and structure. Most of the studies available are focused on bacterial removal and regrowth potential, based on the monitoring of some indicator groups, such as *Escherichia coli* or enterococci, which in spite of their importance to assess treatment efficiency, neglect the complexity of the whole water microbiome (e.g., Bohrerova et al., 2014; Chong et al., 2010; Fiorentino et al., 2015; Giannakis et al., 2015; Rizzo et al., 2014). In fact, the best advanced water treatment technologies would be those capable of selectively removing chemical and microbial contaminants without disturbing the ecosystem; however, it may be difficult to cope with these objectives, since water habitats comprise an impressive chemical complexity and bacterial diversity (Tamames et al., 2010; Vaz-Moreira et al., 2014). Taking into account the structural and physiological diversity of bacteria, it is expected that different populations will respond differently to disinfection and will exhibit different capacity to recover. How clear would be such differences of response, or if they would differ with the type of advanced treatment technology applied, were the underlining questions of this study.

The hypothesis of this study was that water disinfection processes, given the incapability to eliminate the whole water microbiota, may have the potential to select some bacterial groups characterized by a higher resilience to the advanced treatment or higher fitness to recover after disinfection. To test this hypothesis, secondarily treated wastewater samples from two urban wastewater treatment plants (WW) and surface water samples collected in the supply area of a drinking water treatment plant (SW) were treated using UV, O_3 or photocatalytic ozonation ($PhotO_3$). In all replicated experiments, the treatment conditions were controlled, assuring that the water microbiota composition was the major variable. The bacterial community composition was analysed based on 16S rRNA gene barcode 454-pyrosequencing, in disinfected water after storage for 3-days after treatment (T3) and compared with the same samples before treatment (C0) and non-treated stored samples under the same conditions (C3).

2. Materials and methods

2.1. Water samples

Twelve grab water samples of secondary effluents from urban wastewater treatment plants and of surface river water, all located in Northern Portugal, were analysed in this study. The main characteristics