



Impacts of reclaimed water irrigation on soil antibiotic resistome in urban parks of Victoria, Australia[☆]



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ABSTRACT

The effluents from wastewater treatment plants have been recognized as a significant environmental reservoir of antibiotics and antibiotic resistance genes (ARGs). Reclaimed water irrigation (RWI) is increasingly used as a practical solution for combating water scarcity in arid and semiarid regions, however, impacts of RWI on the patterns of ARGs and the soil bacterial community remain unclear. Here, we used high-throughput quantitative PCR and terminal restriction fragment length polymorphism techniques to compare the diversity, abundance and composition of a broad-spectrum of ARGs and total bacteria in 12 urban parks with and without RWI in Victoria, Australia. A total of 40 unique ARGs were detected across all park soils, with genes conferring resistance to β -lactam being the most prevalent ARG type. The total numbers and the fold changes of the detected ARGs were significantly increased by RWI, and marked shifts in ARG patterns were also observed in urban parks with RWI compared to those without RWI. The changes in ARG patterns were paralleled by a significant effect of RWI on the bacterial community structure and a co-occurrence pattern of the detected ARG types. There were significant and positive correlations between the fold changes of the integrase *intI1* gene and two β -lactam resistance genes (KPC and IMP-2 groups), but no significant impacts of RWI on the abundances of *intI1* and the transposase *tnpA* gene were found, indicating that RWI did not improve the potential for horizontal gene transfer of soil ARGs. Taken together, our findings suggested that irrigation of urban parks with reclaimed water could influence the abundance, diversity, and compositions of a wide variety of soil ARGs of clinical relevance.

One-sentence summary: Irrigation of urban parks with treated wastewater significantly increased the abundance and diversity of various antibiotic resistance genes, but did not significantly enhance their potential for horizontal gene transfer.

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1. Introduction

The discovery of antibiotics and their extensive clinical use have made great contributions to treating infectious diseases, promoting livestock's growth, and protecting human and animal health (Hu et al., 2010; Nesme and Simonet, 2015). However, antibiotics are poorly absorbed by the body of humans and animals, and most of these antibiotic compounds and their metabolites are excreted and

finally released into soils and municipal wastewater (Michael et al., 2013), which may exert selective pressure on resident microbial community and contribute to development of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) within the environment. The increasing emergence and propagation of ARGs are threatening the achievement of modern medicine and posing major risks to human health and ecological security in the 21st century (Udikovic-Kolic et al., 2014; Berendonk et al., 2015). In recent years, the magnitude of ARGs has been reported to reach alarming levels in many parts of the world (WHO, 2014), which attracted increasing worldwide concerns (Levy and Marshall, 2004). Therefore, ARGs have been recognized as a new type of emerging environmental contaminant (Pruden et al., 2006).

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In contrast to chemical contaminants, bacterial ARGs might be more persistent in the environment, as ARGs can be not only multiplied in their hosts but also transferred to other microbial populations including human and animal commensals and pathogens through horizontal gene transfer (HGT) mechanisms via mobile genetic elements (MGEs), such as integrons, transposons, and plasmids (Gogarten and Townsend, 2005; Yu et al., 2012). It is well known that integron and transposon are responsible for the acquisition of ARGs and have frequently been found in antibiotic-resistant strains (Carattoli et al., 2001; Scott, 2002). Integrons possess a site specific recombination system which could capture and express mobile gene cassettes (Heuer et al. 2011a), and they are reported to often localize in broad-host range IncP-1 ϵ plasmids with a wide distribution in the environment which further facilitates their mobility potential (Heuer et al. 2012; Wolters et al. 2015). Similarly, transposons include transposase genes such as *tnpA*, *tnpR* and *tnpM* and sites required for transposition (Carattoli, 2001), and carry accessory genes conferring resistance to several classes of antibiotics, and therefore they can horizontally transfer ARGs with them (Reid et al., 2015). Resistance determinants can be horizontally transferred under broad host range through integron and transposon being carried by or incorporated into conjugative plasmids (Carattoli, 2001; Butaye et al., 2003). Horizontal transmission of ARGs via integron, transposon, and plasmid facilitates the dissemination of ARGs in environment and may raise the risk of public health.

Soil is the original habitat for most currently-known antibiotics, and soil microbes might have developed resistance even before the production of modern antibiotics in the 1940s (Wright, 2010; Davelos et al., 2004). Therefore, soil may be a reservoir for novel ARGs that can horizontally transfer to human and animal commensals and pathogens (Dantas and Sommer, 2014). Due to the intensive anthropogenic activities such as aquaculture, land application of manure and biosolids, and large inputs of ARGs from the reuse of reclaimed water (LaPara et al., 2011; Cytryn, 2013; Zhu et al., 2013; Wang et al., 2014a), soil has been recognized as the largest environmental reservoir of antibiotic resistance (Nesme et al., 2014). Reclaimed water irrigation (RWI) is a practical solution for overcoming water resource shortage, and has been widely utilized in arid and semi-arid regions of the world (Fahrenfeld et al., 2013; Berendonk et al., 2015). Given the increasingly exacerbated water scarcity owing to urbanization, population growth and less available freshwater, it is anticipated that RWI will likely be more widely applied in the future (Wang et al., 2014a). However, a large amount of antibiotics, ARB and ARGs can still persist in the reclaimed water after traditional wastewater treatments which are mainly designed to remove organic matter, inorganic nitrogen and phosphorous, and suspended solids, but not for the removal of antibiotics and ARGs (Berendonk et al., 2015; Rodriguez-Mozaz et al., 2015; Xu et al., 2015). The presence of abundant and diverse ARGs of clinical relevance was frequently reported in the effluents of wastewater treatment plants (WWTPs), even after rigorous tertiary disinfection and mixed-media filtration (LaPara et al., 2011; Gatica and Cytryn, 2013). For example, genes conferring resistance to ampicillin, vancomycin, and methicillin were found in wastewater samples collected from five municipal WWTPs in Germany (Volkman et al., 2004). Wang et al. (2015a, b) detected the concentrations of 10 subtypes of ARGs and antibiotics in five pharmaceutical WWTPs in Northern China, and found that the levels of typical ARGs ranged from 2.86×10^3 to 3.68×10^6 copies ml^{-1} and antibiotic residues still remain in the final WWTP effluent. Likewise, Gao et al. (2012) detected high abundances of the *tetO*, *tetW* and *sulI* genes, as well as residues of tetracycline and sulfonamide in the final effluent from a WWTP in Michigan, USA. The continuous release of ARB, ARGs and antibiotic residues from

effluents of WWTPs could cause the dissemination of ARGs in environments receiving these effluents (Czekalski et al., 2014; Wang et al., 2014b; Rodriguez-Mozaz et al., 2015), which has become a global concern (Berendonk et al., 2015).

Although reclaimed water has been recognized as an important reservoir of ARB and ARGs (Fahrenfeld et al., 2013), only a limited number of studies have assessed the fate of reclaimed water-derived ARGs in downstream environments (Negreanu et al., 2012; Fahrenfeld et al., 2013), and people are not aware of the potential health risks they are facing. To date, impacts of treated wastewater on antibiotic resistance have been reported in rivers (LaPara et al., 2011), agricultural soils (McLain and Williams, 2012; Negreanu et al., 2012; Fahrenfeld et al., 2013; Chen et al., 2014) and sediments (Czekalski et al., 2014), but only a few studies focused on the occurrence and prevalence of ARGs in urban park soils irrigated by reclaimed water (Wang et al., 2014a, 2014b). Public urban parks play a vital role in the social life of human beings, and provide a potentially important pathway for the spread of ARGs from soil to human pathogens. Therefore, this study was designed to investigate the impacts of RWI on the patterns of ARGs and the soil bacterial community in 12 public urban parks in Victoria, Australia. Pristine soil samples from two remote national parks without any known exposure to antibiotics and with minimal human-induced selective pressure were collected as control. We tested the following hypotheses: (i) the occurrence and prevalence of ARGs might be strongly affected by RWI, owing to the possible selective pressure of antibiotics and ARGs in treated wastewater; (2) RWI would influence the mobility potential of ARGs in urban parks, as measured by the abundances of the *intI1* and *tnpA* genes, and (iii) RWI might also result in significant changes of the soil bacterial community, which has been suggested as a critical determinant of soil ARGs (Forsberg et al., 2014).

2. Materials and methods

2.1. Sampling sites and soil collection

Soil samples were collected from 12 public urban parks and two remote national parks in Victoria, Australia in January 2015. Of these parks, six urban parks were irrigated with reclaimed water including Werribee Park (WP), Werribee Rose Garden (WRG), Yarra Park (YP), HD Graham Reserve (HDGR), Altona Green Park (AGP) and Werribee Campus of the University of Melbourne (WCUM); six urban parks were irrigated with potable water including Royal Botanic Gardens (RBG), Carlton Garden (CG), Fitzroy Gardens (FZG), Princes Park (PP), Yarra Bend Park (YBP) and Flagstaff Garden (FSG); and the control soil samples were taken from pristine forests in two remote national parks far away from the Melbourne city: Lake Eildon National Park (LENP) and Yarra Ranges National Park (YRNP). The two national parks have no known history of antibiotics exposure and have minimal human-induced selective pressure. The detailed information about the reclaimed water and potable water irrigation in the 12 urban parks is shown in Table S1. In each park, three replicate soil samples (5 cm in diameter) from the upper 10 cm were collected at a distance of 20 m from each other, and each sample was thoroughly homogenized by mixing five subsamples taken within an area of 50 m^2 . All samples were transported on ice to the laboratory, and then gently crumbled to pass through a 2-mm sieve and homogenized thoroughly. Soil samples were stored at 4 °C prior to measurement of soil basic properties, and stored at –20 °C before DNA extraction within two weeks after collection.

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