Impact of reclaimed water irrigation on antibiotic resistance in public parks, Beijing, China

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ABSTRACT

The abundance and distribution of antibiotics and antibiotic resistance genes (ARGs) in soils from six parks using reclaimed water in Beijing, China, were characterized. Three classes of commonly used antibiotics (tetracyclines, quinolones, and sulfonamides) were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The highest concentrations of tetracyclines and quinolones were $145.2 \mu\text{g kg}^{-1}$ and $79.2 \mu\text{g kg}^{-1}$, respectively. Detected *tetG*, *tetW*, *sulI*, and *sulII* genes were quantified by quantitative PCR. ARGs exhibited various abundances for different park soils. The integrase gene (*intI1*) as an indicator of horizontal gene transfer potential was also detected in high abundance, and had significant positive correlation with *tetG*, *sulI*, and *sulII* genes, suggesting that *intI1* may be involved in ARGs dissemination. Both *sulII* and *intI1* clones had high homology with some classes of pathogenic bacteria, such as *Klebsiella oxytoca*, *Acinetobacter baumannii*, *Shigella flexneri*, which could trigger potential public health concern.

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1. Introduction

Antibiotics are probably the most successful family of drugs so far developed for treating human diseases. Antibiotics have also been widely used to prevent/treat animal diseases and promote animal growth in livestock production (Hu et al., 2010). However, most antibiotics are poorly absorbed in both animals and humans, and a large proportion of antibiotics can be excreted in an unaltered state (Kumar et al., 2005) and then dispersed into environment. Indeed, antibiotics have been detected in municipal sewage (Zhang and Zhang, 2011), hospital wastewater (Lindberg et al., 2004), surface water (Luo et al., 2011; Stoll et al., 2012), groundwater (Lindsey et al., 2001), as well as in soil, sediment and sludge samples (Kim and Carlson, 2007). Residual antibiotics may exert selection pressure on environmental microorganisms, contributing to proliferating antibiotic resistance in microorganisms (Martinez, 2009).

Many developing countries including China are facing the challenge of water shortage, and landscape irrigation has to some extent

exacerbated this problem with the acceleration of urbanization, growing demand and declining supplies of freshwater. Reclaimed water from wastewater treatment plants (WWTPs) could partly relieve this issue. Antibiotics in WWTPs are mainly from hospital, pharmaceutical plant wastewater, and domestic wastewater. However, the conventional biological processes in WWTPs could not remove the antibiotics and resistant bacteria completely, which could be influenced by compound-specific properties and treatment processes in specific WWTPs, such as hydraulic retention time (HRT) and solid retention time (SRT) (Rizzo et al., 2013; Lindberg et al., 2005; Li and Zhang, 2010). Numerous studies have reported the occurrence of antibiotics in wastewater, sludge, and the removal efficiency of antibiotics by WWTPs (Leung et al., 2012; Liu et al., 2012; Zhang and Zhang, 2011; Zhang et al., 2011). For example, Gao et al. (2012a) investigated the occurrence of antibiotics in eight sewage treatment plants in Beijing, China, and found that the removal efficiency of antibiotics varied significantly among the eight WWTPs, with average removal efficiency of fluoroquinolones, sulfonamides, and macrolides ranging from 48% to 72%, 39% to 64%, and –34% to 69%, respectively. In addition, although the resistance genes was decreased by 1–3 log units after sewage treatment, tetracycline resistance genes could still be detected from 10^2 to 10^6 copies per milliliter effluent water and 10^8 to 10^9 copies per

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milliliter of biosolids (Auerbach et al., 2007). The continuously release of residual antibiotic compounds, antibiotic resistant bacteria (ARB) and ARGs from WWTPs effluent could result in the dissemination of ARGs in downstream environment (Negreanu et al., 2012; Pruden et al., 2012), which has raised great concerns (Le-Minh et al., 2010). Furthermore, the propagation of ARGs between similar bacteria, gram-positive bacteria and gram-negative bacteria, and even between pathogenic bacteria and non-pathogenic bacteria could be further facilitated through horizontal gene transfer mechanisms via self-transmissible mobile genetic elements, such as plasmids, integrons, transposons, and insertion sequences (Gogarten and Townsend, 2005; Yu et al., 2012), which could pose potential threat to human health.

Until now, only a few studies have concerned about the occurrence and abundance of antibiotics and ARGs in public park soils (Malik et al., 2008; Yang et al., 2010). The hypothesis was that environmental compartments most directly impacted by urban/agricultural activities would have significantly higher concentrations of ARGs than less impacted and pristine environments. Additionally, the integrase gene *intI1* of class I integron was tracked as an indicator of horizontal gene transfer potential. Therefore, soil samples from six public parks using reclaimed water irrigation were collected. Since antibiotic resistance is ancient (Allen et al., 2009; D'Costa et al., 2011), four pristine soil samples from Natural Scenic Resort of Ling Mountain not using reclaimed water were collected as control. The objective of this study was to evaluate whether the soil in public parks receiving reclaimed water acts as a reservoir of ARGs and has the potential for transferring ARGs to human pathogens. Therefore, this study tended to (i) evaluate the occurrence and concentration of 24 antibiotics and ARGs in 29 soil samples; (ii) determine if the pattern of ARGs prevalence was different between each public park; (iii) investigate if there was a link between the ARGs and integrase gene (*intI1*).

2. Material and methods

2.1. Soil sampling and processing

Soil samples were collected from Taoranting Park (TP; $n = 8$), Daguan Yuan Park (DP; $n = 5$), Longtanhu Park (LP; $n = 2$), Beijing World Park (SP; $n = 4$), Olympic Forest Park (OP; $n = 4$), and Research Center for Eco-Environmental Sciences Park (EP; $n = 2$) using reclaimed water irrigation between August and September 2011. Samples were taken from rhizosphere soil, non-rhizosphere soil, and wetland soil according to the condition of each park. At the same time, four samples collected from Natural Scenic Resort of Ling Mountain (LM) not receiving reclaimed water, were used as control. Details about the samples were shown in Table S1. All of the samples were kept on dry ice during transportation and stored at -80°C before DNA extraction and chemical analysis. The soil characteristics were measured using standard methods (Lu, 1999). Soil organic matter was determined by the $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation method. Soil pH was determined with a soil to water ratio of 1:5. Total carbon and total nitrogen were determined by Element Analyzer (Vario EL III, Elementar, USA). All samples were analyzed in triplicate. The average results are summarized in Table 1.

2.2. Antibiotics analysis

The procedures for extraction of tetracyclines, quinolones, sulfonamides, and their degradation products in soils followed methods described by Wu et al. (2011) with some modifications. LC–MS/MS was used to separate and detect the

tetracyclines, sulfonamides, and quinolones following methods described by Zhang et al. (2011). Briefly, Na_2EDTA (0.4 g) and 15 mL of a mixed solution containing phosphate buffer (pH = 2.0) and acetonitrile (2:1, vol/vol) were used as extraction solution. After sonication for 30 min, samples were then centrifuged at 7000 rpm for 5 min. The extraction process was performed three times in each sample. The obtained supernatants were combined and then diluted to 400 mL with deionized water, and acidified to pH 2.5 before SPE extraction. The samples were extracted using Oasis HLB (500 mg, 6 mL) extraction cartridges. Final extracts were transferred to 2 mL amber vials for LC–MS/MS analysis.

Five target tetracyclines, including tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), methacycline (MTC), and doxycycline (DXC), and nine degradation products, including anhydrotetracycline (ATC), 4-epitetracycline (ETC), 4-epianhydrotetracycline (EATC), 4-epioxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), β -apo-oxytetracycline (β -apo-OTC), isochlortetracycline (ICTC), 4-epianhydrochlortetracycline (EACTC), demethylchlortetracycline (DMCTC), four sulfonamides, sulfadimethoxine (SDM), sulfamerazine (SMI), sulfamethizole (SMT), sulfamethoxazole (SMZ), and six fluoroquinolones, including ofloxacin (OFL), enrofloxacin (ENRO), sarafloxacin (SARA), danofloxacin (DANO), ciprofloxacin (CIP), norfloxacin (NOR), were analyzed in this study.

2.3. DNA extraction

Total DNA was extracted from 0.5 g of soil using a FastDNA SPIN Kit for soil (Bio 101 Inc., Vista, CA), following the manufacturer's instructions. The concentration and quality of the extracted DNA was determined by spectrophotometer analysis (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, DE) and agarose gel electrophoresis.

2.4. Detection and quantification of ARGs

Polymerase chain reaction (PCR) detection assays were used for broad-scale screening of the presence/absence of fifteen tetracycline genes (seven efflux pump genes (*tetA*, *tetC*, *tetE*, *tetK*, *tetL*, *tetA/P*, *tetG*), seven ribosomal protection proteins (RPPs) genes (*tetM*, *tetO*, *tetQ*, *tetS*, *tetT*, *tetW*, *tetB/P*), and one enzymatic modification gene (*tetX*)), four beta-lactamases genes (*bla_{CTX-M}*, *bla_{TEM}*, *bla_{SHV}*, *bla_{ampC}*), three sulfonamide genes (*sulI*, *sulII*, *sulIII*), three quinolone genes (*qnrA*, *qnrB*, *qnrS*), three erythromycin genes (*ereA*, *ereB*, *mphA*), and the integrase gene of class I integron (*intI1*). Primers and annealing temperatures are described in Table S2. To ensure reproducibility, triplicate PCR reactions were performed for each sample. Sterile water was used as the negative control in every run. After PCR amplification, gel slices of an agarose gel containing the PCR products were excised and purified using Agarose Gel DNA Purification Kit (Promega). The purified PCR products were ligated into p-GEM T easy vector (Promega) and then cloned into *Escherichia coli* JM109 (Takara). Clones containing ARGs inserts were picked and sequenced. If the gene inserts were verified as the object resistance genes using the BLAST alignment tool (<http://www.ncbi.nlm.nih.gov/blast/>), clones that had the right ARGs inserts were chosen as the positive control and standards for quantitative polymerase chain reaction (Q-PCR). Plasmids carrying target genes were extracted with Plasmid Kit (Takara).

Four ARGs (*tetG*, *tetW*, *sulI*, *sulIII*) that were frequently detected and *intI1* were quantified by Q-PCR using a SYBR Green approach. Eubacterial 16S rRNA genes were quantified according to the TaqMan Q-PCR method described by Suzuki et al. (2000). 10-fold serial dilutions of a known copy number of the plasmid DNA were generated to produce the standard curve. Each sample DNA was diluted for minimizing threshold cycle suppression by inhibitors for that sample type (typically 1:10). All Q-PCR reactions were done in triplicate for both the standards and the microbial community DNA samples. The Q-PCR efficiencies (89.9%–107.5%) were examined to test for inhibition. R^2 values were more than 0.997 for all calibration curves. All Q-PCR analyses were performed using an iCycler IQ5 Thermocycler (Bio-Rad, Hercules, CA).

2.5. Cloning and phylogenetic analysis of *sulII* and *intI1* genes

The clone libraries of *sulII* and *intI1* genes amplicons obtained from six soil samples were constructed to analyze the sequence diversity. *SulII* (191 bp) and *intI1* (473 bp) genes fragments were cloned as described above. Between 20 and 30

Table 1
Soil physicochemical properties of each park (mean values are in brackets).

Samples	pH	Organic matter OM (g kg^{-1})	N (g kg^{-1})	C (g kg^{-1})	S (g kg^{-1})	C/N
TP	8.11–8.32 (8.23)	24.34–72.38 (46.00)	0.98–2.64 (1.92)	19.49–47.07 (33.14)	1.23–3.11 (2.02)	13.68–22.37 (17.84)
DP	7.96–8.63 (8.35)	17.54–44.65 (29.67)	0.95–2.26 (1.48)	25.03–34.20 (28.85)	1.30–1.80 (1.54)	15.12–27.53 (20.69)
LP	8.56–8.61 (8.58)	26.61–27.46 (27.04)	1.06–1.33 (1.20)	24.10–25.81 (24.96)	0.61–0.98 (0.80)	18.07–24.43 (21.25)
OP	8.13–8.57 (8.37)	13.65–73.53 (40.74)	0.42–5.56 (2.56)	20.40–66.49 (38.58)	0.52–1.56 (1.03)	12.08–48.52 (24.55)
SP	8.42–8.82 (8.64)	16.67–35.85 (26.18)	0.91–2.41 (1.79)	17.93–31.44 (26.78)	0.54–0.82 (0.62)	13.07–19.86 (15.94)
EP	8.41–8.58 (8.50)	27.09–32.32 (29.71)	1.37–1.58 (1.48)	24.46–29.19 (26.82)	1.01–1.14 (1.08)	17.91–18.47 (18.19)
LM	7.24–7.57 (7.41)	36.62–61.55 (47.27)	2.15–3.03 (2.58)	24.93–36.77 (31.08)	0.30–0.43 (0.37)	11.57–12.38 (12.02)

representative clones from each library bearing inserts of the correct size were randomly selected for sequencing. Online similarity searching was performed using the BLAST tool. Sequences were subjected to homology analysis using DNAMAN version 6.0.3.48 (Lynnon Biosoft, USA).

2.6. Data analysis

Averages and standard deviations of all data were determined using Microsoft Excel, 2010. Copy numbers were log-transformed as needed to normalize the distributions prior to statistical analysis. Spearman correlations and one-way ANOVA were performed among environmental variables using SPSS software (version 11.5). The ordination analyses of environmental variables and the abundance of functional genes were performed using CANOCO 4.5 software (Microcomputer Power, Ithaca, NY). Redundancy analysis (RDA) was chosen according to the results of detrended correspondence analysis (DCA).

3. Results and discussion

3.1. Antibiotics concentrations

The concentrations of six parent tetracyclines and nine degradation products in soils were summarized in Fig. 1(A). The occurrences of parent antibiotics were higher than their degradations (except EACTC). The total concentration of tetracyclines was in the range of 12.7–145.2 $\mu\text{g kg}^{-1}$. DXC and EACTC were detected in all irrigated soil samples and the concentrations were in the range of 1.8–107.1 $\mu\text{g kg}^{-1}$ and 1.3–5.1 $\mu\text{g kg}^{-1}$, respectively. All of the six quinolones were detected only in sample DR2 and SN1, with a total concentration of 79.2 $\mu\text{g kg}^{-1}$ and 58.5 $\mu\text{g kg}^{-1}$, respectively (Fig. 1(B)). None of the four sulfonamides (SLM, SMI, SMT, and SMZ) were found in all soil samples. Generally, antibiotic compounds were not fully degraded in the WWTPs process; therefore antibiotic degradation products were formed and their concentrations were varied among WWTPs using different treatment processes (Gao et al., 2012a; Leung et al., 2012). In addition, none of the twenty four antibiotics were detected in the control soil samples. The results partly demonstrated that reclaimed water irrigation could increase the concentrations of antibiotics and their degradation products in soil environment through releasing the residues of antibiotics.

Fig. 1. The concentration of tetracyclines (A) and quinolones (B) in irrigated park soils.

Several studies have revealed that the effluent of WWTPs contained various concentrations of antibiotics. For example, 0.12–0.56 $\mu\text{g L}^{-1}$, 0.13–0.46 $\mu\text{g L}^{-1}$, and 0.05–0.30 $\mu\text{g L}^{-1}$ for SD, SMX and ERY, have been detected in the effluents of WWTPs in Beijing, respectively (Gao et al., 2012a). The range of 0.17–5.07 $\mu\text{g L}^{-1}$ and 0.10–9.87 $\mu\text{g L}^{-1}$ for CFX and OFX, respectively, were reported in the effluents of WWTPs in Hong Kong (Leung et al., 2012). In contrast to the wastewater directly irrigated soil (Shi et al., 2012) or the farmlands applied with swine manures (Wu et al., 2010), the concentrations of antibiotics in public park soil were much lower. However, a previous study demonstrated that low concentrations of antibiotics could also induce ARGs and/or stimulates horizontal gene transfer of ARGs (Storteboom et al., 2010). Subinhibitory concentrations of oxytetracycline as low as 20 $\mu\text{g L}^{-1}$ were able to select for tetracycline resistance, as shown by an increasing ratio of *tet* over 16S rRNA gene in surface water mesocosms (Knapp et al., 2008). It was found that the patterns of antibiotics distribution among different parks were different, including parks using reclaimed water from the same WWTPs (Table S1 and Fig. 1). On the one hand, factors, such as irrigation volume, frequency, and removal efficiency of antibiotics in WWTPs may lead to different concentrations of antibiotic residues in soils (Leung et al., 2012; Liu et al., 2012; Zhang et al., 2011). On the other hand, different antibiotic physicochemical property and structure could also influence the existence of antibiotics in environment. For example, sulfonamides with low K_d values (0.9–18.1 L kg^{-1}) can be easily mobile in terrestrial environments due to their strong water-solubility and are easier to be transported into surface water and groundwater through leaching and runoff (Picó and Andreu, 2007).

3.2. Occurrence and abundance of ARGs and *int1* gene

Four ARGs (*tetG*, *tetW*, *sull*, and *sullI*) and *int1* gene were detected in all irrigated soil samples. Other ARGs encoding beta-lactamases, quinolone or erythromycin resistance were not detected in any samples using the current PCR method.

Absolute gene copy numbers of five genes (*tetG*, *tetW*, *sull*, *sullI*, and *int1*) in soil samples were presented in Fig. S1 and exhibited various abundances for different samples. The highest abundance of *tetG*, *tetW*, and *sull* was all occurred in samples from Olympic Forest Park (OP), but different site, *tetG* in sample OR1 (9.90×10^7 copies per g dry soil), *tetW* in sample ON1 (2.64×10^5 copies per g dry soil), and *sull* in sample OR2 (1.69×10^8 copies per g dry soil). It was also found that the highest abundance of *sullI* and *int1* was detected in the same soil sample of TW2 (Taoranting Park), the abundances were 8.43×10^7 copies per g dry soil and 7.62×10^7 copies per g dry soil, respectively. The total number of 16S rRNA gene copies was relatively consistent among different sites at 10^9 – 10^{10} copies per g dry soil (Fig. S1). To minimize variance caused by different extraction and analytical efficiencies and differences in background bacterial abundances, the absolute number of all resistance genes was further normalized to that of ambient 16S rRNA gene (Fig. 2). According to Fig. 2, the relative abundances of these ARGs (*tetG*, *tetW*, *sull*, and *sullI*) ranged from 1.8×10^{-7} to 5.3×10^{-3} copy of ARGs genes/copy of 16S rRNA genes. *TetG* had the highest abundance 5.3×10^{-3} copy of *tetG* gene/copy of 16S rRNA genes, whereas *tetW* had the lowest abundance. Like antibiotics, the distribution of ARGs was different among park soils using the same reclaimed water. In addition, there were no similar change patterns about the abundance of ARGs among different samples in the same park, which were collected from rhizosphere, non-rhizosphere, and wetland, respectively. Multiple factors could result in this phenomenon, such as irrigation volume, frequency, and the abundance of ARGs in reclaimed water as well as

Fig. 2. Detected levels of ARGs and integrase gene (*intI1*) normalized to copies of ambient 16S rRNA gene present in soil samples from different sites. Error bars indicate the standard deviation of three replicates.

soil conditions (Auerbach et al., 2007; Gao et al., 2012b; Liu et al., 2012).

To date, more than forty classes of tetracycline resistance genes have been found, which encode three main resistance mechanisms (efflux pump proteins, ribosomal protection proteins, and inactivating enzymes) (Roberts, 2005). Recent studies have also demonstrated that *tet* genes are often located on the plasmids and can be horizontally transferred among bacterial strains in WWTPs (Szczepanowski et al., 2004). In this study, only *tetG* and *tetW* were detected, and *tetG* had higher gene copy numbers than *tetW*. This may be caused by different ARGs concentrations among the effluents of different WWTPs. For instance, *tetG* had the highest concentration (1.75×10^{-2} copy of *tet* genes/copy of 16S genes) for the fifteen WWTPs across China and other locations (Zhang et al., 2011). While other study demonstrated that *tetW* had higher abundance than other genes along the treatment process of three WWTPs (Kim et al., 2010).

SulI and *sulII* genes were detected with high abundance in this study. Although the use of sulfonamides has been restricted for several years due to its rapid occurrence of resistance and dissemination among treated patients (Skögl, 2000), *sulI* gene was found to be associated with persistent sulfonamide resistance in gram-negative bacteria (Radstöm et al., 1991). The *sulI* gene was detected at high concentrations of 1.82×10^9 and 1.05×10^7 gene copies L^{-1} in raw influent and final effluent, respectively (Gao et al., 2012b). In spite of reduction by more than

two orders of magnitude after treatment, the concentration of *sulI* gene was still very high in the effluent and could enter into downstream environments through reclaimed water discharge and use.

It's worth noting that *sulI* and *intI1* were also detected in pristine soil samples (LM) with the high abundance of 9.08×10^7 copies per g dry soil and 2.61×10^7 copies per g dry soil respectively, indicating the extensive existence of *sulI* and *intI1* genes (Fig. S1). This obvious distinction between *sulI* and other ARGs was that *sulI* was carried within the 3' conserved region of class 1 integron, and such an association may facilitate the establishment of *sulI* within native environment and enhance its persistence (Partridge et al., 2009). Previous study showed that resistance could occur in pristine soil environments even without any record of antibiotic contamination (Martinez, 2008), regarding that most antibiotics used for treating infections are produced by environmental microorganisms. The evidence that a higher diversity and abundance of ARGs were detected in irrigated soil than pristine soil (LM) in this study showed that reclaimed water played an important role in dissemination of antibiotic resistance.

3.3. Relationship between ARGs and environmental factors or *intI1* gene

No significant correlations between antibiotics and ARGs were found in this study (Table S3). It was still inconclusive regarding the relationship between antibiotics concentration and ARGs (Pei et al.,

Fig. 3. Redundance analysis (RDA) of ARGs, *int11* gene, and environmental factors.

2006; Wu et al., 2010; Zhu et al., 2013). This could be related to subinhibitory concentrations of antibiotics. In this study, tetracyclines and quinolones had lower concentrations (Fig. 1), while, sulfonamides were even not detected in any park soils. It was interesting to find that although quinolones had been detected, none of encoding quinolone resistance genes were detected in soil samples, and in spite of no sulfonamides were detected in soil, *sul* genes had been detected with relative high abundances, indicating that antibiotics and ARGs were not always emerging at the same time and ARGs can be persistent even in the absence of antibiotic selection pressure. Furthermore, it was also found that functional genes and 16S rRNA gene copies had negative correlation with soil pH. Soil pH exerted a strong selection pressure on soil microbes and appeared to have a pervasive effect on the abundance of bacteria

(Rousk et al., 2010). In our study, soil pH of all sites was greater than 7.0 (Table 1), and was negatively correlated with the abundance of the ARGs, 16S rRNA gene, and *int11* gene (Fig. 3), indicating that neutral soils were more suitable for the microbes. Meanwhile, Fig. 3 showed that functional genes had positive correlation with C, N, and OM, which could be attributed to the significant relationship between 16S rRNA gene copies and C, N, and OM ($P < 0.01$). The results indicated that various soil physicochemical properties among different park soils, such as C, N, and OM (Table 1), could influence the abundance of ARGs.

In addition, it was found that *tetG*, *sulI*, and *sulII* genes had significantly positive correlation with *int11* gene (Table S3 and Fig. 3). A bivariate correlation analysis further showed that the levels of *tetG*, *sulI*, and *sulII* were correlated with the levels of *int11* ($R^2 = 0.623$, 0.606 , and 0.641 , respectively) (Fig. 4). Although this correlation was not as strong as other study (Liu et al., 2012), the high abundance of *int11* and correlation with abundance of ARGs could suggest that *int11* may play certain role in the dissemination of ARGs, thus indicating that horizontal gene transfer could be an important pathway for ARG proliferation, especially under antibiotic selection pressure (Ochman et al., 2000). Previous study also demonstrated that class 1 integrons are an important vector for the propagation of antibiotic resistance (Gillings et al., 2008).

3.4. Diversity of *sulII* gene and *int11* gene in soil samples

For *sulII* and *int11* genes, BLAST search of a total of 167 (*sulII*) and 157 (*int11*) sequences showed that all of the sequenced clones matched known *sulII* gene and *int11* gene, with sequence identities

Fig. 4. Relationships between log of *int11* gene copy numbers and log of *tetG* (A), *sulI* (B), *sulII* (C), and *tetW* (D) gene copy numbers, respectively. $P < 0.01$ indicates the significant correlation.

ranging from 95% to 100% and 94% to 100%, respectively (Table S4–S5). Most identified bacteria belonged to two phyla: *Proteobacteria* and *Actinobacteridae*. *Proteobacteria* was the dominant bacterial community in soil samples. As for *sullI* gene, at least three types of *sullI* existed in soil samples. The most common one was the type3, which was identical to the sequences found in various plasmids (*Haemophilus parasuis* plasmid pFZ51 (JN202624.1)), transposons (*Escherichia coli* strain J53 transposon SXT (FJ968160.1)), conjugative transposons (*Vibrio cholerae* *lnd5* integrating conjugative element ICEVchind5 (GQ463142.1)), and chromosomes (*Vibrio cholerae* O1 str. 2010 EL-1786 chromosome 1 (CP003069.1)) of both gram-negative and gram-positive bacteria, even in human pathogens such as *Klebsiella oxytoca* E718 (CP003684.1), *Pasteurella multocida* subsp. *multocida* str. HN06 (CP003314.1), *Shigella flexneri* 2002017 (CP001386.1), *Enterobacter cloacae* subsp. *cloacae* ATCC 13047 (CP001918.1) and so on. As for *intl1* gene, at least two types of *intl1* existed in soil samples. The most common one was the type2, which was also carried by several pathogenic bacteria, such as *Enterobacter cloacae* strain ABC104 (JX275775.1), *Salmonella enterica* subsp. *enterica* serovar *Kentucky* strain 19-85 (JQ796373.1), *Klebsiella oxytoca* strain 11005 (JQ407409.1), *Shigella* sp. ER.123 (FJ460182.2) and so on.

Cloning and phylogenetic analysis further demonstrated the association of ARGs with mobile genetic element class I integron. The results showed that *intl1* gene clones were observed on mobile DNA elements, such as plasmids, chromosomes, and transposons, and since integrons have the ability to capture ARGs, this could result in ARGs proliferating more easily and widely. It was also found that both *sullI* gene and *intl1* gene were relatively more conservative, and these two genes could be existed in some pathogenic bacteria, which could facilitate the further spreading of *sullI* gene to pathogenic bacteria, and induce potential public health problem. A previous study demonstrated that a significant proportion of isolated bacteria resistant to sulfonamides carried *intl1* gene, further confirming integrons could play a major role in the epidemiology of sulfonamide resistance genes (Lanz et al., 2003).

4. Conclusions

This study clearly demonstrates the presence of antibiotic residues and high levels of ARGs in public park soils irrigated with reclaimed water. The continually input of reclaimed water from WWTPs might be responsible for the higher levels of antibiotics and ARGs. Sulfonamide resistance genes (*sull* and *sullI*) could be persistent even without antibiotic pressure, indicating that ARGs was more permanent than antibiotics. In addition, integrase gene (*intl1*) as an indicator of horizontal gene transfer potential played an important role in the widely dissemination of ARGs. Although it is difficult to build a direct link between ARGs and public health concerns, the results of this study provide baseline information for understanding the occurrence of ARGs in public parks in urban environments with high population densities. For further study, it is important to investigate the impact of reclaimed water irrigation on antibiotics and ARGs in park soils from many more urban cities. Meanwhile, exploring the transfer of ARGs from soil to human and establishing a suitable risk assessment model for antibiotics and ARGs to evaluate their possible hazard for human health are also urgently needed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2013.08.038>.

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