Environmental Pollution 235 (2018) 525–533

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Spatial and temporal distribution of antibiotic resistomes in a peri-urban area is associated significantly with anthropogenic activities☆

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ARTICLE INFO

Article history:
Received 22 October 2017
Received in revised form
29 December 2017
Accepted 29 December 2017

Keywords:
Antibiotic resistance
Urbanization
Spatio-temporal
Watershed

ABSTRACT

With the rapid development of urbanization and industrialization, the peri-urban areas are often the sites for waste dumps, which may exacerbate the occurrence and spread of antibiotic resistance from waste to soil bacteria. However, the profiles of antibiotic resistomes and the associated factors influencing their dissemination in peri-urban areas have not been fully explored. Here, we characterized the antibiotic resistance genes (ARGs) in peri-urban arable and pristine soils in four seasons at the watershed scale, by using high-throughput qPCR. ARGs in peri-urban soils were diverse and abundant, with a total of 222 genes were detected in the peri-urban soil samples. The arable soil harbored more diverse ARGs compared to the pristine soils, and nearly all the ARGs detected in the pristine soils were also detected in the farmlands. A random forest prediction showed that the overall patterns of ARGs clustered closely with the landuse type. Mantel test and partial redundancy analysis indicated that bacterial community variation is a major contributor to antibiotic resistome alteration. Significant positive correlation was found between the abundance of ARGs and mobile genetic elements (MGEs), suggesting potential mobility of ARGs in peri-urban areas. Our results extend knowledge of the resistomes compositions in peri-urban areas, and suggest that anthropogenic activities driving its spatial and temporal distribution.

1. Introduction

Peri-urban areas constitute a transitional zone where urban and rural activities are juxtaposed (Simon, 2008). Their ecosystems function at the rural-urban symbiotic interface and allow a re-coupling of resource flows, namely mass, energy, and genetic information, leading to resource flows from urban/industrial areas into rural environments (Huang et al., 2009; Lin, 2010; Zhu et al., 2017b). With the rapid development of urbanization and industrialization, the peri-urban areas are not only important for food and materials supply to urban areas, but also the sites for urban and industrial waste disposal (Khai et al., 2007). Therefore, the peri-urban areas are influenced highly by human activity and reflect the most intense conflicts of land ecosystem services.

Soil is easily impacted by humans, biological perturbations, and record environmental changes (Huang et al., 2009; Lin, 2010; Zhu et al., 2017b). In peri-urban areas, increasing application of organic fertilizers produced by urban organic wastes from food, manure, and sewage sludge is a substantial step for the recovery and recycling of nutrients, and ensuring food production under rapid urbanization. However, this practice, without doubt, have a potential risk of introducing heavy metals, pharmaceutical compounds, antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) directly into the human food chain (Chen et al., 2016; Hu et al., 2010; Zhao et al., 2017). In addition, these introduced chemicals can continue to impose selection or co-selection pressures on soil microbes, thereby promoting the proliferation and dissemination of ARGs (Chen et al., 2017a; Heuer et al., 2011; Hu et al., 2016b; Kolpin et al., 2002; Zhu et al., 2013). Therefore, aggressively closed-loop nutrient cycling in peri-urban ecosystems is a potential pathway for the trophic concentration of ARGs, which poses health risks to consumers including both rural and urban populations. Importantly, once antibiotic resistance appears, it is

☆ This paper has been recommended for acceptance by Klaus Kummerer.
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https://doi.org/10.1016/j.envpol.2017.12.119
0269-7491/

difficult to eliminate (Zhu et al., 2017b). However, only few studies have addressed the problems in peri-urban areas (Chen et al., 2016; Sengelov et al., 2003; Zhang et al., 2017), and such studies used small-scale single plots, without taking account of seasonal fluctuations, and they failed to incorporate pristine control environments. It has been suggested that pristine control environments can provide important information regarding background antibiotic-resistance patterns in any experimental design seeking to delineate anthropogenic impacts and assess the risk of ARGs (Allen et al., 2010; Berendonk et al., 2015; Singer et al., 2006; Storteboom et al., 2010b), while seasonal fluctuations could also significantly shift the profile of the antibiotic resistomes. Therefore, a comprehensive understanding of the temporal and spatial distributions of antibiotic resistomes in peri-urban areas at a large scale, such as the watershed scale, is needed to profile the antibiotic resistomes and identify the factors responsible for shaping its distribution.

In the present study, we collected soil samples in four different seasons in 1 year from the Zhangxi watershed, which hosts productive, peri-urban farmland in the Yangtze Delta of Zhejiang Province, China. Using high-throughput qPCR with 296 validated primer sets and Illumina sequencing, our objective was to (1) characterize the profile of the antibiotic resistomes in both pristine (forest) and arable (farmland) soils, and identify the shared/unique ARGs; (2) determine the temporal and spatial distributions of ARGs at the watershed scale; and (3) explore how biotic factors (bacterial compositions), anthropogenic factors (landuse type), and environmental factors (geographic parameters, climatic change, and soil properties) shape the distribution of ARGs and address the key drivers of antibiotic resistance.

2. Material & methods

2.1. Sampling site and sample collection

Soil samples (surface soil, 0–20 cm) were collected from the Zhangxi watershed (Fig. 1), a peri-urban area of Ningbo, Zhejiang Province, China (29.75 °N–29.85 °N, 121.22 °E–121.33 °E). The watershed has a subtropical monsoon climate, warm, humid and windy during April to September, and the rainy season is around June. Zhangxi watershed, with an area of 85 km^2 and elevation range of 3–763 m, is a mixed landuse watershed, including forests (natural secondary forest, artificial forest) and farmlands (vegetable fields, orchard, nursery etc). The soil samples were collected along a gradient of landuse intensity in four sub-watersheds (sub-watershed 4 > sub-watershed 1 > sub-watershed 2 and sub-watershed 3). Sub-watershed 4 located on the downstream of Zhangxi river is the area most affected by agricultural activity. We selected 32 representative sites covering the entire watershed with two types of landuse (farmlands and forest) to investigate the impacts of human activities on the ARG distribution (Fig. 1). The farmland has been treated with manure and inorganic fertilizers in the winter. Since there are no large-scale and intensive farms, the manures were mainly derived from humans or their domestic livestock including pig, chicken, cattle and sheep. While the forest has no known exposure to antibiotics or fertilizers, as well as minimal human-induced selective pressure. To better understand the temporal variations in the ARG burden, the soils were sampled in spring (April, 13–24 °C), summer (July, 27–33 °C), autumn (October, 19–27 °C), and winter (January, 6–15 °C). Each sample was collected in triplicate during each season from April 2016 to January 2017. The collected soil samples were frozen immediately with dry ice, and transported to our laboratory, stored at −20 °C prior to analysis.

2.2. DNA extraction

Each soil DNA was extracted from 0.5 g soil using a FastDNA® Spin Kit for soil (MP Biomedical, Santa Ana, California, USA) according to the manufacturer's protocol. The extracted DNA concentration and quality was checked using NanoDrop ND-1000 (Nanodrop, USA) and agarose gel electrophoresis. Soil DNA was stored at −20 °C until use.

2.3. High-throughput quantitative PCR (HT-qPCR)

High-throughput qPCR reactions were performed to evaluate the relative abundance of ARGs in samples, using the Wafergen SmartChip Real-time PCR system (Warfergen Inc. USA) as described previously (Su et al., 2015). A total of 296 primer sets were used, including 285 primer sets targeting almost all major classes of ARGs, 8 transposase genes, 1 class I integron-integrase gene, 1 clinic integron-integrase gene and 1 16S rRNA gene. The classification and primers of the quantified genes were showed in Supplementary Table S1. A more detailed description about the experimental procedure and data analysis can be found in previous studies (Su et al., 2015). The relative abundance of ARGs and MGEs (Mobile Genetic Elements) was calculated and transformed to absolute abundance (copies per g soil) according to a previous study (Ouyang et al., 2015). To minimize potential variations in DNA extraction, analytical efficiencies and background bacterial abundance, the relative copy number of ARGs was normalized by 16S rRNA gene and represented as ARG copies per bacterial cell (Klappenbach et al., 2001).

2.4. Bacterial 16S rRNA gene sequencing

To profile bacterial communities, the V4–V5 region of 16S rRNA gene was amplified with primers F515: GTGCCAGCMGCCGCGG and R907: CCGTCAATTCMTTTRAGTTT (Ren et al., 2015). The amplicons were purified, quantified, pooled and sequenced on an Illumina Miseq platform at Novogene (Xu et al., 2014). The high-quality sequences were processed and analyzed using the QIIME pipeline. Operational taxonomic units (OTUs) were identified using the UCLUST algorithm with a phylotype defined at 97% sequence similarity (Edgar, 2010). The database from the Ribosomal Database Project was used for taxonomic classification at an 80% confidence threshold (Cole et al., 2009; Wang et al., 2007).

2.5. Statistical analysis

Averages, standard deviations, and Venn diagram were determined using Microsoft Excel 2010. Paired-Sample T-tests and correlation tests were performed using SPSS V20.0 (SPSS Inc., Chicago, USA). Differences were considered significant at $P < .05$. Bar charts were generated by OriginPro 2015 (OriginLab, USA). Boxplots and Scatter diagrams were generated using R3.2.3 (R Core Team, 2016) with the ggplot2 package (Wickham, 2009). Redundancy analysis (RDA), significance test (Adonis test, mantel test) and Random forest prediction analysis were using R3.2.3 with vegan (Oksanen et al., 2014) and randomForest (Liaw and Wiener, 2002) packages. And the ArcGIS was applied to map the antibiotic resistance burden at varying spatial scales.

3. Results

3.1. Diversity and abundance of ARGs

A total of 222 genes (212 ARGs, eight transposase genes, one class I integron-integrase gene, and one clinically associated integron-integrase gene) were detected in soil samples. The number of ARGs