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Occurrence and removal of antibiotic resistance genes in municipal wastewater and rural domestic sewage treatment systems in eastern China

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ABSTRACT

Antibiotic resistance genes (ARGs) are emerging environmental contaminants and pose a threat to public health. In this study, four tetracycline resistance genes (*tet*M, *tet*O, *tet*Q and *tet*W) and two sulfonamide resistance genes (*sul*I and *sul*II) were evaluated in 4 municipal wastewater and 8 rural domestic sewage treatment systems with different wastewater handling abilities and treatment processes using quantitative polymerase chain reaction (qPCR). In the influents, the relative abundance of different ARGs showed significant variations among the sampling sites. In addition, significant correlations (*tet*Q: $R^2 = 0.712$, $P<0.05$; *tet*O: $R^2 = 0.394$, $P<0.05$) between the gene copy numbers and wastewater-receiving capacity were observed. Statistical analysis revealed a positive correlation ($R^2 = 0.756$, $P<0.05$) between the gene copy numbers of *sul*I and *intI*1, whereas the gene numbers of *tet*M and *sul*I were strongly correlated with 16S rDNA. Significant reductions (1–3 orders of magnitude) in ARGs were observed in municipal wastewater treatment systems, but a smaller reduction was found in the rural domestic sewage treatment systems. These results provide insights into the occurrence and removal of ARGs in wastewater treatment systems in both rural and urban areas in eastern China.

1. Introduction

Antibiotics have been widely used in human and veterinary medicines to treat and control many bacterial infections, but misuse or overuse of antibiotics contributes to the emergence and spread of antibiotic resistance genes (ARGs) in the environment. A significant correlation between the emergence of ARGs and the concentration of antibiotics has been reported (Gao et al., 2012a; Luo et al., 2010), suggesting that antibiotics exert selective pressure for these ARGs. The establishment of ARGs may require a long period of antibiotic exposure, but once established, the genes persist, even after the selective pressure is removed (Pei et al., 2006; Tamminen et al., 2010).

To date, at least 40 tetracycline resistance (*tet*) genes, along with three specific mechanisms (i.e., antibiotic efflux pumps, target modification with ribosomal protection protein, and antibiotic inactivation), have been characterized (Roberts, 2005). Four sulfonamide resistance (*sul*) gene types, including *sul*1, *sul*2, *sul*3 and *sul*A, have also been studied (Pei et al., 2006). In both animals and humans, a significant amount of antibiotics (up to 75%) can be excreted in an unaltered state (Elmund et al., 1971). In urban regions, large amounts of antibiotics, as well as ARGs, are excreted and reach wastewater treatment systems. In addition, other factors in wastewater treatment systems, such as gene cassettes, integrons, and heavy metals, which play important roles in the exchange of resistance as well as contribute to resistance retention and dissemination, are also detected in wastewater (Moura et al., 2012; Stepanauskas et al., 2006). Wastewater treatment systems are regarded as important reservoirs for various ARG encoding resistance (Auerbach et al., 2007; Guardabassi et al., 2002; Moura et al., 2012; Zhang et al., 2009a, 2009b), and real-time quantitative polymerase chain reaction (qPCR) has been widely used to explore the occurrence and diversity of *tet* and *sul* genes in such systems (Auerbach et al., 2007; Munir et al., 2011). So far, at least five classes of integrons have been characterized (Mazel, 2006). Due to their ability to capture exogenous gene cassettes and convert them by site-specific recombination, integrons were identified by virtue of their important role in the spread of ARG among bacterial species and multi-drug resistance in environmental bacteria (Mazel, 2006; Ploy et al., 2000), particularly class 1 integrons (Gaze et al., 2011). Generally, class 1 integrons contain an *intI*1 gene encoding a site-specific integrase responsible for integration, and a conserved segment (the *qacE*△1, *sul*I, and *orf*5 genes) encoding resistance to quaternary ammonium compounds and sulphonamide (Gaze et al., 2011; Zhang et al., 2009a). Thus, the efficiency of ARG and *intI*1 gene removal by wastewater treatment systems has recently received increasing research interest (Auerbach et al., 2007; Gao et al., 2012b; Munir et al., 2011; Zhang et al., 2009a).

Research on ARG and *intI*1 removal has focused on municipal wastewater treatment systems. With the development of economy and attention on environmental protection, an increasing number of rural domestic sewage treatment systems, such as onsite and/or cluster systems, have been implemented in many rural areas across China where no municipal sewage pipelines exist. Rural domestic sewage treatment primarily relies on septic tanks associated with biological treatment (anaerobic digester, biological filter, drop-aeration biofilm

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process; Liang et al., 2010), ecological treatment (constructed wetland and multi-soil-layering system; Chen et al., 2009), or combined bio-eco treatment. These systems meet not only the demands for environmental protection but also economic requirements (Chen et al., 2010; Dong et al., 2012). Previous studies on rural domestic sewage treatment systems have mainly focused on the removal of chemical oxygen demand, biochemical oxygen demand, total suspended solid, phosphorus, and nitrogen (Hellström and Jonsson, 2003; Liang et al., 2010). Information about the occurrence and removal of ARGs in rural domestic treatment systems is limited.

In this study, four *tet* genes (*tet*M, *tet*O, *tet*Q and *tet*W) and two *sul* genes (*sul*I and *sul*II) were investigated in 4 municipal wastewater treatment plants (WWTPs) and 8 rural domestic sewage treatment systems using qPCR. The integrase gene (*intI*1) of class 1 integrons, whose genetic element is believed to contribute significantly to the evolution and proliferation of multiple antibiotic resistant bacteria, was also quantified. The objectives of this study were (1) to investigate the occurrence and removal of *tet* and *sul* resistance genes in 12 wastewater treatment systems with different treatment capacities and treatment processes, (2) to assess the factors that contribute to the diversity of ARGs in raw influents, and (3) to explore the correlations between ARGs, integrons, and other environmental factors.

2. Materials and methods

2.1. Sampling sites and sample collection

All field samples were collected from March to May 2012 in Hangzhou, China. The Hangzhou region comprises the Hangzhou metropolitan area (eight districts, including Yuhang) and five county-level cities (including Linan). The sampling sites (Fig. 1) included eight rural domestic sewage treatment systems and four municipal wastewater treatment systems. Detailed information on the sampling sites is shown in Table 1. Sites A, B, and C represented rural domestic sewage treatment systems for single families. Sites D to H represented rural domestic sewage treatment systems for towns or villages. Sites I to L represented municipal wastewater treatment plants. Sites I and J received domestic sewage from the suburbs of Hangzhou, while sites K and L received domestic sewage from the city areas of Hangzhou. There were no factories or livestock farms near the sampling sites. For systems A to H, the effluents discharged into irrigation ditches, and then permeated into nearby land and/or farmland. The effluent of system I was released into Shangtang River, the effluent of system J was discharged into Qingshan Lake, and the effluent of systems K and L were discharged into Qiangtang River.

Table 1
Wastewater treatment characteristics in this study.

Sampling site	Wastewater-receiving capacity (t)	Treatment process	Service scale
A	0.2	Anaerobic digester associated with eco-filter and constructed wetland	One family
B	0.4	Anaerobic digester associated with multi-soil-layering system	One family
C	1	Drop-aeration biofilm process associated with constructed wetland	One family
D	10	Multi-stage anaerobic biological filter	Part of the village
E	20	Anaerobic digester associated with multi-soil-layering system	Part of the village
F	50	Multi-stage anaerobic biological filter associated with constructed wetland	Part of the village
G	180	Multi-stage anaerobic biological filter	The whole village
H	300	Multi-stage anaerobic biological filter	The whole village
I	20,000	Oxidation ditch	Part of the district
J	60,000	Oxidation ditch	Part of the county
K	400,000	Anaerobic oxic	Part of the city
L	600,000	Anaerobic anoxic oxic	Part of the city

Due to the discontinuous nature of rural wastewaters, a composite sampling method was adopted: the samples were collected three times throughout the sampling day (i.e., morning, noon, and evening) and then mixed together. Water samples were stored in amber glass bottles. The influents and effluents from the four municipal WWTPs were collected in equal proportions every hour using a 24-h composite sampler. The samples were delivered to the laboratory on ice and processed within 24 h.

2.2. Sample pretreatment and DNA extraction

Water samples were concentrated using a vacuum filtration apparatus onto 0.22-μm filters until the filter clogged, after which the filters were stored at −80 °C until DNA extraction was performed. Total DNA was extracted using an UltraClean Water DNA Kit (MoBio Laboratories, location?). DNA extraction was performed following the manufacturer's protocol. The quality and concentration of the purified DNA were

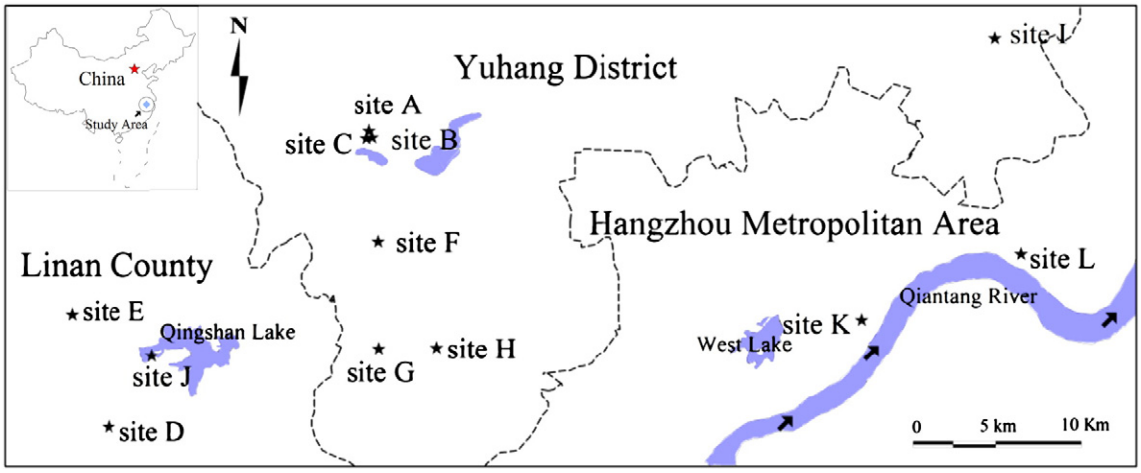

Fig. 1. Sampling sites.