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Simulated discharge of treated landfill leachates reveals a fueled development of antibiotic resistance in receiving tidal river

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ABSTRACT

Around 350 million tons of solid waste is disposed of in landfills every year globally, with millions of cubic meters of landfill leachates released into neighboring environment. However, to date, little is known about the variations of antimicrobial resistance (AMR) in on-site leachate treatment systems and its development in leachate-receiving water environment. Here, we quantified 7 subtypes of antibiotic resistance genes (ARGs), 3 types of culturable antibiotic resistant bacteria (ARB) and 6 subtypes of mobile genetic elements (MGEs) in the effluents from a combined leachate treatment process, including biological treatment (MBR), physical separation (UF), ultraviolet (UV) disinfection and advanced oxidation process (AOP). The contents of ARGs, ARB and MGEs were generally enriched by the MBR, but then decreased significantly along with the tertiary treatment process. However, in the effluent-receiving water samples, the abundance of dominant ARGs (i.e. *ermB*, *sul1*, bla_{TEM}) increased by 1.5 orders of magnitude within 96 h, alongside a general increase of MGEs (~10.0 $\log_{10}$(copies/mL) and total ARB (~1100 CFU/mL). Structural correlation analyses reveal that target ARGs were closely associated with MGEs, particularly in effluent-receiving samples (Procrustes test; $M^2 = 0.49$, $R = 0.71$, $P = 0.001$); and occurrences of ARB were majorly affected by ARG's distribution and environmental conditions (e.g. nitrogen speciation) in effluent and recipient groups, respectively. This study indicates that current treatment technologies and operation protocols are not feasible in countering the development of AMR in effluent-receiving water environment, particularly in tidal rivers that are capable of retaining contaminants for a long residence time.

1. Introduction

There is a growing concern about the development of antimicrobial resistance (AMR) that jeopardizes human health at global scales (Ashbolt et al., 2013; Berendonk et al., 2015). During last 60 years, the increasing levels of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) have been detected in both clinical settings and the natural environment (Knapp et al., 2010; Wellington et al., 2013). Discharge of human waste/wastewater exacerbates this AMR problem (Gaze et al., 2011; Walsh et al., 2011), especially in the case of landfills (Wu et al., 2015), which annually receive the largest portion of, equivalent to 350 million tons (Word Bank, 2012), municipal solid waste (MSW) around the world that spans countries at all levels of economic development. The estimated MSW landfilling rates are 34%, 52%, 60% and > 80% in the European Union (Brennan et al., 2016), United States (Environmental Protection Agency, 2016), China (Zheng et al., 2014), and Northern Africa (Word Bank, 2012), respectively. The high antibiotic consumption rate in emerging countries (e.g. China and India) results in a considerable amount of antibiotic residues being transferred into landfills (Sun et al., 2016; Wu et al., 2015). During the long-term landfill process, microbial populations exposed to these antibiotic residues are likely to build up AMR. Current studies have confirmed that landfills are characterized with high abundance of ARB and ARGs (Threedeach et al., 2012; Wu et al., 2017), which can be readily released through landfill leachates (Wang et al., 2015).

In most of industrialized countries, the treatment of landfill leachates is required by law (Mukherjee et al., 2014). However, conventional wastewater treatment systems and regulatory guidelines are primarily designed and drafted to remove organic carbons and nutrients (Renou et al., 2008), as opposed to contaminants such as pharmaceuticals or ARGs. As a result, these emerging contaminants are reportedly discharged with inefficient treatment (Chen and Zhang, 2013; Di Cesare et al., 2016; Zuccato et al., 2010), which provides a continued entry of AMR to the environment. Compared to other types of wastewater,

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landfill leachates have higher concentrations of AMR selective agents (e.g. antibiotics, metals, biocides) and contain more abundant mobile genetic elements (MGEs) (Wu et al., 2017). These features suggest higher pressures on AMR-selection and higher potentials for horizontal gene transfer (HGT) of ARGs (Yu et al., 2016). Zhang et al. (2016) has shown that the discharge of leachates, with more severity than domestic wastewater, facilitate disseminations of ARGs in neighboring surface water. To date, 2.6 billion people lack access to basic sanitation (Pruden et al., 2013), which lead to the direct release of clinically important ARGs and ARB (resistant pathogens) into ambient waters (Lamba et al., 2017; Pehrsson et al., 2016). The death toll in Europe from ARB is ~25,000 per year (ECDC/EMEA, 2009), and 2,000,000 illnesses are related to AMR in the United States (CDCP, 2013). Thus, WHO (2011) has suggested that containments of antibiotic resistance should be "turned toward factors that are out of the clinical realm".

Our target for this study is the Yangtze Delta Estuary, being the most populous (156 million population) urban agglomeration in the world. Its intensive industrial activity and sprawl of mega-cities drive rivers in this watershed to be the most antibiotic-contaminated ones in China (Zhang et al., 2015). Different from other river networks, tributary tidal rivers in Yangtze Delta Estuary can hold contaminants for a relatively long period before release them to main stream (Zheng et al., 2016). This prolonged residence time facilitates the accumulation of AMR selective agents (Jiang et al., 2013), thereby increasing related risks to neighboring environments and human health. Here, i) to analyze conventional wastewater treatment technologies' removal efficiencies of AMR determinants and ii) to assess treatment system's capabilities of containing AMR development in effluent-receiving water, we sampled MSW landfill leachates from a multi-stage treatment process, including biological treatment physical separation, ultraviolet (UV) disinfection and advanced oxidation process (AOP); and mixed effluents with river water to simulate the contaminant's retention in tidal river courses. The abundances of ARGs, MGEs and the culturable ARB in all samples at different treatment steps were compared before and after discharge, and their inter-structural associations were further studied to reveal the elimination and developmental mechanisms of AMR in treatment and water environment, respectively.

2. Materials and methods

2.1. On-site sampling and lab-simulation treatment

Raw leachate samples were collected from Laogang Landfill (Shanghai Laogang Sanitary Landfill Plant, China), which is located in the eastern suburbs of Shanghai. Leachates discharged from Laogang Landfill (Phase V section that were commenced in 2010) are directly piped to the affiliated wastewater treatment plants, where influents were treated consecutively through high loading membrane bioreactors (MBR) systems, and ultra-filtration (UF) before final discharge. In the present study, raw leachate effluents from MBR (biological treatment) and UF (physical separation) were collected in triplicate once a week for 6 weeks by operating personnel of the treatment plant. To avoid hydraulic loading fluctuations, wastewater was collected every 8 h (hydraulic retention time of MBR) on the sampling day. Wastewater at each sampling site were mixed as one sample, which was considered as the representative sample for the sampling day. Samples (~800 mL) were collected in sterile 1000 mL containers, returned to the laboratory on ice in coolers, and stored at 4 °C for further pretreatment or analysis.

UF effluents (150 mL) were subjected to further UV disinfection and AOP (UV + TiO_2). The disinfection and AOP experiments were performed in glass crystallizing dishes (200 mL volumes in 150 mm diameter). The UV disinfection experiments were conducted by using a collimated beam apparatus (low-pressure, installed on the top of the target crystallizing dishes) that emitted monochromatic light at a wavelength of 254 nm, a commonly selected wavelength for UV-disinfection in domestic wastewater treatment (Guo et al., 2009; McKinney and Pruden, 2012). The UV + TiO_2 based AOP were conducted using a similar beam apparatus, but the emitted monochromatic light was at a wavelength of 365 nm. The irradiation (fluence) rates of 254 nm and 365 nm were measured by a UV radiometer (Yaoshi, Shanghai) with the 230–280 nm spectrum sensor (LS126C) and 315–370 nm spectrum sensor (LS123A), respectively. The radiation density of these two UV lights was approximately 0.15 mW/cm^2 (stable reading after UV-lamps was warmed for ~15 min). Previous investigations show that the required UV dose for the inactivation of pathogenic indicators (i.e. *Escherichia coli*) in domestic wastewater ranged from 15 to 45 mJ/cm^2 (Meckes, 1982). This study adopted the highest dosing rate corresponding to a fluence time of 300 s. The same irradiation time was also applied to AOP experiment. The treatment-simulating experiments were all performed in three parallel to minimize test errors.

2.2. Simulation of the discharge of effluent into tidal rivers

Simulations were expected to directly answer whether biological treatment processes, physical separations, UV-disinfection or AOP could contain the development of antibiotic resistance in effluent-receiving water bodies. Tidal river water was sampled from Huangpu River, sections with less industrialized areas (Binjiang Park), in southern suburbs of Shanghai. 50 mL effluents from MBR, UF, UV and AOP were mixed with 450 mL sampled river water respectively in a 1 L glass beaker. The combined aliquots (effluent-river water) were prepared in triplicate and continuously mixed by magnetic stirrers at 25 °C for 96 h before further analysis. Here, this 96-hour design was based on a previous study which showed that water residence time in tidal rivers in Yangtze Delta ranged from 4 to 11 days (Wang et al., 2004; Yang et al., 2015). Considering the tidal river water was collected in April (raining season of Shanghai), the lowest residence time was adopted in simulation studies.

2.3. DNA extraction and target genes

All liquid samples (150 mL) were directly filtered through sterile 0.22-μm membrane discfilters (Pall Life Science, USA) within 2 h of sampling, and the membranes were stored at −40 °C before DNA extraction. Genomic DNA were extracted by using Ribolyzer (FastPre-24, MP, USA) and Power Soil DNA extraction kit (MOBio, USA), according to manufacture protocols. The yields and quality of extractions were verified by agarose gel electrophoresis (MapBio, Shanghai) and UV–Vis spectrophotometer (Merinton-4000, Beijing). The DNA concentrations were calculated based on the OD_{260} value; the absorption ratio of $OD_{260/280}$ in this study was required no < 1.8. The obtained extractions were diluted to ~5 ng/μL to minimize inhibitions on the polymerase chain reaction (PCR) assays.

There were 14 genes targeted in this study, including 8 subtypes of ARGs and 6 marker genes of MGEs. Specifically, the ARGs were comprised of *sul1* and *sul2* that confer resistance (*sulR*) to a class of outdated antibiotics, sulfonamides (Luo et al., 2010); *ermB* and *mefA* that confer resistance (*MLsR*) to a class of both human and veterinary use antibiotics, macrolides (Knapp et al., 2010); and bla_{OXA}, $bla_{CTX\text{-}M}$, bla_{TEM} and $bla_{NDM\text{-}1}$ confer resistance (*blaR*) to a class of human clinical-use antibiotics, beta-lactams (Ahammad et al., 2014). The targeted MGEs were comprised of *intl1* and *intl2* that represent integrons; *IS-26* and *IS-CR* that represent insertion sequences; *traA* and *trbC* that represent bacterial plasmids (Yu et al., 2016).

Conventional PCR assays were initially conducted based on the MyCycler (BioRad, USA) platform to check the existences of target genes. The conventional PCR mixture (25 μL-volume systems) contains 12.5 μL of Taq MasterMix (Sangon, Shanghai), 0.5 μL of each primer (1 μM, MapBio, Shanghai), 1 μL of template DNA, and 10.5 μL sterile nuclease free ddH_2O. The PCR products were verified by gel electrophoresis (Sanger, MapBio, Shanghai). The DNA extracts from gels were sequenced (Sanger, MapBio, Shanghai) and blasted against GenBank