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Bioaerosol emissions and detection of airborne antibiotic resistance genes from a wastewater treatment plant

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HIGHLIGHTS

- Highest bacterial and fungal aerosol levels were found at sludge thickening basin.
- Fluorescent peaks for aerosol particles were observed around 3–4 μm for most sites.
- Airborne resistant genes including sul2 and class 1 integrase were detected.
- Human pathogens were detected from the air samples.

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ABSTRACT

Air samples from twelve sampling sites (including seven intra-plant sites, one upwind site and four downwind sites) from a wastewater treatment plant (WWTP) in Beijing were collected using a Reuter Centrifugal Sampler High Flow (RCS); and their microbial fractions were studied using culturing and high throughput gene sequence. In addition, the viable (fluorescent) bioaerosol concentrations for 7 intra-plant sites were also monitored for 30 min each using an ultraviolet aerodynamic particle sizer (UV-APS). Both air and water samples collected from the plant were investigated for possible bacterial antibiotic resistance genes and integrons using polymerase chain reaction (PCR) coupled with gel electrophoresis.

The results showed that the air near sludge thickening basin was detected to have the highest level of culturable bacterial aerosols (up to 1697 CFU/m^3) and fungal aerosols (up to 930 CFU/m^3). For most sampling sites, fluorescent peaks were observed at around 3–4 μm, except the office building with a peak at 1.5 μm, with a number concentration level up to 1233–6533 Particles/m^3. About 300 unique bacterial species, including human opportunistic pathogens, such as *Comamonas* Testosteroni and *Moraxella* Osloensis, were detected from the air samples collected over the biological reaction basin. In addition, we have detected the sul2 gene resistant to cotrimoxazole (also known as septra, bactrim and TMP-SMX) and class 1 integrase gene from the air samples collected from the screen room and the biological reaction basin. Overall, the screen room, sludge thickening basin and biological reaction basin imposed significant microbial exposure risks, including those from airborne antibiotic resistance genes.

1. Introduction

Antibiotics are widely used for prevention and treatment of microbial infections in humans, and also applied for hygiene practice in agriculture and farming sectors (Martinez, 2008; Cabello, 2006; Sarmah et al., 2006). For example, sulfonamide and tetracycline have been generally used as therapeutic agents in human and animal medicine since the middle of the 20th century

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(Pastor-Navarro et al., 2009). In recent years, there is a great concern that overuse of antibiotics would promote the development of antibiotic resistant bacteria and genes (Chen and Zhang, 2013a; Pruden et al., 2006). According to Shlaes et al. (2013), the cases of drug-resistant nosocomial infections in 2013 had doubled, even quadrupled since 2008 in the United States. Another greater concern is the possibility of the sharing of antibiotic resistant genes among bacteria through horizontal gene transfer (Stalder et al., 2012; Szczepanowski et al., 2009). For instance, the gene of *Klebsiella* pneumonia resistant to carbapenem was observed to spread to *Escherichia* coli (Yong et al., 2009). Expectedly, airborne resistant bacteria and genes have caused significant human and economic costs. Tuberculosis (TB), which was once considered to be eradicated, is now re-boarding the stage again (WHO, 2013). In 2012, there were about 450, 000 new confirmed cases of multidrug-resistant tuberculosis (MDR-TB) infections in 92 countries (WHO, 2013). The treatment courses MDR-TB requires are much longer and less effective than those for non-resistant TB (WHO, 2014). Another example is that a group of 662 patients including from newborns to 93-year-old men in a hospital in the US from January 2000 to June 2008 were all diagnosed with nosocomial infections due to the antibiotic-resistant Gram-negative bacteria during an investigation (Mauldin et al., 2010). The results showed that the median additional costs for patients infected with resistant bacteria reached US$ 38,121. In conclusion, antibiotic resistant genes are rapidly evolving into a significant environmental problem (Andersson and Hughes, 2010), and have already become a global threat to public health.

Among many others, wastewater treatment plants (WWTPs) are described as one of major anthropogenic contributors to the emission and spread of antibiotic resistant bacteria genes (Rizzo et al., 2013). In previous studies, two sulfonamide resistant (sul) genes (sul1 and sul2) and ten tetracycline resistant (tet) genes (tetA, tetB, tetC, tetD, tetE, tetG, tetM, tetO, tetQ, tetW) were frequently detected in influent wastewater, effluent before advanced treatment, final effluent and activated sludge of WWTPs (Chen and Zhang, 2013b; Wang et al., 2013; Boerjesson et al., 2010; Zhang et al., 2009; Yang et al., 2013; Gao et al., 2012; Su et al., 2014). In a recent study by Chen and Zhang (2013b), the concentrations of tet, sul genes and integrase (int) gene of integrons were detected to range from 10^6 to 10^7 copies/mL in the influent, from 10^4 to 10^6 copies/mL in the effluent, and from 10^8 to 10^{11} copies/g in the biosolids in three Chinese WWTPs, respectively. Integrons are described as genetic units that can capture, integrate exogenous gene cassettes and further convert them via site-specific recombination (Mazel, 2006). Thus, Integrons could play important roles in the exchange, retention and dissemination of antibiotics resistance. The genes of multidrug-resistant (MDR) and pan-drug resistant (PDR) bacteria were also detected from different processes in wastewater treatment plants (WWTPs). Luo et al. (2014) reported the occurrence, persistence, and fate of the New Delhi metallo-β-lactamase (NDM-1) genes through several treatment units (including disinfection by chlorination) in two WWTPs in northern China. The European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) (2011) estimated that patients infected with NDM-1 would be untreatable for at least a decade. Current practices in WWTPs could inevitably pollute the environment in the form of resistant genes via wastewater discharge and land application of biosolids (Brooks et al., 2007; Munir and Xagoraraki, 2011; Reinthaler et al., 2003). The presence and airborne release of these resistant genes can pose significant health problems.

In addition, the WWTP is also observed to release significant amount of airborne human pathogens. Gotkowska-Płachta et al. (2013) identified 25 species of *Enterobacteriaceae* in the air obtained from the premises of a WWTP in Poland, including *Klebsiella* pneumonia, *Escherichia* coli and *Salmonella* spp., which could cause destructive effects to human lungs when inhaled. Viegas et al. (2014) detected a number of human pathogens in the air samples collected above the grit chamber and the biological reaction basin of a WWTP in Portugal, including *Aspergillus fumigatus* which is often blame for pulmonary infections and allergies; and *Stachybotrys chartarum*. Through discharging, mixing, aerating, and spraying of sewage, significant amounts of bioaerosols can be produced during the wastewater treatment process to pose a high health risk to workers and further dispersed over considerable distances, causing adverse effects on humans through inhalation (Gotkowska-Płachta et al., 2013). The WWTP workers have already been shown to be at a higher risk of developing a large variety of work-related symptoms compared with the general population, including respiratory and gastrointestinal (e.g. diarrhoea) effects (Douwes et al., 2001; Rylander, 1999; Thorn et al., 2002; Thorn and Beijer, 2004).

China is facing increasing demand for safe water, while current WWTPs are being challenged with increased health problems, among which aerosol transmitted diseases are on the focus. This work was conducted to investigate biological aerosol contents and airborne antibiotic resistant genes emitted from a typical WWTP in Beijing. Here, a high flow rate sampler (100 L/min)-RCS High Flow was used to study airborne culturable bacterial and fungal aerosol concentration levels in the wastewater treatment plant. In addition, PCR and a real-time bioaerosol sensor (UV-APS) were applied to assessing bioaerosol exposure risks including airborne resistant genes. This work presented evidence for possible release of airborne antibiotic resistant genes and pathogens from a typical wastewater treatment plant, and the results are useful to help manage microbial exposure risk involving wastewater treatment.

2. Experimental methods

2.1. Study sites and sample collection

A WWTP located in the northern part of Beijing, North China was chosen for this study. The treatment technologies (activated sludge) used in the plant is typical in wastewater treatment field. The WWTP covers an area of 40 ha and treats up to 400,000 m^3 of municipal wastewater per day serving a population of 814,000. The schematic diagram of basic wastewater treatment process flow is shown in Fig. 1. It consists of pre-treatment (an indoor screen room), a grit chamber and a biological reaction basin, which includes an anoxic zone, an anaerobic zone and an aerobic zone with a micro bubble aeration system (AAO process), and also a secondary clarifier. Part of excess sludge is returned back to the biological reaction basin, and the rest goes into an indoor sludge thickening basin. The secondary clarifier effluent receives the advanced treatment before discharged.

Air, water and concentrated sludge samples were collected from the WWTP on April 4th, 2014, which was a clear day at the time of sample collection with a temperature from 8 °C to 24 °C. The prevailing wind direction was north with a wind velocity of 5.5–10.7 m/s or 20–38 km/h according to Beijing meteorology report. A high volume air sampler-Reuter Centrifugal Sampler High Flow (RCS) (Biotest, Inc.) was used to collect air samples in 12 sampling sites, including an upwind site outside the plant, 7 intra-plant sites (screen room, grit chamber, biological reaction basin, sludge thickening basin, effluent outlet, office building and the boundary of plant in downwind direction as shown in Fig. 2) and 4 downwind sites (0.2, 1, 5 and 10 km away from plant, respectively). Air samples collected from 12 sample sites were collected on Trypticase Soy Agar (TSA) strips (Becton, Dickson and Company, Sparks, MD) for bacteria and Malt Extract Agar strips (Becton,