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Quantitative and qualitative changes in antibiotic resistance genes after passing through treatment processes in municipal wastewater treatment plants

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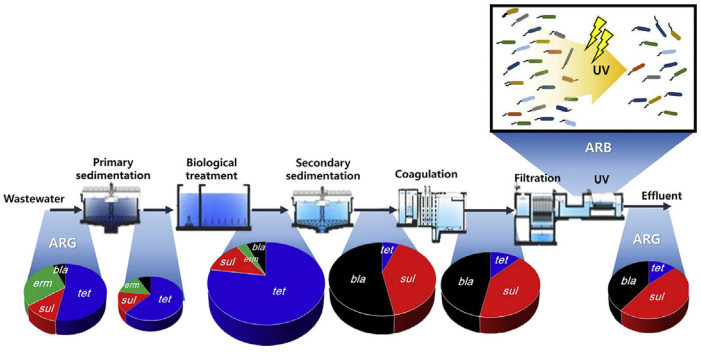
HIGHLIGHTS

- Quantitative and qualitative changes in ARGs were investigated in WWTPs.
- Changes were unique for each ARG community while undergoing treatment processes.
- Variation in ARGs was largest during biological and post-physiochemical processes.
- ARGs showed limited response to UV disinfection.
- ARB were reduced by UV disinfection.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, quantitative and qualitative changes in antibiotics resistance genes (ARGs) were investigated in two municipal wastewater treatment plants (WWTPs) treating pretreated livestock or industrial wastewater as well as municipal sewage. Total eight ARGs (*tet*X, *tet*M, *tet*A, *sul*1, *sul*2, *erm*B, *qnr*D, and bla_{TEM}) were quantified, and their relative abundance was assessed by ARGs copies/16S rRNA gene copies. The fate of ARGs was observed to be different between two WWTPs: *sul*, *qnr*D, and bla_{TEM} were proliferated during the treatment processes only in the WWTP1 which received pretreated livestock wastewater. Furthermore, dynamic shifts in patterns of ARGs occurrence were observed during biological, secondary sedimentation and coagulation processes. During biological treatment in both WWTPs, relative abundance of *tet* and *erm*B changed: *tet* increased significantly by 211.6–357.6%, while *erm*B decreased by 70.4–92.0%. Little variation was observed in *sul*, *qnr*D and bla_{TEM}. Subsequently, the relative abundance of *tet* decreased during the secondary sedimentation and coagulation in both WWTPs: *tet* decreased by 56.0–86.3% during sedimentation and by 48.2–75.7% during coagulation, respectively. During the final treatment, different responses of antibiotic resistance bacteria (ARB) and ARGs to ultraviolet (UV) disinfection were found: removal efficiencies of ARB were observed in the range of 34–75%, while obvious reduction in ARGs was not observed at the UV dose of 27 mJ/cm^2. Although ARGs underwent various treatment processes, considerable levels of ARGs remained at discharge amounting to 4.2×10^{18} copies/day from WWTP1 and 5.4×10^{16} copies/day from WWTP2, respectively.

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1. Introduction

Proliferation of antibiotic resistance genes (ARGs) is recognized by the World Health Organization (WHO) and the Centers for Disease Control (CDC) (CDC, 2001; WHO, 2000) to be a serious concern for public health. ARGs proliferation occurs via the following possible mechanisms (Lee et al., 2017): (1) antibiotics are not fully metabolized but are significantly (30–90%) excreted into the environment (Daghrir and Drogui, 2013; Sharma et al., 2016); (2) antibiotics exert selection pressure facilitating the proliferation of antibiotic resistant bacteria (ARB) (Sharma et al., 2016); (3) horizontal gene transfer between bacteria promotes the spread of ARGs in the environment (Rizzo et al., 2013; Sharma et al., 2016). Current reports describe the widespread use of antibiotics for medical, veterinary, and agricultural purposes in many countries which stimulates further spread of ARGs (Rizzo et al., 2013; Sharma et al., 2016).

Municipal wastewater treatment plants (WWTPs) have been identified as one of the main sources of ARGs and ARB (Rizzo et al., 2013). In many cases, various antibiotic residues and co-selection factors (e.g. metals, and surfactants) responsible for ARB/ARG proliferation enter WWTPs (Pruden et al., 2013). Furthermore, environmental conditions in WWTPs allow ARB to flourish which in turn leads to gene transfer between ARB and non-ARB, even though the fates of ARGs and ARB could differ depending on treatment plant design and operating conditions (Bouki et al., 2013). Accordingly, ARB and ARGs have been frequently detected in WWTP effluents even after disinfection (Pruden et al., 2013). For instance, diverse ARGs belonging to the tetracycline, sulfonamide, macrolide, and quinolone resistance genes (*tet*, *sul*, *erm*, and *qnr*, respectively) were detected in the effluents from WWTPs in Italy (Di Cesare et al., 2016) and from WWTPs in China (Mao et al., 2015). Munir et al. (2011) detected tetracycline and sulfonamide-resistant bacteria in the effluents from WWTPs in the United States. Therefore, a growing number of scientists have paid attention to determining the role of WWTPs in ARGs and ARB regulation. The occurrence of ARGs resulting from each treatment process has been investigated in WWTPs in China (Mao et al., 2015; Wen et al., 2016) and Italy (Di Cesare et al., 2016). In U.S. WWTPs, occurrence of ARB and ARGs in effluents before and after disinfection was analyzed, and the role of disinfection in the reduction of ARB/ARGs was investigated (Munir et al., 2011).

Despite growing attention, antibiotic resistance has not been fully explored in WWTPs in South Korea. Overuse of antibiotics in South Korea is a serious issue; for instance, 723 tons of tetracycline are consumed annually, second only to the United States (3200 tons/year) and much more than in European countries (Daghrir and Drogui, 2013; Lee et al., 2017). The characteristics of antibiotic resistance depend on types of wastewater: for example, the occurrence of ARGs and ARB has been found to be different among various wastewater (Zhang et al., 2009). The livestock wastewater is appointed to one of the most serious sources of antibiotics because >70% of total antibiotics are used for livestock in United States and Australia (Pruden et al., 2013). The type of wastewater could affect the fate of ARGs through treatment processes, but it has not been fully explored, especially for livestock and industrial wastewater.

In this study, quantitative and qualitative changes in ARGs having passed through treatment units were investigated in representative municipal WWTPs in the metropolitan city of Gwangju. One of the largest cities in South Korea, Gwangju serves a total population of 1.5 million inhabitants. The Yeong-san river, the fourth longest river in South Korea, flows through the city, presenting a means to disseminate the effects of WWTPs' discharge across the broad population inhabiting the river basin. Each WWTP receives not only municipal sewage but also its unique type of wastewater (pretreated livestock or industrial wastewater). The research objectives of this study are: 1) to investigate quantitative and qualitative changes in ARGs undergoing wastewater treatment processes 2) to compare the effect of wastewater type on the fate of ARGs between two WWTPs and 3) to analyze impact of ultraviolet (UV) disinfection on ARGs and ARB.

2. Materials and methods

2.1. Descriptions of WWTPs

Two full-scale municipal WWTPs in the greater Gwangju, South Korea area were studied. WWTP1 receives domestic sewage mainly from the districts of Dong-gu, Seo-gu, Nam-gu, and Buk-gu, which comprise almost 72.6% of the total population of Gwangju. In addition, the plant receives wastewater mainly from poultry farms (97.3% of total livestock) in Gwangju. WWTP2 receives pretreated industrial wastewater from major industrial clusters consisting of machinery, steel and electronic manufacturing plants, as well as domestic sewage mainly from the district of Gwangsan-gu making up 27.4% of the total population (Table S1). The two WWTPs employ traditional wastewater treatment processes of primary sedimentation, biological treatment, chemical treatment (coagulation), filtration, and tertiary treatment using UV disinfection (Fig. 1). The biological treatment of WWTP1 constitutes a sequential process consisting of anaerobic, anoxic and oxic tanks (A2O). The WWTP2 is operated in parallel via two types of biological treatment: one, a biological process consisting of anoxic and oxic tanks (Modified Ludzack-Ettinger; MLE); the other, an A2O process (Fig. 1). During chemical treatment, polyaluminum chloride (PAC) is used as a coagulant in both WWTPs. Daily usage was up to 20,000 kg/day in the WWTP1 and 7493 kg/day in the WWTP2, respectively. The tertiary treatments of both WWTPs are UV disinfection using 27 mJ/cm^2 of the UV dose (a fluence rate of 13.5 mW/cm^2 and 2 s of assumed contact time) suggested by the manufacturer, EcoSet, Inc. (Seoul, South Korea). Total treatment capacities of WWTP1 and WWTP2 are 600,000 tons of sewage influent per day (tons/day) and 120,000 tons/day, respectively. The major operational information summarizing the two WWTPs can be found in Table S1.

2.2. Wastewater sampling and genomic DNA extraction

Four liters of wastewater samples were obtained from each treatment process (primary sedimentation, biological treatment, secondary sedimentation, coagulation, filtration, and UV disinfection) shown in Fig.1. The samples were stored in a portable, cool box after the sampling and brought to the laboratory within 4 h. Upon arrival at the laboratory, each sample was immediately distributed to a 50 mL sterilized centrifuge tube (SPL Life Science, South Korea) to analyze the ARB plate count.

Microbial samples for DNA extraction were prepared using the following procedures: each sample was prepared in a 1.5 mL microcentrifuge tube (Watson Biolab, Japan) with 0.5 mL volume from the biological treatment process (E ~ G for WWTP1, e ~ g for WWTP2, Fig. 1) and with 1.0–3.0 mL volume for the points prior to primary sedimentation (A ~ D for WWTP1, a ~ d for WWTP2, Fig. 1) in such a way that similar size (100 μL) of pellet could be produced; the sample was then centrifuged at >13,000 rpm for 10 min; the supernatant was replaced by nuclease-free deionized and distilled water; the wastewater samples (150 mL) after secondary sedimentation process (H ~ L for WWTP1, h ~ l for WWTP2, Fig. 1) were concentrated by being filtered through a 0.45 μm cellulose nitrate membrane filter (CHMLAB, Japan) (Munir et al., 2011). Subsequently, DNA was extracted from all the prepared samples using a GeneAll Exgene™ Soil SV Kit (GeneAll Biotechnology, South Korea) following the manufacturer's instruction. The extracted DNA samples were refrigerated at −20 °C until further analyzed.

2.3. ARG pre-screening and gene quantification

Samples underwent a conventional polymerase chain reaction (PCR) run and typical agarose gel electrophoresis to detect the following twelve ARGs: three tetracycline resistance genes (*tet*X, *tet*M, *tet*A), two