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Fate of antibiotic resistance genes and antibiotic-resistant bacteria in water resource recovery facilities

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Received 30 January 2018; Revised 19 September 2018; Accepted 24 September 2018

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DOI: 10.1002/wer.1008

Abstract

Many important diseases are showing resistance to commonly used antibiotics, and the resistance is potentially caused by widespread use of antibiotics for maintaining human health and improving food production. Antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) are associated with this increase, and their fate in water resource recovery facilities is an important, emerging area of research. This literature review summarizes current findings of worldwide research on the fate of ARB and ARGs in various types of treatment plants. Twenty-five published studies were reviewed which contained 215 observations in activated sludge, membrane bioreactors, anaerobic digestion, constructed wetlands, coagulation–filtration, and three types of disinfection. We found 70% decreased observations, 18% increased observations, and 12% unchanged observations of all observations in all treatment processes. Resistance genes to tetracycline were most often observed, but more studies are needed in other antibiotic resistance genes. The causes for increased abundance of ARGs and ARB are not well understood, and further studies are warranted. © 2018 Water Environment Federation

Practitioner points

- Antibiotic resistance is increasing with concern that treatment plants may acclimate bacteria to antibiotics.
- A literature survey found 215 resistance observations with 70% decreased, 18% increased, 12% unchanged after treatment.
- The type of treatment process is important with activated sludge showing the greatest reductions.

Key words

antibiotic resistance genes; antibiotic-resistant bacteria; wastewater treatment

Introduction

Antibiotic failure due to increasing antibiotic resistance is a worldwide threat to public health. According to the Center for Disease Control (CDC), in 2013 in the United States alone, about 2,000,000 people were infected with antibiotic-resistant bacteria, and 23,000 died as a result (CDC, 2013). Globally, current antibiotic resistance accounts for at least 700,000 lives lost per year, and it is projected that an unabated rise in antimicrobial resistance may lead to 10 million deaths per year by 2050 (Liu et al., 2016; May, 2016; O'Neill December, 2014). Antibiotic overuse is one of the most important causes for the loss of antibiotic effectiveness. Even in developed countries, where antibiotic usage in medicine is strictly supervised, they are still widely overused in human daily life and on farms. In 2014, for example, outpatient healthcare providers in the United States wrote over 266 million antibiotic prescriptions, amounting to 835 antibiotic prescriptions for every 1,000 people. However, it is believed that at least 30 percent of oral antibiotics prescribed may be unnecessary (CDC, 2017).

In food animal production, approximately 9.7 million kilograms, or 21.4 million pounds, of antibiotics considered important for human use were sold for use in animal agriculture in 2015, which is 26 percent increased since 2009 (CDC, 2017). According

Table 1. Antibiotic consumptions as a function of meat production in different countries

COUNTRY	ANTIBIOTIC CONSUMPTION (MG)[a]	RATIO (MG/KG)
USA	9.7×10^{12}	266.2
EU	6.7×10^{12}	152.0
Cyprus	2.9×10^{10}	396.5
Italy	1.2×10^{12}	341.0
Hungary	2.0×10^{11}	245.5
Spain	6.5×10^{11}	242.0
Germany	1.1×10^{12}	204.8
Belgium	2.9×10^{11}	161.1
UK	2.2×10^{11}	62.0

Note. [a]The meat consumption data are cited from OECD data (https://data.oecd.org/agroutput/meat-consumption.htm) and statistical data (https://www.statista.com/statistics/679528/per-capita-meat-consumption-european-union-eu/).

to the report of Statista, for European Union's countries, Cyprus had the greatest antibiotics usage in 2015, which was almost 400 mg of antibiotics for every kilogram of meat produced, followed by Italy with 341 mg/kg and Hungary with 246 mg/kg in Europe. The average consumption for the European Union counties was 152 mg/kg in livestock production. Table 1 shows the total antibiotic consumption and the consumption per kg of meat (beef, pork, chicken, and lamb) in some developed countries and indicates the antibiotics used in the meat production. The United States had an annual antibiotic consumption of 266 mg/kg from meat consumption, which was 100 mg/kg more than the average of the European Union countries. Meat consumption is not the only source of antibiotics, and large amounts of antibiotics are utilized in a clinical setting. In 2015 in the United States, healthcare providers wrote 269.4 million antibiotic prescriptions, which is equivalent to 838 antibiotic prescriptions per 1,000 persons (CDC, 2015). Assuming one adult weighs 70 kg and a typical amoxicillin dosage of 500 mg (because amoxicillin was the most commonly used antibiotic in the United States in 2015, according to Outpatient Antibiotic Prescriptions—United States, 2015), the consumption is 5.98 mg/Kg per 500 mg dosage. Assuming a typical prescription instruction every 8 hr for 1 week, the consumption is 125.6 mg/kg per prescription (MAYO CLINIC, 2017). The average number of prescriptions was one prescription for one person in the United States in 2015. Therefore, an adult consumed on average 8,792 mg of antibiotics in 2015. However, the annual possible antibiotic intake from meat production was approximately 30.5 g/person in 2015, which is three times more than consumption from antibiotic prescriptions.

In the EU, the level of usage exists in spite of their 20-year history of bans or partial bans on the use of antimicrobials for growth promotion. The reduced use of antibiotics may be the reason for the decreasing bacterial resistance to antibiotics in some European countries. For example, *Enterococcus faecium* from broiler chickens in Denmark are showing decreased resistance to avilamycin and macrolide antibiotics (tylosin is one type of macrolide antibiotic; Maron, Smith, & Nachman, 2013). In the United Kingdom, studies following the ban on antibiotic growth promoters in pigs showed that resistance to erythromycin in *Campylobacter coli* and *Enterococcus faecium* decreased from 85% in 1999–2000 to 36% in 2007 (Carol, Herman, & Christina, 2011). These results are very encouraging and may be a good example for the United States in anticipation of future regulations. Apart from chemical pollution caused by antibiotics themselves, widespread usage accelerates the development of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB), which can influence the health of both humans and animals. The World Health Organization in 2017 published its first ever list of antibiotic-resistant "priority pathogens," which is a catalogue of 12 families of bacteria that pose the greatest threat to human health (Table 2), which reinforces the urgency and need for the study of the emergence and fate of antibiotic resistance genes and bacteria and their threats to the environment and human health (Tacconelli et al., 2018). This list is guided by both small and large pharmaceutical companies applying a multicriteria decision analysis, a method that incorporates both expert opinion and evidence-based data in

Table 2. WHO priority pathogens list for Research & Development (R&D)

PRIORITY[a]	BACTERIA	ANTIBIOTIC-RESISTANT
Critical	*Acinetobacter baumannii*	Carbapenem-resistant
	Pseudomonas aeruginosa	Carbapenem-resistant
	Enterobacteriaceae	Carbapenem-resistant, ESBL-producing
High	*Enterococcus faecium*	Vancomycin-resistant
	Staphylococcus aureus	Methicillin-resistant, vancomycin-intermediate and resistant
	Helicobacter pylori	Clarithromycin-resistant
	Campylobacter spp.	Fluoroquinolone-resistant
	Salmonellae	Fluoroquinolone-resistant
	Neisseria gonorrhoeae	Cephalosporin-resistant, fluoroquinolone-resistant
Medium	*Streptococcus pneumoniae*	Penicillin-non-susceptible
	Haemophilus influenzae	Ampicillin-resistant
	Shigella spp.	Fluoroquinolone-resistant

Note. [a]The priority level is according to the urgency of need for new antibiotics.

a transparent, explicit, and deliberative fashion (Tacconelli & Magrini, 2017). The following ten criteria were selected to perform pairwise comparison: all-cause mortality, healthcare and community burden, prevalence of resistance, 10-year trend of resistance, transmissibility, preventability in hospital and community settings, treatability, and current pipeline. ARGs and ARB are clearly starting to be a serious concern.

Antibiotic-resistant bacteria spread via physical forces, such as wind, water environment, and soil, or biological forces, including human activities, animals, insects, and birds (Allen et al., 2010). These bacteria may also be transported from the environment to humans via direct or indirect contact (Allen et al. 2010, Davies & Davies, 2010; Zhang, Zhang, & Fang, 2009). Therefore, knowing the origins of ARGs to the environment plays an important role in understanding transport mechanisms. Hospitals and farms are two major sources (Baquero, Martinez, & Canton, 2008; Timraz, Xiong, Al Qarni, & Hong, 2017) introducing ARGs and ARB to the environment. The discharge of antimicrobial agents, detergents, disinfectants, and residues from industrial pollution such as heavy metals also contributes to the evolution and spread of such resistant organisms in the water environment (Baquero et al., 2008). Water resource recovery facilities are major receptors of ARGs and are potentially major sources of ARGs to the environment. Treated effluents are usually discharged to lakes, rivers, and oceans, or reused for human activities such as agricultural or landscape irrigation and reclamation with potential human contact. The City of Los Angeles proposed an indirect potable reclamation program that will eventually result in deliberate introduction of highly treated domestic wastewater into drinking water in order to reduce dependence on imported water. Thus, water resource recovery facilities play an important role in the water field. Water resource recovery facilities are rarely equipped with the processes specifically for removing antibiotics, ARGs, or ARB. For this reason, many current studies focus on the efficiency of the existing processes to remove ARGs or ARB (Gerrity, McLain, Rock, Dickenson, & Batista, 2018), which is also a major objective of this literature review.

This paper summarizes what has been reported about the performance of different types of water resource recovery facilities in removing ARGs and ARB, with particular emphasis on fate of ARGs and ARB in different processes. In addition, the factors potentially affecting the removal efficiency of water resource recovery facilities are discussed.

Types of environmental antibiotic resistance genes

Long-term applications of antibiotics in the protection of humans and animals and in agricultural growth promotion have exerted a major impact on bacterial communities. These applications have resulted in bacteria possessing various resistances to antibiotics that are generally controlled by ARGs. The following mechanisms create increasing resistance in a bacterial community: (a) target bypass, which allows some bacteria to become refractory to specific antibiotics by bypassing the inactivation of a given enzyme; (b) efflux pump, which is the mechanism that prevents the antibiotic from penetrating the outer and/or cytoplasmic membrane by decreasing the uptake of the antimicrobial molecule (Munita & Arias, 2016); (c) antibiotic inactivation, which inactivates the active antibiotic molecule directly; and (d) target modification, which modifies action sites of antibiotics (Džidić, Šušković, & Kos, 2008; Munita & Arias, 2016; Zhang et al., 2009). Furthermore, the resistance of certain antibiotics might be associated with more than one mechanism.

Generally, several types of antibiotic resistance genes (ARGs) have been found in the environment, including genes resistant to tetracycline, sulfonamide, aminoglycoside, macrolide–lincosamide–streptogramin (MLS), chloramphenicol, vancomycin, and β-lactam genes. Each type has its own characteristics and is discussed in detail.

ARGs related to tetracycline

Tetracycline-resistant bacteria emerge in environments with the introduction of tetracycline, and more than 22 tetracycline *(tet)* or oxytetracycline *(otr)* resistance genes have been found in bacterial isolates from water environments. There have been at least 38 different *tet* genes and *otr* resistance genes characterized to date. Of these genes, 23 tetracycline resistance genes code for efflux pump mechanism, 11 genes contain ribosomal protection proteins (target modification mechanism), and three genes inactivate enzymes (Zhang et al., 2009). Most environmental *tet* genes code for transport proteins, which pump the antibiotics out of the bacteria cell and keep the intercellular concentrations low to make ribosomes function normally (Roberts, 2002). Studies showed that tetracycline resistance genes were detected in the sediments near a fish farm, even after antibiotic usage had stopped 6 years before sampling, suggesting the high persistence of tetracycline resistance genes in aquatic environments (Gao, Munir, & Xagoraraki, 2012). They determined that 8% and 6.7% of tetracycline-resistant bacteria are found in the pre- and postchlorinated samples of a water resource recovery facility, respectively, and are discharged into a nearby river water where similar percentages of bacteria were found to be resistant to tetracycline (Munir, Wong, & Xagoraraki, 2011). Recently, the tetracycline resistance genes including *tetA*, *tetB*, *tetBP*, *tetG*, *tetM*, *tetO*, *tetQ*, *tetS*, *tetT*, *tetW*, *tetX*, and *tetZ* have been detected in anthropogenic or pristine environments; of these, *tetM*, *tetO*, *tetS*, *tetQ*, and *tetW* contain coding for ribosomal protection proteins and have been detected in microbial communities of water resource recovery facility, hospital or animal protection wastewaters, and even in natural water environments (Munir et al., 2011). Additionally, Pruden, Pei, Storteboom, and Carlson (2006) reported that *tetB*, *tetP*, *tetO*, *tetS*, *tetT*, and *tetW* were detected in the presence of ditch water and dairy lagoon water in Northern Colorado in the United States. Tetracycline-resistant bacteria have been frequently detected in many water sources.

ARGs related to sulfonamide

Sulfonamides are the first antibiotics developed for large-scale clinical use, which target dihydropteroate synthase (DHPS), a catalytic enzyme in the folic acid biosynthesis pathway (Alekshun & Levy, 2007). They found that sulfonamides compete with the

structural analog *p*-aminobenzoic acid for binding to DHPS, thus preventing bacterial growth by inhibiting the formation of dihydrofolic acid. Resistance to sulfonamides in *Escherichia coli* can result from mutations in the chromosomal DHPS gene (*folP*) or more frequently from the acquisition of an alternative DHPS gene (*sul*), whose product has a lower affinity for sulfonamides (Perreten & Boerlin, 2003). Different mechanisms have been found to confer sulfonamide resistance, mostly based on changes in the *sul* genes and mediation by mobile elements (Zhang et al., 2009). Four *sul* genes (*sulI*, *sulII*, *sulIII*, and *sulA*) have been found in bacteria of environmental origin. *SulI* and *sulII* have been detected in bacteria isolates from fecal slurry of dairy farms, water or sediments of aquaculture areas, and even from the river or seawater without evidence pollution. In China, *sulI* and *sulIII* were detected in both rural wastewater treatment and municipal water resource recovery facilities in Zhejiang province (Chen & Zhang, 2013). This suggests that sulfonamide genes are worthy of concern.

ARGs related to macrolide

Antibiotics of macrolide are often investigated simultaneously for microbial resistance, because certain macrolide resistance genes (*erm*) encode resistance to at least two macrolide, lincosamide, and streptogramin antibiotics, although they are structurally unrelated to macrolide antibiotics (Roberts et al., 1999). Until 2009, more than 60 different genes, which confer total resistance to one or more of the macrolide–lincosamide–streptogramin (MLS) antibiotics, have been identified, including the genes associated with ribosomal RNA (rRNA) methylation, efflux, and inactivation. MLS resistance is mostly mediated by rRNA methylases (encoded by *erm* genes), which methylate the adenine residues to prevent the three antimicrobials from binding to ribosomal protein (Zhang et al., 2009). The *erm* genes can easily be transferred from one host to another, since they are usually acquired and associated with mobile elements, such as plasmids and transposons. Chen and Zhang (2013) detected several *erm* genes in *Enterococcus spp.* isolated from poultry wastewater and environmental DNA extracted from livestock manures. Six classes of *ermA, B, C, F, T, and X* genes have been detected and quantified in the samples from animal production manures, lagoons, and a biofilter system treating hog house wastewater. Among the macrolide resistance determinants, *ermB* is considered the most prevalent gene in environmental microorganisms, especially in the strains of *Enterococcus* and *Streptococcus spp* (Zhang et al., 2009).

ARGs related to other types

Other types of ARGs, including aminoglycoside, vancomycin, and β-lactam genes, exist in the environment as well, which are potential pollutants to human and animals. More than 50 modification enzymes have been found so far (Chen & Zhang, 2013). These enzymes are divided into three groups based upon their biochemical actions on the aminoglycoside substrates. These include acetyltransferases, phosphotransferases, and nucleotidyltransferases (adenylyltransferases), which are encoded by three types of genes, namely, *aac*, *aph*, and *ant* (*aad*), respectively. Vancomycin resistance emerged first in *enterococci*. Recently, the resistance has also been detected in *Staphylococcus aureus*. So far, six types of vancomycin resistance genes (*van*) are known, and *vanA* and *vanB* are the most prevalent ones in water environments. Thirty-five ARGs are listed in Table 3 and discussed in the following sections, although there are more ARGs that have been found in the water resource recovery facility and natural environment. Major biological sources for individual ARGs are summarized as suggestions for future studies of antibiotic-resistant bacteria detection. However, other bacteria that are not listed in the table may contain these ARGs as well.

Techniques for the detection of ARGs

Selecting methods to determine treatment efficiency as well as methods for gene detection is a critical research decision to positively obtain results of the antibiotic resistance genes from water resource recovery facilities. Wastewater samples may be collected before and after treatment processes and stored at appropriate temperatures for transportation to laboratories. Wastewater sources and types of water resource recovery facilities are important factors to understand the results. Samples are usually concentrated with vacuum filtration apparatus onto different filters with various pore sizes or materials depending on water quality and study objectives. In addition, DNA extraction is usually performed, and there are several alterative DNA extraction instruments used in previous research with their own specific protocols (Guo, Li, Yang, Yang, & Yin, 2014; Munir et al. 2011). There are a number of methods for detecting and characterizing ARGs and ARB, but the four most commonly used methods are DNA hybridization, PCR (simple and multiplex PCR), quantitative PCR, and DNA microarray. A less commonly used method is Illumina HiSeq 2000 (Aydin, Ince, & Ince, 2015). Molecular hybridization has been used to detect the presence or absence of specific ARGs for three decades and is still often applied to distinguish different ARGs within one group or to identify the presence of specific genes in certain environments (Zhang et al., 2009). Fluorescence in situ hybridization (FISH), an important nonradiolabeled method, has been established and applied to detect clinical microbial resistances and for the rapid identification of macrolide resistances caused by ribosomal mutations (Russmann et al., 2001). PCR, including simple and multiplex PCR, has been widely used in both pure cultures and mixed environmental samples to detect different kinds of ARGs, such as resistance encoded by tetracycline and β-lactam (Jacobs & Chenia, 2007; Taviani et al., 2008). The multiplex PCR method has been often used to simultaneously detect more than one ARG, which can reduce time and effort compared to simple PCR. DNA microarray is a hybridization application that analyzes gene expression or screens samples for single nucleotide polymorphisms (SNPs). This allows DNA and/or RNA hybridization analysis to be carried out in microminiaturized highly parallel formats (Heller, 2002). One advantage of DNA microarray is that microarray allows detection of antibiotic resistance determinants within a few hours, saving time and being used as a convenient tool supporting conventional resistance detection methods (Antwerpen, Schellhase, Ehrentreich-Foerster, Witte, & Nuebel, 2007). The core and one

Table 3. Antibiotic resistance genes and their biological sources

ARG	CATEGORY	BIOLOGICAL SOURCE	ARG	CATEGORY	BIOLOGICAL SOURCE
tetA		*Aeromonas, Alcaligenes, Arthrobacter, Comamonas, Escherichia, Listeria, Pseudomonas, Salmonella, and Vibrio; Plasmids pB10, pTB11 and pRSB101*	*blaTEM*	β-Lactam resistance genes	*Escherichia*
tetB		*Afipia, Alcaligenes, Arthrobacter, Burkholderia, Escherichia, Pseudomonas, Serratia, Staphylococcus, and Vibrio*	*blaVIM*		*Pseudomonas aeruginosa*
tetC		*Aeromonas, Alcaligenes, Arthrobacter, Brevibacterium, and Pseudomonas*	*blaSHV*		*Klebsiella pneumoniae*
tetE		*Aeromonas, Pseudoalteromonas, and Vibrio*	*blaCTX-M*		*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa*
tetG		*Pseudomonas; microbial community*	*amp(A)*		*N/A*
tetM		*Aeromonas, Bacillus, Escherichia, Lactococcus, Pseudoalteromonas, and Vibrio; microbial community*	*mecA*		*Staphylococcus*
tetO	Tetracycline resistance genes	*Paenibacillus, Pseudoalteromonas, Shewanella, Sporosarcina, and Vibrio; microbial community*	*dfrA1*	Dihydrofolate reductase-encoding genes	*Aeromonas, Escherichia, and Salmonella*
tetQ		*Microbial community*	*dfrA12*		*Aeromonas, Escherichia, and Salmonella*
tetS		*Lactococcus and Vibrio; microbial community*	*ermB*		*Bacillus and Enterococcus*
tetT		*Microbial community*	*ermF*		*Microbial community*
tetW		*Microbial community*	*mefA*	Macrolide resistance genes	*Streptococcus pyogenes, Enterococcus faecalis, Bacteroides ovatus, Neisseria gonorrhoeae, Exiguobacterium sp.*
tetX		*clostridium tetani, bacteriodes fragilis, clostridium sp. RKD, riemerella anatipestifer (Moraxella anatipestifer), actinoplanes sp., corallococcus coralloides*	*ereA*	Erythromycin encoding genes	*Escherichia coli, Achromobacter denitrifican, Zobellia galactanivorans, Proteus mirabillis, Salmonella choleraesuis, Aeromonas hydrophila, Vogesella fluminis*
tetZ		*Actinomycetales, Afipia, Brevibacterium, Burkholderia, Dietzia, Leucobacter, and Microbacterium*	*marA*	Multiple antibiotic resistance genes	*Salmonella enteritidis*
sul1	Sulfonamide resistance genes	*Aeromonas, Escherichia, and Listeria; Plasmids pB2, pB3, pB8, and pB10; Microbial community*	*qnrS*	Fluoroquinolone resistance genes	*Escherichia coli, Providencia rettgeri, Morganella morganili, pseudomonas guangdongensis*
			vanA	Vancomycin resistance genes	*Enterococcus and Staphylococci*
sul2		*cinetobacter, Escherichia, Salmonella, and Vibrio; Microbial community*	*strA*	Aminoglycoside resistance genes	*Listeria, Salmonella and Vibrio; Plasmids pB4 and pB10*
sul3		*Escherichia; Microbial community*	*strB*		*Salmonella and Vibrio; plasmids pB4 and pB10*
sulA		*Microbial community*	*aphA2*		*Escherichia*

Note. Biological sources are cited from http://www.uniprot.org/.

of the most widely used methods is quantitative polymerase chain reaction (qPCR) utilizing fluorescent DNA-intercalating dyes (e.g., SYBR Green) and/or fluorophore-tagged hybridization dyes (e.g., TaqMan probes) to determine the presence and absence of targeted ARGs after extraction and normalization (Dodd, 2012). It is an enabling technology par excellence of molecular diagnostics with a capacity to detect and measure minute amounts of nucleic acids in a wide range of samples from numerous sources (Bustin et al., 2009). Primers may be selected to detect targeted genes with corresponding melting temperature applied during the thermal cycles determined based on genes for running qPCR. Other methods may also be applicable, but qPCR has been the major application in previous studies (Li, Li, & Zhang, 2015). Most of the selected papers in this review used qPCR to amplify targeted DNA molecules. Significant statistical analysis of the experimental results is required, and techniques have been demonstrated (Liu et al., 2014; Narciso-da-Rocha, Varela, Schwartz, Nunes, & Manaia, 2014; Pruden et al., 2006). Measurement units are generally in gene copies/16S. Correlations between several factors are assessed using redundant analysis.

Antibiotic resistance genes in water resource recovery facilities

Effluents from water resource recovery facilities are an important, direct receptor of ARGs and ARB. Wastewater influents come from various genetic reactors, such as the human and animal microbiota, hospitals, long-term healthcare facilities, farms, or other places where susceptible individuals are in close proximity and exposed to bacteria exchange. Farms and hospital/household wastewater are two major sources of influents. Municipal treatment plants are one of the treatment systems where ARGs are most frequently detected, where wastewaters mostly originate from household wastes and toilets. Guo et al. (2014) showed that incomplete metabolism in humans and improper disposal of antibiotics have been a main source of antibiotics released into the environment through municipal water resource recovery facilities. There are some other types of water resource recovery facility that have been optimized for different treatment purposes, such as industrial water resource recovery facilities and agricultural water resource recovery facilities. However, there are currently no water resource recovery facilities specifically designed to remove ARGs and ARB, due to many factors such as unknown mechanisms for removing ARGs and ARB and potentially high costs of facility improvements. Fortunately, many previous studies have reported that some current treatment plant designs may remove ARGs and ARB, at least partially, and different processes have their own characteristics for different types of ARGs. For example, studies have found that solids retention time may be an important factor to the treatment removal efficiency of ARGs in activated sludge processes (De Sotto et al., 2016; Neyestani, Dickenson, McLain, Obergh, et al., 2017; Neyestani, Dickenson, McLain, Robleto, et al., 2017). Characteristics of different treatment processes are discussed below in detail.

The presence of ARGs in treatment plants is often documented by comparing the detections in both influents and effluents. Naquin, Shrestha, Sherpa, Nathaniel, and Boopathy (2015) detected ARGs in a treatment plant in Thibodaux, Louisiana. ARGs were detected in the raw wastewater influent and the treated wastewater after being treated with UV disinfection from February 2014 until November 2014 (Naquin et al., 2015). ARGs, including *ermB, sulI, tetA, tetX,* and *mecA,* were all detected in both the raw and treated wastewater, and were coded for resistance to erythromycin, sulfonamide, tetracycline, and methicillin, respectively. Their results showed that the levels of these ARGs from raw wastewater were higher than those from effluents of the water resource recovery facilities, and they hypothesized that the UV disinfection might play an important role in reducing the ARGs.

Munir et al. (2011) also studied five water resource recovery facilities and found that the concentrations of two tetracycline resistance genes (*tetW* and *tetO*) and one sulfonamide resistance gene (*sulI*) declined significantly in the treated effluent compared to the raw influents. They also concluded that water resource recovery facilities are a potential source of ARGs as well as a potential way to remove ARGs and ARB (Munir et al., 2011).

The other main potential source of ARGs flowing into water resource recovery facilities, especially in urban areas, is hospital wastewater where antibiotics are frequently prescribed and toilets containing excreta with antibiotics are flushed into wastewater systems. Thus, detection of ARGs in municipal water resource recovery facilities is frequent. Narciso-da-Rocha et al. (2014) detected three ARGs in a hospital-urban water resource recovery facility system: *blaTEM* that is associated with Enterobacteriaceae; *vanA* that is frequently used in clinical isolates, and *marA* that is multiple antibiotic-resistant. They observed that the relative abundance of *blaTEM* (ratio of *blaTEM* to 16S rRNA) decreased, while the ratio of *vanA* to 16S rRNA did not vary and the ratio of *marA* to 16S rRNA increased (Narciso-da-Rocha et al., 2014). Although the overall results are not conclusive, the impacts of water resource recovery facilities in municipal areas are a concern for ARGs release to the environment.

Although many studies have found that water resource recovery facilities may remove a fraction of ARGs and ARB, the study of individual processes is more useful and practical to determine mechanisms of removal and potentially to optimize removal. Processes found and reported are described in the following sections.

Antibiotic resistance genes in physicochemical treatment processes

ARGs in coagulation, sedimentation, and clarification

Physicochemical treatment processes at water resource recovery facilities are nonbiological processes and include coagulation, sedimentation, disinfection, and clarification. According to Guo et al. (2014) in the Yangtze River Delta, China, these processes only slightly reduced the relative abundance of ARGs, indicating poor ARG removal, although another study (Li, Sheng, Lu, Zeng, & Yu, 2017) in a full-scale water resource

recovery facility found that coagulation with FeCl3 or polyferric chloride removed certain amount of ARGs. Generally, these nonbiological treatment processes appear to be less able to remove antibiotic resistance genes and resistant bacteria than other processes.

ARGs in filtration process

Filtration is a widely used process in water resource recovery facilities and is capable of removing a variety of contaminants. Nevertheless, research is needed to understand its removal efficiency for ARGs. One study (Tawfik El-Zanfaly, Reasoner, & Geldreich, 1998) found that both sand filters and granular activated carbon columns reduced certain amounts of ARGs in a pilot water resource recovery facility. Other workers (Breazeal, Novak, Vikesland, & Pruden, 2013) showed that membranes of 100 kDa or smaller achieved significant removal of ARGs. They found that membrane filtration of deionized water spiked with ARGs removed them, and interestingly, the addition of wastewater containing protein, polysaccharide, and total organic carbon improved removal efficiency. They also found that 0.1-um alumina membranes removed ARGs from spiked deionized water more effectively than the more common 0.1-um polyvinylidene fluoride membranes. The addition of wastewater materials enhanced removal efficiency of polyvinylidene membranes more than alumina membranes. Interestingly, however, this improvement was only observed in the presence of wastewater materials. Filtration can be regarded as a potential ARGs and ARB removal processes.

ARGs in chlorination process

Chlorination, one of the most widely used processes in water resource recovery facilities, disinfects the effluent from upstream processes, by oxidation of nucleic acids and cell membranes of microorganisms (Zhang, Zhuang, et al., 2015). Owing to its ability to destroy microorganisms, it can remove ARGs as well.

Guo et al. (2014) in seven municipal water resource recovery facilities in China found that chlorine was effective in attenuating total absolute quantities for most resistance genes, especially for *sulI*, *sulII*, *tetG*, and *tetA*, with the removal percentage of more than 98%. Similarly, Zhang, Han, et al. (2015) and Zhang, Zhuang, et al. (2015) had the same results for the reduction of ARGs after chlorination with free chlorine under laboratory conditions. Furthermore, they investigated the significant ARG removal at a concentration-contact time (CT) value of 450 mg (Cl_2) min/L, (30 mg/L free chlorine added from NaClO with 15 min contact time), especially for tetracycline and sulfonamide genes. They found that chlorination selectively removed certain ARGs but did not remove others including *sulI*, *sulII*, *tetG*, and *tetA*. Low-level genes (*tet*O, *tetM*, and *tetW*) were difficult to eliminate, and interestingly, these three genes, which were present in the influent wastewater, encoded ribosomal protection proteins, became much more prevalent after chlorination compared to their nonchlorinated abundance. They hypothesized that chlorination was effective for the majority of the bacteria community, but might function as a selector for some chlorine-tolerant bacteria. Zhang, Han, et al. (2015) and Zhang, Zhuang, et al. (2015) hypothesized chlorination may affect bacterial populations differently based upon water properties and original ARG concentrations. Furthermore, they investigated the effect of NH3-N on chlorination disinfection in removing ARGs and found that higher NH3-N concentration (15 mg/L) resulted in lower ARGs removal, which may be attributed to the form of the chlorine, which is more likely to be combined chlorine at higher NH3-N. They also stated that in order to obtain ARG reduction in the presence of ammonia the application of Cl2: NH3-N mass ratio should be greater than 7.6:1, or beyond the break point. Chlorination may be an effective process for the removal of ARGs.

Similarly, Guo et al. (2014) also found that chlorine dose significantly impacts the removal efficiency of ARB. They found that low chlorine CT (up to 40 free chlorine mg-min/L) highly promoted the frequency of conjugative transfer (one of the three major mechanisms for horizontal gene transfer) by twofold to fivefold for *E. coli*. The chlorine dose induced more pilus on the surface of conjugative cells, which acted as pathways for ARGs transfer. Also, the generated chloramine stimulated the bacteria and improved the cell permeability (Guo et al. 2014). High-chlorine contact time (>80 free chlorine mg-min/L) greatly suppressed the frequency of ARG transfers. Chlorination enhanced the transfer of ARGs at low CT and decreased the transfer at high CT.

ARGs in ozonation process

Ozonation is a treatment process that can improve the removal of organic compounds as well as disinfecting bacteria and viruses. Molecular ozone is a strong oxidant and can form hydroxyl radials, another strong oxidant, as the ozone decomposes. Different researchers have observed widely varying results, often with contradictory conclusions. Ozonation effectively removed *ermB*, but it increased the amount of *blaVIM* and *vanA* at the same time (Alexander, Knopp, Dotsch, Wieland, & Schwartz, 2016). Another research group found that treatment with ozone decreased the total number of bacteria but increased the percentage of antibiotic-resistant bacteria, especially such as bacteria *E. coli* isolates and *staphylococci* isolates (Luddeke et al., 2015). However, they found that ozonation followed by filtration led to an additional reduction of total ARB of these species by 0.8 and 1.1 log-units, as compared with the currently established removals in tertiary wastewater treatment (flocculation and filtration). Some researchers (Czekalski et al., 2016) also found that ozonation reduced selective ARGs with different doses suggesting that some ARGs may be more resistant to ozonation than others. Therefore, dosage may be one of the important considerations for future application of ozonation for target genes. Additionally, a research group (Sousa et al., 2017) compared the removal efficiency of ozonation under different contact times (15, 30, and 60 min) and found that the largest reduction occurred in the first 30 min and no quantifiable reduction of ARGs was observed after 60 min. Thus, they hypothesized that a contact time of 30 min might be adequate but further studies should confirm this hypothesis.

The reason that ozonation inefficiently removes or even increases antibiotic resistance genes may be due to the environment of ozonation process. One research group (Alexander et al., 2016) found that a sublethal concentration of bacterial antibiotics in batch experiments in the presence of ozone stimulated the formation of intracellular, highly reactive hydroxyl radicals that contributed to the killing efficiency of bacterial antibiotics. Nevertheless, they also found that the induction of oxidative stress by bactericidal antibiotics may induce sublethal stress response mechanisms in bacteria that not only deal with the adaptation to the original drug target (antibiotic resistance development), but also activate antioxidative mechanisms and oxidative damage-associated responses, resulting a considerable advantage in surviving oxidative wastewater treatment. Experimental verification in full-scale treatment plants is needed to further validate their results.

ARGs in UV disinfection process

UV radiation is a disinfection process growing in popularity because it reduces or eliminates the need to transport and store chlorine gas. It has been reported to remove ARGs or inactivate ARB in wastewaters. UV light can penetrate UV-transparent structures in cells and be sorbed by the nucleobases comprising DNA and RNA, removing ARGs. It has the additional advantage of producing few disinfection by-products (DBPs; Zhang, Zhuang, et al., 2015). Exposure to UV produced no or very small change in an *E. coli* strain's resistance to 384 amoxicillin (AMX; minimum inhibiting concentration (MIC) > 256 mg/L) and sulfamethoxazole (SMZ; MIC > 1,024 mg/L; Rizzo et al., 2013). This result is consistent with other ARB as well. Another research group (Lee et al., 2017) found that several antibiotic-resistant bacteria were reduced by 34%–74% in two water resource recovery facilities after UV disinfection (27 mJ/cm^2), but found the amount of ARGs was not significantly reduced. McKinney and Pruden (2012) found that a UV dose of 200–400 mg/cm^2 was required to reduce ARGs in the magnitude of 3–4 orders in a filtered-wastewater matrix. In order to improve removal of ARGs at smaller dosage, it is possible to add certain chemicals in the wastewater to promote removal efficiency. For instance, H2O2 improved UV disinfection efficiency. The researchers found that UV/H2O2 at the dose of 50–130 mJ/cm^2 could achieve 4-log reduction of ARG concentration at pH 7 following first-order kinetics (Yoon et al., 2017). Because of ˙OH (from H2O2), the ARGs' structural integrity of extracellular plasmid changed more rapidly in UV/H2O2 than without H2O2, which enhanced ARG destruction during UV/H2O2. Zhang, Han, et al. (2015) and Zhang, Zhuang, et al. (2015) also studied the sequential combination of chlorination and UV disinfection, resulting in the higher removal efficiency of ARGs than chlorination or UV disinfection alone. The improvement might be due to the decreased bioactivity affected by UV irradiation, causing the chlorine to react with the cells more readily. Furthermore, the sequential UV/chlorination disinfection process has the advantages of reducing the demand dosages of chlorine and the possibility of reducing DBPs formation at the same level of removal efficiency (Zhang, Zhuang, et al., 2015). Therefore, combined UV with other oxidants may be better than UV alone for the future applications.

ARGs in biological treatment

Biological treatment processes use organisms to break down organic substances in wastewater and are often used as secondary treatment processes. They are the major processes of either enriching or removing antibiotic-resistant bacteria. Aerobic treatment processes and anaerobic treatment processes are two major categories for biological treatment. For example, researchers studied the potential proliferation of ARGs in a sequencing batch reactor operated in a successive arrangement of aerobic and anoxic cycles and membrane bioreactor (MBR) in the full-scale treatment plants in China (Wang, Mao, Mu, & Luo, 2015). Other biological treatment processes, such as anaerobic digesters, have also been found to influence the amount of some kinds of antibiotic resistance genes under the positive influence of the influent sludge microbial composition (Miller, Novak, Knocke, & Pruden, 2016). Activated sludge process, anaerobic digestion, and constructed wetland are discussed in detail.

ARGs in activated sludge process

The activated sludge process (ASP) is the most widely used process in water resource recovery facilities, especially for large treatment plants. The activated sludge process reduces organic matter in wastewater by using a complex biological community in the presence of oxygen to convert the organic matter to new cell mass, carbon dioxide, and energy. It also produces solids capable of bioflocculating and settling out in a clarifier to produce an effluent low in biological oxygen demand (BOD) and total suspended solids (TSS). Removal of microcontaminants and their fates is an increasing concern, and researches are investigating the removal of ARGs and ARB in activated sludge process (Korzeniewaska & Harnisz, 2018). Previous work has shown that certain ASP processes, those with longer solids retention time (SRT), are much better in removing trace contaminants than short SRT processes (Leu, Chan, & Stenstrom, 2012; Soliman et al., 2007). They identified more than 100 contaminants in literature and their experiments that are better or completely removed at longer SRT. Contradictory results from different researchers, as well as the impacts of other factors such as food-to-mass ratio (F/M), are discussed in the following sections and show the need for more research to better understand the removals of ARGs and ARB.

In order to observe the ability of activated sludge processes to remove ARGs and ARB, some researchers simulated mechanisms in laboratory-scale reactors. Huang, Tang, Zhang, Xu, and Ren (2014) sampled activated sludge from a water resource recovery facility in Nanjing, China, and detected the abundance of tetracycline and sulfonamide resistance genes. They found that 11 *tet* genes were present in the sludge, including *tetA*, *tetB*, *tetC*, *tetG*, *tetK*, and *tetP(A)* encoding tetracycline efflux proteins; *tetM*, *tetO*, *tetS*, and *tetW* encoding ribosomal protection proteins; and *tetX* encoding enzymatic modification protein. The amount of all of the *tet* genes increased, while *sulII* was decreased (Huang et al., 2014). Similarly, in a bench-scale activated sludge process with 500 μg/L tetracycline influent, an increase in the proportion of *tetA* and *tetB* as well as both tetracycline-intermediate-resistant and tetracycline-resistant heterotrophic bacteria occurred (Yu, Liu, & Li, 2015). Yu

et al. (2015) hypothesized that the tetracycline-resistant genes created by efflux pump spread earlier and more quickly to encode resistance to tetracycline in activated sludge, because efflux pump mechanisms generally show an easier response to resistance of tetracycline.

Interestingly, when tetracycline resistance genes exist in activated sludge together with other ARGs such as *sulII*, the occurrence and diversity of nontetracycline ARGs decreased (Huang et al., 2014). They found that the considerable decrease in the abundance of *sulII* (from 83.49% to 14.76%) might be attributed to the domination of tetracycline in the sludge, owing to the higher abundance in the sludge without tetracycline stress than with tetracycline stress. They hypothesized that the tetracycline treatment reduced the abundance of *sulII. SulII* is located on the genome of *Salmonella enterica* plasmid pCVM19633_110 and the genome *Pasteurella multocida* plasmid pCCK381; tetracycline reduces the predominance of these two genomes in the biomass. In full-scale water resource recovery facilities, the observed removal ability of ARGs in the activated sludge process also varies. For example, full-scale wastewater treatment plants with activated sludge processes showed partial removal of 13 tetracycline-, sulfonamide-, streptomycin-, and β-lactam resistance genes in three water resource recovery facilities in Jiangsu, China (Zhang, Han, et al., 2015). They observed that the presence of 13 ARGs (*sulI, sulII, sulIII, sulA, tetA, tetB, tetE, tetA, strA, strB, TEM, SHV,* and *CTX-M*) was detected at a higher frequency in ARBs from the influent than the effluent samples, except for *sulA* and *CTX-M*. In another wastewater treatment plant using an anaerobic–anoxic–oxic configuration activated sludge process (sometimes called A^2O), eight *tet* genes (*tetA*, *tetB*, *tetC*, *tetE*, *tetM*, *tetO*, *tetS*, and *tetX*) were removed after the treatment process, especially for *tetC*, *tetM*, *tetO*, and *tetX* with removal efficiencies of 94.9%, 88.5%, 77.7%, and 74.9%, respectively (Huang, Zhang, Liu, & Hu, 2015).

The activated sludge plants in Jiangsu, China, consists of anoxic, anaerobic, and aerobic compartments (Zhang, Han, et al., 2015). They found that the abundance of the ARGs increased in aerobic sludge as compared to anaerobic sludge. They also observed that the level of *tetA* was higher in the aerobic tank than the anoxic tank, while *tetB* showed a decreased abundance. *tet* gene concentrations were similar among anaerobic, anoxic, and aerobic tanks for the improved anaerobic–aerobic–oxic treatment plant (Huang et al., 2015). They also observed that there was no significant difference between aqueous and biosolids samples from these tanks. The results from different activated sludge processes removing ARGs vary more widely and not just among the different compartments of process modifications. Those results are summarized in Table 4. For example, different removal efficiencies were observed for *tet* and *sul* genes in the same treatment plants in different seasons (Zhang, Han, et al., 2015), indicating possible effects of temperature on ARGs removal. Furthermore, other parameters play an important role in ASP removal efficiency for ARGs and ARBs, such as pH, F/M, hydraulic retention time (HRT), and solids retention time (SRT).

The F/M is considered to be one of the most important factors that influence organic degradation and bacterial growth in the activated sludge process, and is inversely related to the SRT. Yuan, Guo, and Yang (2015) evaluated the effect of the F/M on the fate of six antibiotic-resistant bacteria (tetracycline-, sulfadiazine-, cephalexin-, vancomycin-, erythromycin-, and gentamicin-resistant heterotrophic bacteria) in a bench-scale activated sludge treatment process. They observed that the growth rates of most ARB were increased with increasing F/M from 0.24 to 0.42 kg TCOD/(kg MLSS day) but did not increase beyond 0.42. A similar result was also observed in the batch-scale sequencing batch reactors (SBRs) for tetracycline-resistant bacteria (Kim, Jensen, Aga, & Weber, 2007).

Two possible reasons might account for this ARB amplification with an increased F/M or decreasing SRT (Yuan et al., 2015). First, Yuan et al. (2015) hypothesized that most antibiotic-resistant bacteria in activated sludge at high F/M were surrounded by an environment with higher antibiotic concentrations, which required them to become resistant for survival. Also, nutrients are essential for horizontal gene transfer (HGT), resulting in more frequent transfer of ARGs among bacteria in the presence of higher nutrient concentrations that exist at higher F/M (Yuan et al., 2015). Nevertheless, other possibilities may explain this amplification and the influence of F/M on ARGs and ARB is needed further study. In brief, the F/M is an important factor for controlling and removing the amount of ARGs and ARB in the activated sludge process.

Another important factor used to operate and control activated sludge processes is solids retention time (SRT) which is inversely related to the F/M and is usually expressed in days. The SRT has been observed to be significant to the removal of most of the organic chemicals (Leu et al., 2012). Leu et al. (2012) found that removals of trace organics such as ibuprofen (almost 100%) and several other antioxidants (the variety between 43% and 99%) increased at higher SRTs. In laboratory-scale research, the proliferation of antibiotic resistance genes and antibiotic-resistant bacteria had decreased and sometimes increased at higher SRT (Neyestani, Dickenson, McLain, Obergh, et al., 2017). Neyestani et al. found that the prevalence of ARB in the activated sludge process increased at higher SRTs (2,7, and 20 days) and was more pronounced at higher temperatures. De Sotto et al. (2016) found similar results in laboratory-scale SBRs for the treatment of carcass leachate on the fate of tetracycline resistance. Neyestani, Dickenson, McLain, Robleto, et al. (2017) suggested that longer SRTs might select for resistant bacteria and/or result in false positives for antibiotic resistance. They also hypothesized that the increase in ARB might also be due to high antibiotic concentrations that facilitate selective pressure. However, there are few researchers focusing on the influences of SRTs for the removal of ARGs and ARB in full-scale water resource recovery facilities. Therefore, the detection and fate of ARGs in full-scale water resource recovery facilities and the influence of operating parameters such as SRT are import objectives of future research.

ARGs in anaerobic digestion

Anaerobic digestion (AD) is a series of biological processes in which microorganisms break down biodegradable material in the absence of oxygen. It degrades and stabilizes organic materials under anaerobic conditions by microbial organisms and forms biogas (a mixture of carbon dioxide and methane, a renewable energy source) and microbial biomass (Kelleher et al., 2002). Anaerobic digestion has been

Table 4. Summary of ARGs treatment in different treatment processes

PROCESS	SCALE	TOTAL ARG DETECTED		LOCATION	COMMENTS	REFERENCES
		DECREASED/TOTAL	INCREASED/TOTAL			
AAA	F	10/12	1/12	China	No significant change for CTX-	Zhang, Han, et al. (2015) and Zhang, Zhuang, et al. (2015)
AAO	F	8/8	0/8	China	–	Huang et al. (2015)
CASP	F	3/3	0/3	China	–	Gao et al. (2012)
ASP	F	1/1	0/1	USA	–	Munir et al. (2011)
ASP	F	2/2	0/2	Sweden	Non-nitrifying ASP	Börjesson, Matussek, Melin, Löfgren, and Lindgren, (2010)
ASP	F	1/2	1/2	Saudi Arabia	On-site Hospital water resource recovery facility	Timraz et al. (2017)
SBR	F	8/8	0/8	China	A sequencing batch reactor operated in a successive arrangement of aerobic and anaerobic stages	Wang et al. (2015)
PFR	F	8/8	0/8	China	–	Wang et al. (2015)
CASP	F	8/8	0/8	China	–	Wang et al. (2015)
AAO	F	6/8	2/8	China	–	Wang et al. (2015)
SBR	L	0/2	2/2	China	Factory wastewater	Yu et al. (2015)
ASP	L	0/3	3/3	China	Activated sludge was sampled from full-scale water resource recovery facility	Huang et al. (2014)
AD	F	11/11	0/11	China	T and long SRT improved efficiency	Sui et al. (2016)
AD	L	8/14	5/14	China	*sul2* showed no significant change	Wu et al. (2016)
AD	L	4/4	0/4	UK	Mesophilic AD was more susceptible to ARG intrusion than thermophilic AD.	Miller et al. (2016)
AD	L	3/3	0/3	Brazil	AD may be influenced by the ambient temperature.	Resende et al. (2014)
AD	L	4/4	0/4	USA	T higher than 55°C did not offer better removal.	Burch et al. (2010)

Notes. AAA: anoxic, anaerobic, and aerobics; AAO: anaerobic, anoxic, and oxic; AD: anaerobic digestion; ARGs: Antibiotic resistance genes; ASP: activated sludge process; CASP: conventional activated sludge process; F: full scale; L: laboratory scale; PFR: Plug-flow reactor; SBR: sequencing batch reactor.

widely used for the treatment of municipal sludge and limited application in the treatment of other organic industrial wastes (Parkin & Miller, 1983). Anaerobic digestion processes are critical for reducing residual sludge from other processes, reducing viral and bacterial pathogens, as well as dewatering post-treatment sludge. Digested biosolids can be further treated by composting or utilized for dairy bedding, applied to cropland, or converted into other products. Because of the potential widespread to use of anaerobically digested sludge in agricultural applications, it is possible that antibiotic resistance genes and antibiotic-resistant bacteria may be released to the environment (Munir et al., 2011). Therefore, the performance of anaerobic digestion processes in reducing ARGs and ARB is an important concern.

The performance of AD for removal of antibiotic resistance genes and antibiotic-resistant bacteria varies, according to the types of ARGs and ARB. Sui et al. (2016) studied the removal efficiency of full-scale mesophilic anaerobic digesters (35°C) in two water resource recovery facilities treating wastewater from swine farms, where antibiotic resistance genes and antibiotic-resistant bacteria are frequently found. ARGs of tetracycline (*tet*G, *tet*M, and *tet*X), sulfonamide (*sulI* and *sulII*), and macrolide (*erm*B, *erm*F, *ere*A, and *mef*A); class 1 integron gene (*intI1*); and bacterial 16S rRNA gene were detected in the biosolids and partially decreased after anaerobic digestion (Sui et al., 2016). Resende et al. (2014) obtained similar results in the laboratory-scale mesophilic digester showing that *erm*B, *aph*A2, and *bla*TEM-1 decreased. The authors documented their methods to quantify removal or increase of ARGs and also noted the effects of process conditions. Ambient temperature may influence the performance of anaerobic digestion processes when treating ARGs and ARB, and anaerobic digestion was found to be more effective in reducing ARGs during the summer as compared to winter (Resende et al., 2014).

Thermophilic anaerobic digestion is an alternative to mesophilic digestion and usually operates at approximately 55°C (Iranpour et al., 2002). In the United States, thermophilic digestion is increasing in popularity due to its ability to produce Class A biosolids and has additional benefits such as a higher degree of waste stabilization and more thorough destruction of pathogens and biosolids. There are previous studies focused on the ARG removal performance of thermophilic digesters. Burch, Sadowsky, and LaPara (2010) studied the performances of laboratory-scale thermophilic anaerobic digesters on treating three antibiotic resistance genes (*tet*W, *tet*X, and *qnr*A) in untreated municipal wastewater solids. They found removal efficiency of approximate 99% at 56°C for these three antibiotic resistance genes Wu et al. (2016) also found a two-stage batch-scale thermophilic digestion process (55°C) reduced the presence of *tet*A, *tet*G, *tet*X, *sulI*, *erm*B, *dfr*A1, *dfr*A12, and *intl*1 by 0.1-0.72 log unit removal (approximately 20.57 to 80.95%). In contrast, they found that genes copies of *tet*O, *tet*W, *sulIII*, *erm*F, and *bla*TEM increased in comparison with number in the feed (untreated samples before anaerobic digestion) and *sulII* showed no significant change (Wu et al., 2016).

Interestingly, Burch et al. (2010) also studied the influence of temperature operations on the thermophilic anaerobic digestion system, and they found that the removals of these three ARGs were increased with increasing temperature up to approximately 55°C. Over 55–63°C, the removal rate was not consistently better than that at 55°C (Burch et al., 2010). Unfortunately, there were no further studies explaining the reasons of these results Additionally, longer hydraulic residence times (HRTs) may also improve the reduction efficiency of ARGs in the full-scale anaerobic digestion process (Sui et al., 2016). However, the optimal HRTs for removing ARGs are still unknown. Therefore, appropriate operating temperatures and HRTs for the anaerobic digestion process to remove ARGs is a topic for future study.

ARGs in constructed wetlands

Constructed wetlands consist of an artificial wetland created for the purpose of treating anthropogenic discharges using the natural functions of vegetation, soil, and organisms to treat different water streams. Although constructed wetlands are not normally used for treating large volumes of wastewater due to land requirements, they can be an effective process for removing antibiotic resistance genes.

In Zhejiang, China, Chen and Zhang (2013) identified previous research documenting removal of ARGs in constructed wetlands and in their own work found that constructed wetlands played a major role in the removal of ARG genes, especially *sul* genes, and also provided 2 log unit removals of fecal coliforms. Similarly, in Xiamen, China, Liu et al. (2013) found similar results that constructed wetlands significantly reduced the wastewater antibiotics content with the following sequence of elimination rates—oxytetracycline HCl > ciprofloxacin HCl > sulfamethazine (Liu et al., 2013). Additionally, they detected a higher concentration of antibiotics accumulated in the soil than in the media and vegetation, indicating that soil might be the dominant sink for antibiotics removal from wastewater in vertical flow constructed wetlands. The media and vegetation exhibited lower removal efficiency of 50% and one order of magnitude, respectively. This finding is consistent with Chen and Zhang's mechanism, indicating that plant roots in constructed wetlands encourage microbial attachment, which may be regarded as a mini aerobic/anoxic biological treatment system (Chen & Zhang, 2013). Removals in constructed wetlands vary with season, and temperature is significant factor affecting the removal efficacy of sulfonamide and tetracycline resistance genes, which range from 2.98×10^{-5} to 1.27×10^{-1} for *sul* genes and 4.68×10^{-6} to 1.54×10^{-1} for *tet* genes after treatment (Liu et al., 2014). Constructed wetlands are a potentially viable process for ARG removal, with possible advantages of inexpensive construction cost and easy operation, but further study is recommended.

Summary

Antibiotic resistance genes are contaminants of increasing concern, and their fate in water resource recovery facilities is

Table 5. Treatment processes in 25 studies and their ARGs treatment performances

Treatment Process	Scale	References	Country	*tetA*	*tetB*	*tetC*	*tetE*	*tetG*	*tetM*	*tetO*	*tetQ*	*tetS*	*tetT*	*tetW*	*tetX*	*tetZ*	*sulI*	*sulII*	*sulIII*	*sulA*	bla_{TEM}	bla_{SHV}	bla_{CTX-M}	*ermB*	*ermF*	*ereA*	*marA*	*mecA*	*mefA*	*qnrS*	*vanA*	*strA*	*strB*	*amp(A)*	*dfrA1*	*dfrA12*	*aphA2*
Secondary Treatmet Processes																																				
ASP[1]	F[13]	Zhang et al. (2015)	China	↓	↓		↓										↓	↓	↓	→	↓		↓		↑							↓	↓				
ASP	F	Huang et al. (2015)	China	↓	↓	↓	↓		↓	↓		↓			↓		↓																				
ASP	F	Gao et al. (2012)	USA							↓				↓			↓																				
ASP	F	Timraz et al. (2017)	Saudi Arabia													↓	↑																				
ASP	F	Wang et al. (2015)	China						↓	↓	↓		↓	↓			↓	↓						↓													
ASP	F	Wang et al. (2015)	China						↓	↓	↓		↓	↓			↓	↓						↓													
ASP	F	Wang et al. (2015)	China						↓	↓	↓		↓	↓			↓	↓						↓													
ASP	F	Wang et al. (2015)	China						↓	↑	↑		↓	↓			↓	↓						↓													
ASP	F	Timraz et al. (2017)	Saudi Arabia													↑	↓	↑																		
ASP	F	Munir et al. (2011)	USA														↓																			
ASP	L[14]	Huang et al. (2014)	China	↑		↑		↑																												
MBR[2]	F	Munir et al. (2011)	USA							↓				↓																						
MBR	F	Wang et al. (2015)	China						↓	↑	↑		↓	↓			↓	↓						↓													
CW[3]	L	Liu et al. (2014)	China						→	→				→			↑	↑																		
CW	L	Liu et al. (2013)	China							↓																										
CW	L	Liu et al. (2013)	China						↓	↓				↓																						
Post-Secondary Treatment Prcesses																																				
SF[4]	F	Czekalski et al. (2016)	Switzerland														↓																			
GSF[5]	F	Wang et al. (2015)	China						↑	↓	↑		↓	↑			↑	↑						↓													
Coagulation	F	Li et al. (2016)	China							↓	↓			↓			↓	↓																		
Disinfection Treatment Processes																																				
UV	L	McKinney and Pruden (2012)	USA	↓																								↓		↓			↓			
UV	L	Huang et al. (2016)	China																																	
UV	L	Zhang et al. (2015)	China					↓							↓		↓																			
UV	L	Sousa et al. (2017)	Portugal														↓				↓								↓	↓						
UV/H_2O_2	L	Ferro et al. (2016)	Italy											↓							→								→							
AD[6]	L	Resende et al. (2014)	Brazil																		↓			↓												↓
AD	L	Wu et al. (2016)	China	↓				↓		↑				↑	↓		↓		↑		↑			↓	↑									↓	↓	
AD	L	Miller et al. (2016)	USA					↑																												
AD	F	Sui et al. (2016)	China					↓	↓						↓		↓	↓						↓	↓	↓			↓							
AD	F	Munir et al. (2011)	USA							↓				↓																						
O_3	L	Czekalski et al. (2016)	Switzerland														↓																			
O_3	L	Sousa et al. (2017)	Portugal														↓				↓										↓					
O_3	L	Sousa et al. (2017)	Portugal														↓				↓									↓	↓					
O_3/GAC[7]	L	Alexander et al. (2016)	Germany																			↑		↓							↑					
O_3	F	Czekalski et al. (2016)	Switzerland														↓																			
O_3	F	Guo et al. (2014)	China		↑			↓	↑	↑				↑			↓	↓																		
Cl_2[8]	L	Zhang et al. (2015)	China					↓							↓		↓																			
Cl_2	F	Gao et al. (2012)	USA							↓				↓																						
Cl_2	F	Guo et al. (2014)	China	↓↑→				↓↑→	↑	↑				↑			↓↑→	↓↑→																		
Others																																				
WRRF[9]	F	Naquin et al. (2015)	USA	→										→	→		→							→				→							
Pchem.[10]	F	Guo et al. (2014)	China	→	→	→		→	→	→				→	→		→	→																		
DWTP[11]	F	Guo et al. (2014)	China	↓	↓	↓		↓	↓	↓				↓	↓		↓	↓																		
N/S[12]	F	Chen et al. (2013)	China						↓	↓	↓			↓			↓	↓																		
N/S	F	Narciso-da-Rocha et al. (2014)	Portugal																		↓						↓				↓					

Notes. Arrows indicate the fate of ARGs in the treatment processed ("↓": decreased; "↑": increased; "≡": unchanged).

AD: anaerobic digestion; ASP: activated sludge process; Cl2: chlorination; CW: constructed wetland; DWTP: drinking water treatment plant (similar processes were used with wastewater treatment); F: full scale; GAC: granular activated carbon; GSF: granular sand filter; L: lab scale; MBR: membrane bioreactor; N/S: not specified (Either wastewater treatement plant or treatment processes were not introduced in the reference); Pchem.: physicochemical treatment process; SF: sand filter; WRRF: water resource recovery facility (no specific treatment processed studied).

not fully understood. Many studies, both laboratory scale and full scale, have found that treatment processes may in many but not all cases decrease the amount of some ARGs. A few other studies show an increase in ARGs and ARB. Table 5 summarizes the previous research found in this review in both laboratory- and full-scale studies. Thirty-five ARGs were detected and quantified in 25 independent studies with 215 observed results. Among the 215 results, 152 showed a decrease, while 26 showed no change, and 37 showed an increase. All of the results were reported in relative counts by per 16S. Table 6 summarizes nine different treatment processes that were categorized into three general groups, including secondary treatment processes (activated sludge, membrane bioreactor, and constructed wetlands), postsecondary treatment processes (filtration and coagulation), and disinfection treatment processes (UV disinfection, anaerobic digester, ozonation, and chlorination). Note that genes colocated on a plasmid and transferred together were both counted in this analysis. Thus, transfer of plasmids carrying multiple genes resulted in a higher score in this analysis. The activated sludge process was the most often studied (64/215 observations) and showed the most removal observations, with 54 observations of decreases in 35 ARGs. Anaerobic digestion showed 21 removal results, followed by 13 observations for ozonation. Only one coagulation study was found, and it showed a decrease in ARGs. The results are confusing because some researchers documented increases in ARGs in the same processes. Nine of the 63 activated sludge observations showed increases in ARGs. There were other observations of increasing ARGs in treatment processes. The decreases, increases, and unchanged observations are shown in Figure 1.

For all processes, there were more decreases than increases in ARGs levels relative to the 16S gene levels, which indicates that wastewater treatment processes tend to reduce ARG release to the environment. The results are still confusing, and more research is needed. For example, the activated sludge process decreased ARGs in 54 of 64 observations, but increased in 9 of 64 observations. However, the factors that cause the proliferation of ARGs are still unknown, because of the design factors of treatment processes as discussed previously, horizontal gene transfer, or other natural influence. One potentially important factor is SRT. SRT is correlated with the growth rate of microorganisms; therefore, higher SRT allows enriching the process with slowly growing bacteria and establishing a more diverse microbial community with broader physiological capabilities for better removal or biodegradation

Table 6. Summary of ARG fates in wastewater treatment processes

ARGs	*tetA*	*tetB*	*tetC*	*tetE*	*tetG*	*tetM*	*tetO*	*tetQ*	*tetS*	*tetT*	*tetW*	*tetX*	*tetZ*	*sul1*	*sulII*	*sulIII*	*sulA*	*bla*$_{TE}$	*bla*$_{VIM}$	*bla*$_{SHV}$	*bla*$_{CTX-M}$	*ermB*	*ermF*	*ereA*	*marA*	*mecA*	*mefA*	*qnrS*	*vanA*	*strA*	*strB*	*amp(A)*	*dfrA1*	*dfrA12*	*aphA2*	Total
Secondary Treatment Processes																																				
ASP[1]	2	2	1	2	0	5	5	3	1	4	5	1	1	8	5	1	0	1	0	1	0	4	0	0	0	0	0	0	0	1	1	0	0	0	0	54
	1	0	1	0	1	0	1	1	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
MBR[2]	0	0	0	0	0	1	1	0	0	1	2	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	8
	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
CW[3]	0	0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Total	2	2	1	2	0	7	8	3	1	5	8	1	1	9	6	1	0	1	0	1	0	5	0	0	0	0	0	0	0	1	1	0	0	0	0	66
	1	0	1	0	1	0	2	2	0	0	0	0	1	2	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
	3	2	2	2	1	7	10	5	1	5	8	1	2	11	8	1	0	1	0	1	1	5	0	0	0	0	0	0	0	1	1	0	0	0	0	79
Post Secondary Treatment Processes																																				
Filtration	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	0	0	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Coagualtion	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	2	1	0	1	1	0	0	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	9
	0	0	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
	0	0	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	14
Disinfection Treatment Processes																																				
UV	1	0	0	0	1	0	0	0	0	0	1	1	0	2	0	0	0	1	0	0	0	0	0	0	0	1	0	1	2	0	0	1	0	0	0	12
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AD[4]	1	0	0	0	2	1	1	0	0	0	1	2	0	2	1	0	0	1	0	0	0	3	1	1	0	0	1	0	0	0	0	1	1	1	21
	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	6
O_3	0	0	0	0	1	0	0	0	0	0	0	0	0	5	1	0	0	2	0	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0	13
	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	6
Chlorination	1	0	0	0	2	1	2	0	0	0	1	1	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
	1	0	0	0	1	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Total	3	0	0	0	6	2	3	0	0	0	3	4	0	12	3	0	0	4	0	0	0	4	1	1	0	1	1	2	4	0	0	1	1	1	1	58
	1	1	0	0	2	2	3	0	0	0	3	0	0	1	1	1	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	19
	4	1	0	0	8	4	6	0	0	0	6	4	0	13	4	1	0	5	1	0	0	4	2	1	0	1	1	2	5	0	0	1	1	1	1	77

Notes. Green zone indicate numbers of decreased observations; red zone indicates numbers of increased observations.
AD: anaerobic digestion; ASP: activated sludge process; CW: constructed wetland; MBR: membrane bioreactor.

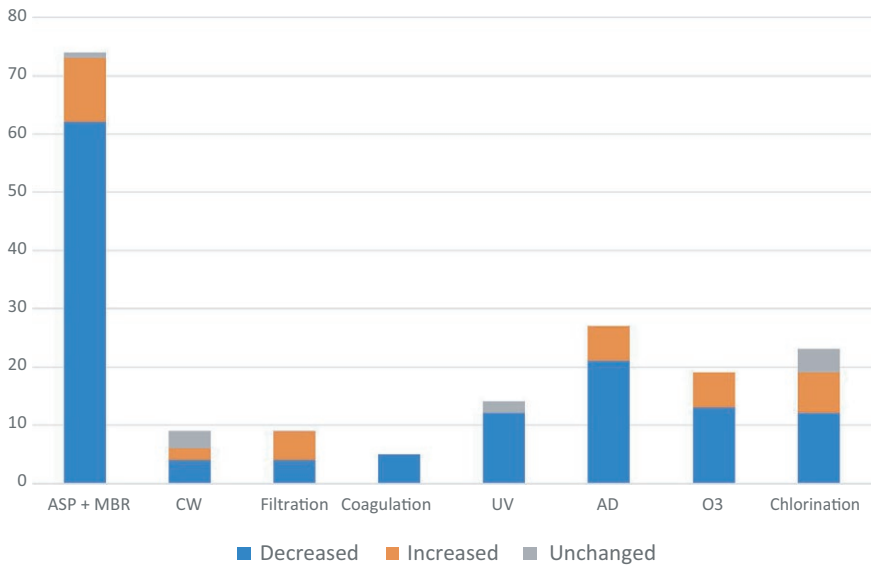

Figure 1. Comparison of ARGs decreased, increased, and unchanged observations in treatment processes, including activated sludge process (ASP) and membrane bioreactor (MBR), constructed wetland (CW), filtration, UV disinfection, anaerobic digester (AD), ozonation (O_3), and chlorination.

in activated sludge processes (Kreuzinger, Clara, Strenn, & Kroiss, 2004).

As discussed above, longer SRT showed greater removal efficiency for more than 100 contaminants, such as ibuprofen, oxybenzone, and benzophenone (Leu et al., 2012). We hypothesize that longer SRT may have a similar influence on the abundance of ARGs and ARB. Unfortunately, few studies have reported and verified this hypothesis. All of these hypotheses need further confirmation and study, and they will be further studied in our future research. Different treatment processes may also have specific influences on the amount and types of antibiotic resistance genes. Figure 2 shows the comparison of tetracycline (*tet*), sulfonamide (*sul*), β-lactam resistance genes, and the rest of the 12 ARGs that are decreased or increased in different treatment processes. It shows that proliferation of ARGs specifically in the activated sludge processes was studied and reported most often. The previous researchers found both increases and decreases for all three types of ARGs, with tetracycline resistance genes exhibiting the most increased results, 23 of 37 observations. Microbiological mechanisms in

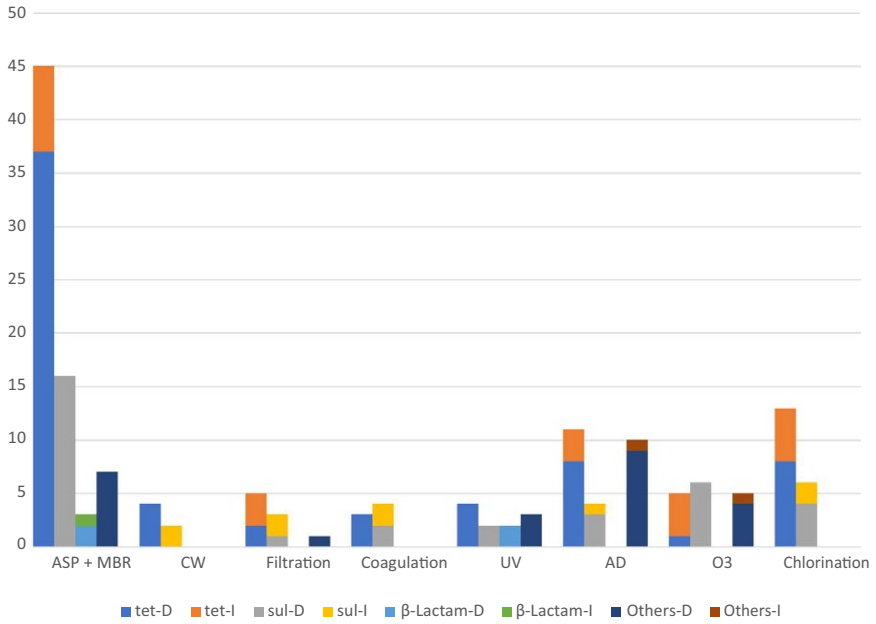

Figure 2. Comparison of tetracycline (*tet*), sulfonamide (*sul*), β-lactam, and other antibiotic resistance genes that are decreased and increased in treatment processes, including activated sludge process (ASP) and membrane bioreactor (MBR), constructed wetland (CW), filtration, UV disinfection, anaerobic digester (AD), ozonation (O3), and chlorination. (Note: D indicates decrease, and I indicates increase. For example, tet-D indicates numbers of decreased observations for tetracycline resistance genes and tet-I indicates numbers of increased observations for tetracycline resistance genes.).

the biological treatment processes are complex, and any result showing an increase in ARG level indicates the proliferation of an antibiotic resistance gene. Other ARGs may also be similarly affected. Therefore, the optimal removal for any water resource recovery facilities should be no proliferation of ARGs.

UV disinfection was the only process studied that decreased ARGs for all observations (Figure 2). UV disinfection typically efficiently eliminates enteric bacteria, viruses, bacterial spores, and parasite cysts with the advantages of reduced disinfection by-product formation and not requiring transportation or storage of a hazardous chemical, for example, chlorine gas (Koivunen & Heinonen-Tanski, 2005). Organisms grown in the presence of antibiotics can develop resistance mechanisms, but they have no exposure to UV light during their growth processes and therefore would not have a selective pressure to develop resistance to UV. Therefore, UV disinfection may have an additional advantage over other disinfection methods and may be valuable for reducing ARGs.

Anaerobic digestion showed increased results of different ARGs in only 5 of 24 observations. Anaerobic digestion is an important process for reducing exposure to ARGs because waste activated sludge or trickling filter sludge would otherwise be an important vector for spreading ARGs. Previous studies detected high concentrations of ARGs in biosolids (Munir et al., 2011). The efficiency of anaerobic digestion in reducing ARGs is important because it may reduce the ARGs not eliminated or reduced in the activated sludge process or other biological treatment processes. In addition, anaerobic digesters can be fed with purpose-grown energy crops as a source of renewable energy, which may increase the possibility of spreading ARGs. Therefore, anaerobic digestion is important and merits more study.

Conclusions

Antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) are of great concern because of the growing loss of effectiveness of important antibiotics. The World Health Organization has published a list of 12 pathogens that need urgent new antibiotics due to the loss of current antibiotic effectiveness. Water resource recovery facilities have recently been implicated as vectors for increasing the occurrence of ARGs and ARB. To understand the potential contribution of treatment plants to the spread of ARGs and ARB, a survey was made of current literature with the specific objective of understanding their fates in treatment processes. References were carefully screened to understand the processes involved, which was difficult because many of the investigators reported only changes throughout an entire treatment plant.

Thirty-five ARGs were detected and quantified in 25 independent studies with 215 observed results. Among the 215 results, 152 showed a decrease, while 26 showed no change, and 37 showed an increase. All of the results were reported in relative counts by per 16S. The group of thirteen tetracycline resistance genes was studied the most, with 118 observations. Sulfonamide resistance genes were also frequently studied with 53 total observations of four kinds of *sul* genes. Other genes such as β-lactam and multidrug-resistant genes were studied less frequently with only 44 observations in total.

Nine different treatment processes were categorized into three general groups, including secondary treatment processes (activated sludge process, membrane bioreactor, and constructed wetlands), postsecondary treatment processes

(filtration and coagulation), and disinfection treatment processes (UV disinfection, anaerobic digester, ozonation, and chlorination). Their treatment efficiency for reducing ARGs and ARB varied depending on the processes and its operating conditions.

The *sulI* gene, associated with resistance to sulfonamide antibiotics, was the gene most studied with 32 observations including 25 decreased observations, four increased observations, and three unchanged observations in all treatment processes. Thirteen of the 32 observations were in disinfection treatment processes, and 12 of the 13 showed reductions. The activated sludge process with its variants such as MBRs was effective in reducing the *sulI* gene in 9 of 10 observations. There were only four observations for *sulI* gene in postsecondary treatment processes and constructed wetlands with two increasing and two decreasing.

There were 118 studies of 13 tetracycline-resistant genes among all treatment processes. In the activated sludge process and its variants such as MBRs, resistant genes decreased in 37 of 45 observations and increased in 8 of 45 observations. In disinfection processes, resistant genes decreased in 21 of 33 observations. Observations in plant-wide studies, provided without specific process information, showed decreases in 12 of 23 observations. Among all processes, tetracycline and sulfonamide resistance genes declined in approximately 70% of the observations and increased in 20% of the observations. ARGs and ARB for all genes decreased in approximately 70% of the observations and increased in 18% of observations, and 12% were unchanged.

The conclusion of this review is that treatment plants decreased the prevalence of ARGs and ARB in approximately 70% of the previous studies. Activated sludge and disinfection are the most effective. There is limited understanding as to what aspects of the activated sludge process are the most effective. Activated sludge plants with long SRT are more effective at removing trace organics and emerging contaminants, but there is no evidence for improved or decreased ARGs and ARB removal by long SRT plants. An important topic for future research is the fate of ARGs and ARB in long SRT processes, since the need for nutrient and improved trace organics removals is increasing. It is important for investigators examining the fate of ARGs and ARB in treatment plants to report as much as possible on the type of treatment process and the parameters of operation, since it is possible that treatment variations may have major impacts.

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